

รายงานสรุปผลการดำเนินงาน
โครงการวิจัยทุนส่งเสริมกลุ่มวิจัย สาขาเคมี
สำนักงานกองทุนสนับสนุนการวิจัย
ประจำปี 2540
(ธันวาคม 2540 – กุมภาพันธ์ 2544)

โครงการวิจัย เรื่อง

1. การสังเคราะห์และปฏิกิริยาสารอัลคาลอยด์ และสารประเภท Oxygen Heterocycle ต่าง ๆ
The syntheses and reactions of various alkaloids and oxygen heterocycles
2. การศึกษาเกี่ยวกับสมุนไพรไทยบางชนิด
Studies of some Thai medicinal plants

หัวหน้าโครงการวิจัย

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รหัสโครงการ : RTA/01/2540

ชื่อโครงการ : การสังเคราะห์และปฏิกิริยาสารอัลคาลอยด์และสารประเภท Oxygen
Heterocycle ต่าง ๆ และการศึกษาเกี่ยวกับสมุนไพรไทยบางชนิด

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วัตถุประสงค์ :

ภาพรวมของจุดประสงค์ของโครงการแรก คือ การหาวิธีการสังเคราะห์สารประเภทอัลคาลอยด์ เช่น Protoberberine Pavine Erythrina Benzazepine และ Oxygen Heterocycles ต่าง ๆ เช่น Wrightiadiene และ Diospyrol ให้ได้วิธีที่ดีกว่า มีประสิทธิภาพที่ดีกว่า และสามารถประยุกต์ในการสังเคราะห์อนุพันธ์ต่าง ๆ เพื่อให้ได้สารจำนวนมากขึ้นเพื่อใช้ในการทดสอบฤทธิ์ทางชีวภาพ.

สำหรับโครงการที่สองเป็นการวิจัยเกี่ยวกับสมุนไพรไทยบางชนิด เช่น ต้นสารภีบ้าน และ ต้นหัวร้อยรู นอกจากการแสวงหาความเป็นเลิศทางวิชาการแล้ว ยังต้องการหาตัวยาจากสมุนไพรเพื่อเป็นการ validate ข้อมูลต่าง ๆ ที่ใช้กันมาและเสริมสร้างให้มีผลผลิตจากภายในประเทศ เพื่อลดการนำเข้าและเพิ่มความสามารถในการพึ่งพาตนเองในอนาคต

ขอบเขตการวิจัย:

โดยทั่ว ๆ ไป ระเบียบวิธีการวิจัยของการสังเคราะห์ คือใช้ความรู้ ประสบการณ์และความสามารถเฉพาะบุคคล คิดถึงวิธีการที่จะใช้สังเคราะห์ด้วยวิธีการทางปฏิกิริยาเคมีต่าง ๆ หลังจากนั้นก็จะทดสอบปฏิกิริยาต่าง ๆ นั้น ด้วยการทดลองในห้องปฏิบัติการ ในการทำปฏิกิริยาต่าง ๆ นั้น สารที่ได้จากปฏิกิริยาก็จะนำมาแยก ทำให้บริสุทธิ์ด้วยวิธีการต่าง ๆ หลังจากนั้นก็จะพิสูจน์โครงสร้างของสารที่ได้ด้วยวิธีการทาง Spectroscopy เช่น IR , NMR (^1H , ^{13}C) , MS เมื่อพิสูจน์ว่าเป็นสารที่ต้องการแล้ว ก็จะต้องพยายามหากระบวนการต่าง ๆ ที่จะทำให้ได้สารมากที่สุด (highest yield) เพื่อนำไปทำปฏิกิริยาต่อ ๆ ไปจนได้สารที่ต้องการ ในกรณีที่ไม่ได้สารที่ต้องการหรือได้น้อยมาก งานวิจัยก็ต้องปรับเปลี่ยนไปเพื่อให้ได้สารที่ต้องการ

ในการศึกษาปฏิกิริยาของสารต่าง ๆ ก็เช่นเดียวกัน เมื่อสารทำปฏิกิริยากันแล้วก็ต้องแยกทำให้บริสุทธิ์ หาสูตรโครงสร้างต่าง ๆ หลังจากนั้นก็จะหาค่าอธิบายกลไกของปฏิกิริยา

งานวิจัยในด้านสมุนไพรจะประกอบด้วย การเก็บสมุนไพร (collection) หาชื่อต้นไม้ (identification) สกัด (extraction) แยกสารและทำให้บริสุทธิ์ (isolation & purification) หาสูตรโครงสร้าง

สร้าง (structural elucidation) และหาฤทธิ์ทางยา (pharmacological activity) ในบางกรณีจะทำการหาฤทธิ์ทางยาเป็นจุดชี้ในการหา fraction ที่น่าจะศึกษาโดยละเอียด

ผลที่ได้จากศึกษาวิจัย : ผลของการวิจัยได้บรรลุวัตถุประสงค์ ดังนี้ คือ

1. สามารถสังเคราะห์อัลคาลอยด์ประเภทต่าง ๆ เช่น Protoberberine Pavine Erythrina Benzazepine และ Lamellarin ได้ โดยใช้กระบวนการทางเคมีอินทรีย์ มีผลผลิตเป็นที่น่าพอใจ มีขั้นตอนสั้นหรือหลายขั้นตอนแต่ไม่ยุ่งยาก โดยสารอัลคาลอยด์เหล่านี้ มีความน่าสนใจทั้งในด้านกระบวนการที่ใช้สังเคราะห์กลไกของปฏิกิริยา และยังแสดงผลทางชีวภาพที่ดีหลาย ๆ ด้าน เช่น เป็นสารต้านเชื้อมะเร็ง และต้านเชื้อมาเลเรีย เป็นต้น
2. สามารถสังเคราะห์สารประเภท Oxygen Heterocycles เช่น Wrightiadione และอนุพันธ์ของ Diospyrol โดยประยุกต์ใช้กระบวนการทางเคมีอินทรีย์ และเป็นสารที่แสดงผลทางชีวภาพที่น่าสนใจ
3. สามารถสกัด แยกให้บริสุทธิ์ หาสูตรโครงสร้าง รวมทั้งการทดสอบฤทธิ์ทางชีวภาพของสมุนไพรไทย เช่น ต้นสารภีบ้าน ต้นหัวร้อยรู พบว่าสารสกัดจากต้นสารภีบ้าน มีฤทธิ์ยับยั้งเซลล์มะเร็งบางชนิดได้

การนำไปใช้ประโยชน์ :

การวิจัยนี้ได้ประสบความสำเร็จด้านการสังเคราะห์อัลคาลอยด์และสารประเภท Oxygen Heterocycles รวมถึงการสกัดสารสำคัญจากสมุนไพรไทยบางชนิด และการทดสอบฤทธิ์ทางชีวภาพของผลิตภัณฑ์ที่ได้ ซึ่งผลของการวิจัยนี้อยู่ในระดับอินทรีย์เคมีพื้นฐาน และทำในห้องปฏิบัติการ อย่างไรก็ตามก็สามารถใช้เป็นรากฐานของการสังเคราะห์และวิเคราะห์สารสำคัญที่มีประโยชน์ในด้านต่าง ๆ เช่น ทางการแพทย์เป็นยารักษาโรค หรือสามารถนำมาปรับใช้กับการวิจัยและพัฒนาในด้านต่าง ๆ ต่อไปในอนาคตได้

Keywords : Alkaloids
Organic Synthesis
Natural Products
Oxygen Heterocycles
Nitrogen Heterocycles

The Thailand Research Fund
Senior Research Scholar (Chemistry) 1997
(December 1997 – February 2001)

Project Code : RTA/01/2540

Project Title : The Syntheses and Reactions of Various Alkaloids and Oxygen
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Project Period : December 1997 – February 2001

Objectives :

The aim of our project involving the studies of various alkaloids is to develop novel, versatile, and efficient synthetic methodologies for (1) different classes of alkaloids such as protoberberine, pavine, erythrina, benzazepine and lamellarin and (2) oxygen heterocycles such as wrightiadione and diospyrol. The methodologies allow us to obtain numerous derivatives of these bioactive compounds in the amount suitable for establishing their biological profiles.

The other part of our project involves the studies of some Thai medicinal plants such as *Mammea siamensis* and *Hydrophytum formicarum* Jack. Aside from the academic interests in Thai herbs, we have focused on identifying the active ingredients of these plants to validate the available data involving their uses. In addition, it is our ultimate goal that these findings will result in more herbal production in Thailand to reduce the amount of imports while increasing the capability of our economic self-reliance.

Methodology :

In general, methodology in organic synthesis involves utilizing individual's knowledge, experiences, and ability to consider analytically and globally all available chemical reactions in organic chemistry. Following that, these carefully thought-out reactions will be performed in a laboratory. Various techniques in chemical isolation and

purification are crucial prior to spectroscopic identification of these compounds via infrared spectroscopy (IR), nuclear magnetic resonance spectroscopy (NMR: ^1H and ^{13}C), and mass spectrometry. Optimization for each chemical step is then carried out. Certain modifications may be necessary when some reactions give little or no desired products.

Our work in the studies of some Thai medicinal plants involves collecting the samples of interest. Following the identification of the plants, the samples are extracted and the compounds isolated into different fractions and each can be purified. Structural elucidation is then performed prior to screening the compounds through various biological assays for their pharmacological activities. In some cases, their pharmacological activities are the important guide and warrant further studies.

Results and Discussion :

- 1) We have successfully synthesized protoberberine, pavine, erythrina, benzazepine and lamellarin alkaloids. The synthetic plans are concise and simple. The syntheses present chemical challenges and some interesting mechanistic aspects of these reactions. Moreover, these alkaloids exhibit numerous biological activities including anticancer and antimalarial.
- 2) We have accomplished the synthesis of oxygen heterocycles such as wrightiadione and diospyrol derivatives by applying organic chemical processes and again, these compounds exhibit interesting biological activities.
- 3) We have extracted various dry samples of, isolated numerous compounds from, and elucidated structures of these compounds of some Thai medicinal plants such as *Mammea siamensis* and *Hydrophytum formicarum* Jack. Their biological evaluation was also performed and revealed that certain components from *Mammea siamensis* can inhibit the growth of some cancer cells.

Suggestions :

Our research has been successful both in the synthesis of alkaloids and oxygen heterocycles and in the extraction and isolation of bioactive compounds from some Thai medicinal plants. These compounds, both synthetic and natural, have been tested for their biological activities. The outcome of our research projects is mainly the application of basic and advanced knowledge in organic chemistry while the experiments can be performed in a basic research laboratory setting. In addition, it is our ultimate goal

that the knowledge gained from our research is among the essential fundamentals for further development in the synthetic and analytical work, especially in the development of novel potent therapeutic agents in the medicinal field.

Keywords : Alkaloids
Organic Synthesis
Natural Products
Oxygen Heterocycles
Nitrogen Heterocycles

รายงานสรุปโครงการวิทยุทุนส่งเสริมกลุ่มวิจัย สาขาเคมี
สำนักงานกองทุนสนับสนุนการวิจัย
ประจำปี 2540 – 2544
(ธันวาคม 2540 – กุมภาพันธ์ 2544)

โครงการวิจัยแบ่งเป็น 2 โครงการใหญ่ คือ

1. การสังเคราะห์และปฏิกิริยาสารอัลคาลอยด์ และสารประเภท oxygen heterocycle ต่าง ๆ

The syntheses and reactions of various alkaloids and oxygen heterocycles

2. การศึกษาเกี่ยวกับสมุนไพรไทยบางชนิด

Studies of some Thai medicinal plants

โดยโครงการแรกแบ่งเป็นโครงการย่อยอีก 6 โครงการดังนี้

1. การสังเคราะห์และปฏิกิริยาสารอัลคาลอยด์และสารประเภท oxygen heterocycle ต่างๆ

The syntheses and reactions of various alkaloids and oxygen heterocycles

1.1 การนำเอาปฏิกิริยาของ Heck และการปิดวงด้วย free radical มาประยุกต์ใช้ในการสังเคราะห์สารอัลคาลอยด์ประเภท Protoberberine, Pavine และ Erythrina

The applications of Heck reaction and free radical cyclization to the syntheses of protoberberine, pavine and erythrina alkaloids

1.2 การสังเคราะห์สารอัลคาลอยด์ประเภท Benzazepine และ Lamellarin

The syntheses of benzazepine and lamellarin alkaloids

1.3 การศึกษาปฏิกิริยาการสังเคราะห์ Wrightiadione และ Diospyrol

Synthetic studies towards the syntheses of Wrightiadione and Diospyrol derivatives.

1.4 ปฏิกิริยาระหว่างสารอัลคาลอยด์บางประเภทและอนุพันธ์กับสารประเภท hypervalent iodine รวมทั้งตัวออกซิไดซ์อื่น ๆ

The reactions of some alkaloids and derivatives with the hypervalent iodine compounds and other oxidising agents.

1.5 การศึกษาปฏิกิริยาการเปลี่ยนรูปของสารอัลคาลอยด์ประเภท α -hydroxy และ oxo isoquinoline

The transformations of α -hydroxy and oxo-isoquinoline alkaloids.

Investigation on synthetic process of some antibacterial fluoroquinolones

2. การศึกษาเกี่ยวกับสมุนไพรไทยบางชนิด

Chemical and pharmacological investigations of *Mammea siamensis*

1. รศ. ดร. สมศักดิ์ รุจิรวัดณ์ ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
สถาบันวิจัยจุฬาภรณ์ และ
2. รศ. ดร. จิตต์กวี ปวโร คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล

3. รศ. ดร. สุภาลักษณ์ ปรัชญาสิทธิกุล ภาควิชาเคมี คณะวิทยาศาสตร์
มหาวิทยาลัยศรีนครินทรวิโรฒ ประสานมิตร
4. รศ. ดร. อภิชาติ สุขสำราญ ภาควิชาเคมี คณะวิทยาศาสตร์
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มหาวิทยาลัยรามคำแหง
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มหาวิทยาลัยเทคโนโลยีพระจอมเกล้า ธนบุรี
7. ผศ. ดร. สุภัตรา โพธิ์พ่วง ภาควิชาวิศวกรรมเคมี คณะวิศวกรรมศาสตร์
สถาบันเทคโนโลยีราชมงคล
8. ดร. เพ็ญศรี บุญสุวรรณค์สง ภาควิชาเคมี คณะวิทยาศาสตร์
มหาวิทยาลัยเกษตรศาสตร์
9. ดร. พูลศักดิ์ สหกิจพิจารณา ห้องปฏิบัติการเภสัชเคมี สถาบันวิจัยจุฬาภรณ์
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14. นายนพพร ทศนา ห้องปฏิบัติการเภสัชเคมี สถาบันวิจัยจุฬาภรณ์
15. น.ส. วิไลลักษณ์ ปรัชญาวรากร ห้องปฏิบัติการเภสัชเคมี สถาบันวิจัยจุฬาภรณ์
16. นายสานิตย์ ทองเนตร ห้องปฏิบัติการเภสัชเคมี สถาบันวิจัยจุฬาภรณ์

นอกจากนี้ กลุ่มวิจัยยังได้ผลิตนักศึกษาในระดับปริญญาเอก ปริญญาโท ปริญญาตรี และนักวิจัย
อื่นๆ ที่ร่วมโครงการ ดังนี้

ชื่อ - นามสกุล	ต้นสังกัด		
	ภาควิชา	คณะ	มหาวิทยาลัย/สถาบัน
1. น.ส. จุติมา วงศ์จรรยา			สถาบันวิจัยจุฬาภรณ์
2. น.ส. ศิริพร วงษ์บัณฑิต			สถาบันวิจัยจุฬาภรณ์
3. น.ส. วิรงรอง กวีไตรภพ			สถาบันวิจัยจุฬาภรณ์
3. น.ส. ปฎิมา ไพบาล			สถาบันวิจัยจุฬาภรณ์
4. นายรัชชัย โลกะนัง	เคมี	วิทยาศาสตร์	มหิดล
5. น.ส. วรรณมา ผู้สุหา	เคมี	วิทยาศาสตร์	มหิดล

ชื่อ-นามสกุล	ต้นสังกัด		
	ภาควิชา	คณะ	มหาวิทยาลัย/สถาบัน
6. น.ส. รัชนก ปิ่นแก้ว	เคมี	วิทยาศาสตร์	มหิดล
7. น.ส. วิดา จินากิ่ง	เภสัชเคมี	เภสัชศาสตร์	มหิดล
8. น.ส. วันทนา มงคลวิสุทธิ	เคมี	วิทยาศาสตร์	รามคำแหง
9. นายประสิทธิ์ บุรพาเรืองแสง	เคมี	วิทยาศาสตร์	ศรีนครินทรวิโรฒ
10. นายอณูชา น้ำสอาด	เคมี	วิทยาศาสตร์	มหิดล
11. น.ส. สุดา จักรทอง	เคมี	วิทยาศาสตร์	มหิดล
12. นายสุพจน์ จันทร์เจริญ	เคมี	วิทยาศาสตร์	มหิดล
13. น.ส. อุมพร หวองเจริญพาณิชย์	เคมี	วิทยาศาสตร์	มหิดล
14. น.ส. พรรณรินทร์ เชยกลิ่น	เคมี	วิทยาศาสตร์	มหิดล
15. น.ส. ธนินี เพชรมณี	เคมี	วิทยาศาสตร์	มหิดล
16. น.ส. เกศแก้ว บุตรวงศ์	เคมี	วิทยาศาสตร์	ศรีนครินทรวิโรฒ
17. นายสุรัชย์ รัชพันธ์	เคมี	วิทยาศาสตร์	เกษตรศาสตร์
18. นางสาวภัทริยา เกษสี	เคมี	วิทยาศาสตร์	เทคโนโลยี พระจอมเกล้าธนบุรี
19. นายคมสันต์ สัจจสันต์	เคมี	วิทยาศาสตร์	เทคโนโลยี พระจอมเกล้าธนบุรี
20. น.ส. ศิรประภา ดิกะโกศล	เคมี	วิทยาศาสตร์	เทคโนโลยี พระจอมเกล้าธนบุรี
21. น.ส. วณิดา ลำซัด	เคมี	วิทยาศาสตร์	ศรีนครินทรวิโรฒ
22. นายเจษฎา ทวีกาญจน์	เคมี	วิทยาศาสตร์	มหิดล
23. น.ส. จรรยา บัวน่วม	เคมี	วิทยาศาสตร์	มหิดล
24. นายคงศักดิ์ โชติกุลสุวรรณ	เคมี	วิทยาศาสตร์	เทคโนโลยี พระจอมเกล้าธนบุรี
25. น.ส. เนติยา ตันทชุนห์	เคมี	วิทยาศาสตร์	เทคโนโลยี พระจอมเกล้าธนบุรี
26. นายธิตี จันทร์ภิรมย์	เคมี	วิทยาศาสตร์	มหิดล
27. น.ส. พุฒิรัตน์ สารบรรณ	เคมี	วิทยาศาสตร์	ศรีนครินทรวิโรฒ

จากการวิจัยมาเป็นลำดับ ผลงานวิจัยของโครงการต่าง ๆ ได้บรรลุวัตถุประสงค์ตามเป้าหมาย เป็นที่น่าพอใจ ดังรายงานความก้าวหน้าที่น่าสนใจไปเรียบร้อยแล้ว โดยผลงานส่วนหนึ่งได้เผยแพร่ในรูปโปสเตอร์และการบรรยายในการประชุมสัมมนาทางวิชาการทั้งในและต่างประเทศ ดังนี้

การเสนอผลงานในที่ประชุมวิชาการนานาชาติ

1. Bhavakul, V., Sahakitpichan, P. and Ruchirawat, S. Efficient Syntheses of the Polycyclic Isoindolone alkaloids. **21th IUPAC International Symposium on the Chemistry of Natural Products**. Beijing, China, October 11-16, 1998.
2. Sahakitpichan, P., Pisutjaroenpong, S. and Ruchirawat, S. Synthesis of Naphthol and 1,3-Dimethylisoquinoline Derivatives. **17th International Congress of Heterocyclic Chemistry**, Vienna, Austria.,1999.
3. Pisutjaroenpong, S., Predapitukkun, S., Mutarapat, T., Ruchirawat, S. Synthesis of C-13 Substituted Protoberberine Alkaloids. **17th International Congress of Heterocyclic Chemistry**, Vienna, Austria.,1999.
4. Wongjunya, T., Namsa-aid, A., Tepayakool, W., Lokanung, T. and Ruchirawat, S. The Synthetic Utilities of Free Radical Reaction in The Syntheses of Alkaloids. **The Fourth Princess Chulabhorn International Science Congress "Chemicals in the 21st Century"**, Bangkok, Thailand, 1999.
5. Sahakitpichan, P., Pisutjaroenpong, S., Thasana, N. and Ruchirawat S. Diospyrol Revisited : New Synthetic Studies. **The Fourth Princess Chulabhorn International Science Congress "Chemicals in the 21st Century"**, Bangkok, Thailand, 1999.
6. Popuang, S., Pisutjaroenpong, S., Pavaro, C., Ruchirawat, S., Watcharasit, P., Khamkong, P. and Satayavivad J. Synthesis and Pharmacology of a Potent New Antimalarial. **The Fourth Princess Chulabhorn International Science Congress "Chemicals in the 21st Century"**, Bangkok, Thailand, 1999.
7. Sahakitpichan, S., Bhavakul, V., Namsa-aid, A., Tontoolarug, S. and Ruchirawat S. The Synthetic Utilities of Organo Palladium in the Syntheses of Alkaloids. **The Fourth Princess Chulabhorn International Science Congress "Chemicals in the 21st Century"**, Bangkok, Thailand, 1999.
8. Bunsawansong, P., Pisutjaroenpong, S., Sahakitpichan, S. and Ruchirawat, S. The Unusual NMR Characteristics of Isoindolobenzazepine Alkaloids. **The Fourth Princess Chulabhorn International Science Congress "Chemicals in the 21st Century"**, Bangkok, Thailand, 1999.
9. Thasana, N. and Ruchirawat, S. Efficient Synthesis of The Tetracyclic Isoflavone : Wrightiadione. **The Fourth Princess Chulabhorn International Science Congress "Chemicals in the 21st Century"**, Bangkok, Thailand, 1999.
10. Panichakul, T., Chaisuriya, P., Wongbundit, S., Prawat, H., Ruchirawat, S. and Sirisinha, S. Cytotoxicity of Colchicine and Its Derivatives Against Human Cholangiocarcinoma Cell Line : Structure Activity Relationship. **The Fourth Princess Chulabhorn International Science Congress "Chemicals in the 21st Century"**, Bangkok, Thailand, 1999.
11. Siripong, P., Kanokmedakul, K., Wongsamran, T., Piyaviriyakul, S., Sangkajang, S., Hitotsuyanagi, Y., Takeya, K., Itokawa, H., Ruchirawat, S. and Cordell G.A. New Cytotoxic Prenylated Xanthenes from *Maclura amboinensis* BL. **The Fourth Princess Chulabhorn International Science Congress "Chemicals in the 21st Century"**, Bangkok, Thailand, 1999.

12. Buraparungsang, P., Prachayasittikul, S. and Ruchirawat, S. Investigation of *Hydnophytum formicarum* Jack. And *Polyalthia cerasoides* (ROXB) Benth. Ex BEDD. **The Fourth Princess Chulabhorn International Science Congress "Chemicals in the 21st Century"**, Bangkok, Thailand, 1999.
13. Kaweetripob, W., Mahidol, C., Prawat, H. and Ruchirawat, S. Chemical Investigation of *Mammea siamensis*. **IOCD International Symposium as Satellite Meeting of The Fourth Princess Chulabhorn Science Congress "Chemistry and Pharmacology of Asian Plants and Validation of Phytopharmaceuticals"**, Bangkok, Thailand, 1999.
14. Prachyawarakorn, V., Mahidol, C. and Ruchirawat, S. Two New Coumarins from the Twigs of *Mammea siamensis*. **IOCD International Symposium as Satellite Meeting of the Fourth Princess Chulabhorn Science Congress "Chemistry and Pharmacology of Asian Plants and Validation of Phytopharmaceuticals"**, Bangkok, Thailand, 1999.
15. Prawat, H., Mahidol, C. and Ruchirawat, S. Reinvestigation of *Derris reticulata*. **IOCD International Symposium as Satellite Meeting of the Fourth Princess Chulabhorn Science Congress "Chemistry and Pharmacology of Asian Plants and Validation of Phytopharmaceuticals"**, Bangkok, Thailand, 1999.

การเสนอผลงานในที่ประชุมวิชาการในประเทศ

Buraparungsang, P., Prachayasittikul, S. and Ruchirawat, S. Constituents from Ethyl Acetate Extracts of *Hydnophytum formicarum* Jack. **Science Symposium**, Bangkok, Thailand, 2000.

นอกจากนี้ยังมีผลงานตีพิมพ์ในวารสารระดับนานาชาติดังนี้

1. Mahidol, C., Ruchirawat, S., Prawat, H., Pisutjaroenpong, S., Engprasert, S., Chumsri, P., Tengchaisri, T., Sirisinha, S., and Picha, P. Biodiversity and Natural Product Drug Discovery. **Pure&Applied Chemistry**, 70 : 2065-2072, 1998.
2. Ruchirawat, S., Mutarapat, T., Sahakitpichan, S., Bhavakul V., and Mahidol, C. The Syntheses of Lamellarins and Isoindolobenzazepine Alkaloids. **Pure & Applied Chemistry** URL: <http://www.IUPAC.ORG/symposia/proceedings/phuket97/ruchirawat.html> 1999 IUPAC 6 pages, 70: 2128, 1999
3. Ruchirawat, S. and Sahakitpichan, P. A novel synthesis of isoinodolobenz-azepine alkaloids: Application to the synthesis of lennoxamine. **Tetrahedron Lett.**, 41: 8007-8010, 2000.
4. Mahidol, C. , Ruchirawat, S., Prawat, H. and Wongbundit, S. Cytotoxic Natural Products From Thai Plants: A Recent Study. **Pharmaceutical Biology**, 38: 6-15, 2000.
5. Prawat, H., Mahidol, C. and Ruchirawat, S. Reinvestigation of *Derris reticulata*. **Pharmaceutical Biology**, 38: 63-67, 2000.
6. Prachyawarakorn, V., Mahidol, C. and Ruchirawat, S. NMR Study of Seven Coumarins From *Mammea siamensis*. **Pharmaceutical Biology**, 38: 58-62, 2000.
7. Kaweetripob, W., Mahidol, C., Prawat, H. and Ruchirawat, Chemical Investigation of *Mammea siamensis*. **Pharmaceutical Biology**, 38: 55-57, 2000.
8. Ruchirawat, S. and Thasana, N. The First Synthesis of Wrightiadione. **Synthetic Commun.** In press.
9. Sutthivaiyakit, S., Nareeboon, P., Ruangrangsri, N., Ruchirawat, S., Pisutjaroenpong, S. and Mahidol, C. Labdane and pimarane diterpenes from *Croton joufra*. **Phytochemistry**, 56: 811-814, 2001.

10. Ruchirawat, S. and Predapitakkun, S. A Synthesis of Bipowine and Bipowinone. **Heterocycles**, 55: 371-376, 2001.
11. Ruchirawat, S. and Namsa-aid, A. An Efficient Synthesis of Argemonine, a Pavine Alkaloid. **Tetrahedron Lett.** 42, 1359-1361, 2001.
12. Ruchirawat, S. and Mutarapat, T. An Efficient Synthesis of Lamellarin Alkaloids : Synthesis of Lamellarin G. Trimethyl Ether. **Tetrahedron Lett.** 42,1205-1208, 2001.
13. Ruchirawat, S., Tontoolarug, S. and Sahakitpichan. Synthesis of 4-Aryltetrahydroisoquinolines : Application to the Synthesis of Cherylline. **Heterocycles**, in press. (No. 4, 2001)

ผลงานตีพิมพ์ในวารสารวิชาการในประเทศไทย

1. Ruchirawat, S.; Prachayasittikul, S. Stereospecific reduction of papaveraldine and papaverinol derivatives. **Songklanakarin J.Sci.Technol.**, : 57-64, 1998.
2. Mahidol, C.; Ruchirawat, S. Biodiversity conservation and Drug Discovery. **Songklanakarin J.Sci.Technol.**, 1-10, 1998

อย่างไรก็ตามยังมีผลงานวิจัยอีกหลายชิ้น ที่ได้รับการสนับสนุนจากสำนักงานกองทุนสนับสนุนการวิจัย ซึ่งอยู่ในระยะเตรียมข้อมูลเพื่อจะนำออกเผยแพร่ในลำดับต่อไปอีกด้วย

นอกจากนี้ กลุ่มวิจัยได้เชิญผู้เชี่ยวชาญจากต่างประเทศและในประเทศ มาบรรยายพิเศษเพื่อเป็นการแลกเปลี่ยนความคิดเห็นและเพิ่มพูนความรู้ความชำนาญในวิชาการใหม่ ๆ ทางด้านอินทรีย์เคมี และทำให้นักวิชาการไทยมีการติดต่อกับนักวิทยาศาสตร์ที่มีชื่อเสียงในต่างประเทศมากขึ้น

Title	Speaker	Date
Synthetic Implications in Natural Products Chemistry	Prof. G. Adam Department of Natural Product Chemistry Institute of Plant Biochemistry, Germany	October 6, 1998
Bioactive Natural Products from Plants	Prof. Dr. Wolfgang Kraus Department of Chemistry University of Hohenheim Stuttgart, Germany	October 22, 1998
Anionically Induced Domino Reactions : Some Recent Results	Prof. Dietrich Spitzner Department of Chemistry University of Hohenheim Stuttgart, Germany	October 22, 1998

Title	Speaker	Date
Novel Generation of 1,3-Dipoles Utilizing Group-14 Metals and Its Application to Heterocyclic Synthesis	Prof. Dr. Mitsuo Komatsu Department of Applied Chemistry Faculty of Engineering, Osaka University, Japan	November 10, 1998
Asymmetric Total Synthesis of Taxol	Prof. T. Mukaiyama Science University of Tokyo Japan	November 18, 1998
Combinatorial Biosynthesis : the Biosynthesis of Erythromycin and Rapamycin	Dr. James Staunton F.R.S Department of Chemistry University of Cambridge England	January 18, 1999
Some Aspects of Solid Phase Organic Synthesis	ดร. ชีรยุทธ วิไลวัลย์ ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	4 มีนาคม 2542
Molecular Genetics of Isoquinoline Alkaloid Biosynthesis	Prof. Dr. Toni Kutchan Institute of Pharmaceutical Science, University of Munich , Germany	April 7, 1999
Benzyne Zirconocene Complexes in Organic Synthesis	Dr. Roderick W. Bates Department of Chemistry Faculty of Sciences Chulalongkorn University	April 8, 1999
Synthetic Studies on (5R)-ABC Segment of Ciguatoxin	ดร. รุ่งนภา แซ่เอ็ง ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยบูรพา	6 พฤษภาคม 2542
Glycosidation on solid support glycosyl donor linked with a sulfonate traceless linker	อาจารย์ บุญเอก ยิ่งยงณรงค์กุล ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยรามคำแหง	6 มกราคม 2543

Title	Speaker	Date
The Secret of Helicene Resolution	ดร. เทียนทอง ทองพันชั่ง ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล	2 มีนาคม 2543
From antimalarial to anticancer : Artemisinin Derivatives and their mode(s) of action	ดร. พูนศักดิ์ พลอยประดิษฐ์ ห้องปฏิบัติการเภสัชเคมี สถาบันวิจัยจุฬาภรณ์	8 มิถุนายน 2543
A Biorational Approach to New Antibacterial Leads : Importance of The Nitrogen Heterocycles	Prof. John Bremner Department of Chemistry University of Wollongong Australia	June 20, 2000
[2 + 2] - , [2 + 3] - and [2 + 4] - Annulation Reactions of Quinones	Prof. William S. Murphy Department of Chemistry University College Cork Ireland	June 27, 2000
Diastereoselective Cyclization of Allene Using Organocobalt Reagent	นางสาวชिरาภรณ์ สัตย์เจริญ ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	14 กันยายน 2543
Stereocontrolled Synthesis of Indolizidine Rings	นางสาวจุฑาทิพ บุญสมบัติ ภาควิชาเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย	14 กันยายน 2543

การสนับสนุนจากสำนักงานกองทุนสนับสนุนการวิจัยนี้ นับว่าเป็นประโยชน์อย่างยิ่งต่อวงการวิจัยของประเทศ เป็นการส่งเสริมและให้กำลังใจแก่นักวิจัยให้มีความคล่องตัวในการทำงาน ทำให้เกิดความร่วมมือเป็นสหวิทยาการ ผลงานการวิจัยที่ได้นอกจากจะสามารถสร้างชื่อเสียงให้แก่นักวิจัยและประเทศชาติ เป็นประโยชน์โดยตรงกับความเป็นเลิศทางวิชาการแล้ว ยังสามารถนำมาปรับใช้กับการศึกษาพัฒนาในด้านต่าง ๆ เพื่อให้มีผลิตผลจากภายในประเทศ เพิ่มความสามารถในการพึ่งตนเองในอนาคตอีกด้วย

ผลงานที่ตีพิมพ์ในวารสารวิชาการนานาชาติ

Biodiversity and natural product drug discovery

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Abstract: The threat to biodiversity through the destruction of terrestrial and marine ecosystems coupled with the urgent need to find novel, new chemotherapeutic agents as well as active chemotypes as leads for effective drug development, make natural products research in drug discovery and development a top priority. The paper will highlight the research of selected natural products aiming at the discovery of therapeutic agents from Thai plants. Attention will be focussed on selected plants that possess cytotoxic properties.

INTRODUCTION

Biodiversity - the diversity of living forms - has lately attracted a great deal of interest and concern since biological resources constitute an asset with a great deal of immediate as well as potential benefit for the quality of life. Ironically, just as we begin to recognize some of the potential benefits that might accrue from our having a large number of species, we are also coming to a realization that there is a current decline in the number of species and that this may have catastrophic consequences (1). This decline in biodiversity is largely the result of human activities such as drastic transformation of natural landscapes or deforestation. These phenomena pose a serious threat to sustainable development since species diversity may well be our planet's most important and irreplaceable resource. Once depleted, species regeneration, if at all possible, might take 5 to 10 million years. Its loss would thus have profound negative effects on the overall quality of life on our planet and on our potential development. The consequences of a reduction in biodiversity through loss of species constitute a serious threat to human survival. The loss of species could reduce the availability of natural products used as raw materials for manufacturing and industry. It could also diminish the future availability of new genetic resources and wild germ plasm essential for breeding crop varieties with higher productivity and with greater resistance to insects, diseases, and adverse climatic conditions. In medical science, the loss of species could reduce the opportunity for treatment of diseases through the loss of medical models and new medicines as a result of reduction of the availability of natural products which have potential medicinal properties. Animals and human beings share certain similarities as well as differences in a number of biochemical processes and

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mechanisms. It is through the knowledge and research findings obtained from studies in animals that many physiological and biochemical mechanisms are unravelled, particularly those underlying the etiology of diseases that lead to development of therapeutic methods and discovery of agents currently employed.

Some animal species that are currently at risk have been shown to be valuable medical models offering windows for greater understanding of human physiology and biochemistry which may lead to successful treatments of diseases that are at present incurable. An example of medical model developed from animals species that would appear to be far apart in the biosystem is highlighted by research on the active constituents of frog toxins. The research undertaken by John Daly of the National Institutes of Health, USA, and colleagues since the early 1970's deserves special mention (2). Certain types of frogs found in tropical rain forests in Central and South America produce a wide range of biologically active alkaloids, which make the dart-poison frogs (*Dendrobatidae*) an extremely important medical model in our understanding of cross-species biochemistry. Some of these alkaloids are important scientific tools in the study of the basic unit of membrane function and neurophysiological research. The study of the mechanism of action of some of these frog toxins may provide clues for the production of new therapeutic agents for treatment of diseases such as Alzheimer's disease and other neurological disorders. The remarkably potent analgesic epibatidine was also discovered during these investigations. Unfortunately, continued deforestation will likely result in the extinction of the dart-poison frog because of the destruction of their habitat and the resulting loss of a possible tool for studying neurological disorders and other diseases.

The loss of other species could have immediate negative effects on the use of medicines derived from these endangered species. The rapid dying out of the periwinkle plant illustrates the negative result of deforestation. Both vinblastine and vincristine (3), derivatives of the periwinkle plant, have proved to be effective against tumor growth. Vinblastine has been shown to be a very effective treatment for Hodgkin's disease, and has also been used to treat breast cancer, Kaposi's sarcoma, and other diseases. Vincristine is well known for its 90% success rate in treating different types of childhood leukemia. Extinction of the periwinkle plants as a result of deforestation would thus result in a loss of treatments which have proven effective in treating certain illnesses. Throughout the ages, humans have exploited the cornucopia of nature as a source of medicines for the treatment of a variety of diseases. Plants have formed the basis for traditional medicine for thousand of years.

It is clear that demand for drugs, disposable consumer products, biological agents and insecticides will continue to increase for the foreseeable future. But unless we take specific action to protect and develop our environment under sustainable conditions, the window of opportunity for the discovery of new medicinal and biological agents will be shut forever.

IMPORTANCE OF NATURAL PRODUCTS IN DRUG DEVELOPMENT

In industrialized nations at the present time, some fifty percent of all prescribed drugs are derived or synthesized from natural products, the only available sources for which are animals, marine, plants, and micro-organisms. It is considered that because of the structural and biological diversity of their constituents, terrestrial plants offer a unique and renewable resource for the discovering of potential new drugs and biological entities. However, only 5-15% of the world's approximately 250,000 flowering plants have as yet been analysed for their possible medicinal uses (4). The most alarming

cause for concern is that, by the turn of this century, it is expected that some 25,000 species of plants will have ceased to exist. This represents about five plant species a day between now and the year 2000.

Moreover, in developing countries, medicinal plants continue to be the main source of medication. In China alone, 7,295 plant species are utilized as medicinal agents. The World Health Organization has estimates that for some 3.4 billion people in the developing world, plants represent the primary source of medicine. This represents about 88% of the world's inhabitants who rely mainly on traditional medicine for their primary health care (5). It is thus a matter of utmost concern to public health and indeed to human life that urgent action is taken to prevent further diminution of actual and potential availability of medicinal and biological agents. Natural remedies that, although undocumented, may have been used for many thousands of years by the human race must be appropriately catalogued to ensure that vital ethnomedical information is not lost for ever. Farnsworth et. al. reported that at least 119 compounds derived from 90 plants species can be considered as important drugs currently in use in one or more countries, with 77% of these being derived from plants used in traditional medicine (5). The importance of natural products is also evidenced by the fact that in 1991 nearly half of the best selling drugs were either natural products or their derivatives (6).

NATURAL PRODUCTS AS ANTICANCER AGENTS

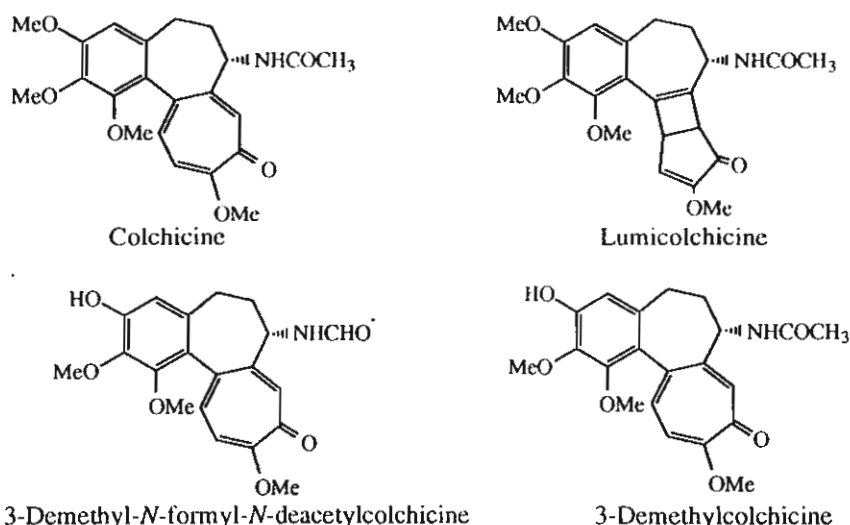
Apart from being an excellent source of anti-infectious drugs (8,13,14) plants are also good source of anticancer agents (7-12). National Cancer Institute (NCI), USA has launched an extensive program for the development of natural products for the treatment of various forms of cancer. Many clinically useful drugs have been discovered from various plants. These include vinblastine and vincristine from *Catharanthus roseus* and the semisynthetic, etoposide, as well as the recently discovered taxol and the semi synthetic taxotere. Taxol (paclitaxel) has been isolated from the bark of the Pacific or American yew tree, *Taxus brevifolia*. The discovery of taxol has indeed heightened the interest in plant-derived anticancer drug.

The addition of taxol to the list of anticancer drug is testimony to the synergism of broadly based contributions from multidisciplinary scientific endeavour. The isolation and structure elucidation led the way to the pharmacological and toxicological testings. The finding of baccatin III from other phytochemical sources coupled with the synthetic organic chemistry make the drug available for human trials. The finding that taxoids act through the stabilization of microtubules has led to the search of new agents that function by a comparable mechanism. Towards this end, new compounds have been discovered. Epothilones (15) are a new class of macrocyclic natural products which were first isolated from myxobacteria (16). Epothilones are more potent than taxol in some cell lines and they hold great promise for further investigation. In addition to the above mentioned clinically approved drugs and promising drug candidates, some other plant-derived compounds show a great deal of promise for future use as anticancer agents. Camptothecin (17) was originally isolated from the Chinese tree *Camptotheca acuminata* and a number of camptothecin analogues (18) are currently being developed as anticancer agents. Camptothecin was established as having *in vivo* activity against the murine leukemia and rat Walker carcinosarcoma 256 models. While early clinical trials on the parent alkaloid and camptothecin sodium were not particularly successful due to toxicity

problems, interest in camptothecin intensified once it was discovered that it exhibits a novel mechanism of action by inhibiting the enzyme DNA topoisomerase I. Accordingly, a number of camptothecin analogues have been developed in an attempt to reduce toxicity, optimize efficacy, and improve water solubility without opening the lactone ring present in the parent molecule. Topotecan is one of the camptothecin analogue which is under clinical trial. Another plant derived alkaloid which is also under clinical trial is homoharringtonine (19,20), a cephalotaxine alkaloid. The compound was originally isolated from *Cephalotaxus harringtonia*, it shows antineoplastic activity, especially against murine lymphocytic leukemias. It was found to be more active than vincristine against mouse leukemias and melanomas.

Apart from our study of *Phyllanthus amarus* (21), we have also investigated *Gloriosa superba* Linn. for anticancer activity. *Gloriosa superba* Linn. is known in Thai as "Dong Dueng" or "Dao Dueng", a climber plant in the family "Colchicaceae", which is widely distributed in the tropical part of Asia and Africa, with many varieties presented in Thailand.

The active principle of *Gloriosa superba* is colchicine which has long been used for the treatment of arthritis. From the dried tuber of *Gloriosa superba*, four tropolone alkaloids were isolated and identified as colchicine, lumicolchicine, 3-demethyl-*N*-deformyl-*N*-deacetylcolchicine and 3-demethylcolchicine (22).



The structures were elucidated using various spectroscopic techniques including UV, IR, MS, and one- and two dimensional NMR.

The biological activity testings of various extracts of *Gloriosa superba* for cytotoxicity were carried out using the P 388 cell line, with 5-fluoro-uracil (5-FU) as the positive control. The ED₅₀ of the different "Gloriosa" extracts are shown in the table.

Colchicine exhibits very low ED₅₀ value suggesting the potent cytotoxicity of the compound. The chloroform, methanol and petroleum ether extracts of *Gloriosa superba* also show very low ED₅₀ value which could result from the presence of colchicine in these extracts.

Compounds	ED ₅₀ values ($\mu\text{g/ml}$)
5-Fluorouracil	0.0189
Colchicine	0.0071
Methanolic extract	0.49
Chloroform extract	0.02
Petroleum ether extract	2.07
β -Lumicolchicine	>20
3-Demethyl- <i>N</i> -formyl- <i>N</i> -deacetyl-colchicine	0.0252

It is interesting to note that the ED₅₀ value of 3-demethyl-*N*-formyl-*N*-deacetylcolchicine is very close in value to the ED₅₀ of 5-FU which is a widely used anticancer agent, suggesting the strong cytotoxic potency of this compound. In this particular test, β -lumicolchicine was found to be inactive, suggesting that the tropolone ring is crucial for the anticancer activity.

Methanol extract was tested against other cancer cell lines, such as KB-3, KB-V-1, BCA-1, HT-1080, LUC-1, MEL-2, A-431, LNCaP, Lul and ZR-75-1 cell lines. Some relevant results are shown in the table.

Cell lines treated	Percentage of cell growth*
KB-3	0
KB-V1 (- VLB)	100
KB-V1 (+VLB)	100
Lul	38.0
LNCaP	30.9
ZR-75-1	6.8

* represent the percent growth of extract-treated cell at concentration 20 $\mu\text{g/ml}$

The results are expressed as the percent growth of the cells treated with the extracts at the concentration of 20 microgram per ml. The extracts exhibited a very potent activity against the drug-sensitive KB-3 cell line, however, it did not mediate a cytotoxic response in the multidrug-resistance KB-V1 cell line, in the presence or the absence of vinblastine. In addition, the extract showed potent activity against the human lung cancer, prostate cancer and breast cancer cell lines.

Another cancer cell line of our interest is the cholangiocarcinoma cell lines. Cholangiocarcinoma (23), a form of bile duct cancer, is a rare type of cancer in the western world but it is highly prevalent in Thailand and in many other Asian countries in our geographical region. The cause of the disease is believed to be associated with infestation of *Opisthorchis viverrini*(O.V.) or liver fluke and exposure to a chemical carcinogen in food or in the environment, presumably dimethylnitrosamine (DMN).

We have been interested in the evaluation of the effectiveness of some new anticancer agents against cholangiocarcinoma. This process was carried out by the *in vitro* testing of our established cholangiocarcinoma (HuCCA-1) cell line (24). Some colchicine derivatives have been subjected to cytotoxicity testing, using microculture protein assay and the results are as shown.

The ED₅₀ values ($\mu\text{g/ml}$) against KB and HuCCA-1 cell lines

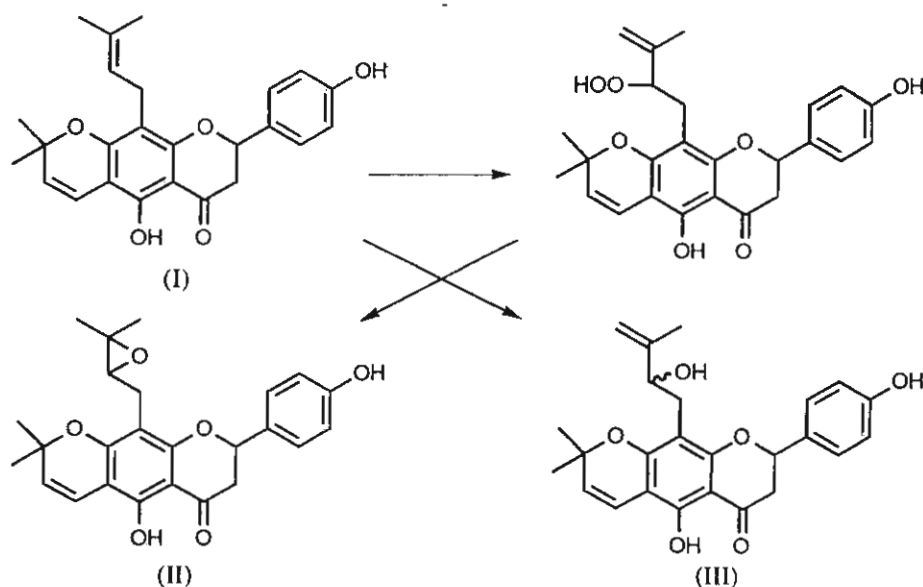
<i>Gloriosa superba</i>	KB	HuCCA-1
Methanol extract	0.5	2.5
3-Demethyl- <i>N</i> -formylcolchicine	0.03125	0.0625
3-Demethyl- <i>N</i> -formyl- <i>N</i> -deacetylcolchicine	0.03125	0.0625

HuCCA-1 = Human cholangiocarcinoma cell line

The ED₅₀ values for the cholangiocarcinoma cell line for the methanol extract was found to be 2.5 micrograms per ml, while both the ED₅₀ of 3-demethyl-*N*-formylcolchicine and 3-demethyl-*N*-formyl-*N*-deacetylcolchicine was found to be 0.0625 microgram per ml, in contrast with the ED₅₀ value of about 0.5 microgram per ml of colchicine. These values were approximately two times higher than the ED₅₀ values for the KB cell line, in all tests conducted. These results showed that the cholangiocarcinoma cell line is highly susceptible to the derivatives of the tropolone alkaloids, at least when testing *in vitro*. Whether or not these agents will be effective *in vivo* remains to be determined in further experiments.

We have recently investigated another plant called *Derris reticulata* locally known as Cha-aim thai (25). Cha-aim thai is a medicinal plant of Thailand used for the relief of thirst and as an expectorant. Our studies led to the isolation of two new pyranoflavanone compounds. Lupinifolin (I), a known flavanone was isolated as the major constituent of this plant.

Detailed analysis of NMR spectra through COSY, NOSEY, APT, HETCOR and selective INEPT confirmed the structure of lupinifolin (I) as the flavanone derivative as shown. The first unknown isolated, named epoxyepinifolin (II), was proved to be the 2,3 epoxide of lupinifolin by spectroscopic methods. The structure of the epoxide was further confirmed by successful epoxidation of lupinifolin with magnesium monoperoxyphthalate hexahydrate (MMPP). The other new compound isolated was named dereticulatin (III) and the structure was found to be hydroxy derivatives through the analysis of the NMR spectra of the corresponding triacetate.



With regard to the biogenetic relationship of these flavanones, dereticulin could be considered to be derived from epoxylupinifolin by the opening of the epoxide ring. Epoxylupinifolin could be formed from oxidation of lupinifolin. Alternatively, it is appealing to speculate the Ene reaction of the double bond in the lupinifolin to give the hydroperoxide intermediate which could then react with lupinifolin again to give directly the epoxylupinifolin and dereticulin.

We have also carried out the *in vitro* bioassay evaluation of lupinifolin, epoxylupinifolin and dereticulin triacetate. Each of the compounds inhibited the P-388 cell line at 0.4-0.5 microgramme per ml, but were inactive against the KB cell line.

ACKNOWLEDGMENTS

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REFERENCES

1. E. Chivian. In *Critical Condition: Human Health and the Environment* (E. Chivian, M. McCally, H. Hu and A. Haines, eds.) M.I.T. Press (1993).
2. J.W. Daly. *J. Nat. Prod.* **61**, 162-172 (1998).
3. M. Neuss, M. Gorman, W. Hargrove, N.J. Cove, K. Biemann, G. Buchi and R.E. Manning *J. Am. Chem. Soc.* **86**, 1441 (1964).
4. M.F. Balandrin, A.D. Kinghorn and N.R. Farnsworth. In *Human Medicinal Agents from Plants* (A.D. Kinghorn and M.F. Balandrin, eds.), Symposium Series No. 534, American Chemical Society, Washington, D.C. 2-12 (1993).
5. N.R. Farnsworth, O. Akerele, A.S. Bingel, D.D. Soejarto and Z. Guo, *Bull. WHO* **63**, 965-981 (1985), N.R. Farnsworth and A.S. Bingel. In *New Natural Products and Plant Drugs with Pharmacological, Biological or Therapeutical Activity* (H. Wagner and P. Wolff, eds) 61-73 (1977). Springer, New York.
6. M.J. O'Neill and J.A. Lewis. In *Human Medicinal Agents from Plants* (A.D. Kinghorn and M.F. Balandrin, eds.), Symposium Series No. 534, American Chemical Society, Washington, D.C. 48-55 (1993).
7. K. Hostettmann, O. Poterat and J.L. Wolfender. *Chimia* **52**, 10-17 (1998), M. Hamburger and K. Hostettmann. *Phytochemistry* **30**, 3864-3874 (1991).
8. G.M. Cragg, D.J. Newman and K.M. Snader. *J. Nat. Prod.* **60**, 52-60 (1997).
9. M. Suffness, G.M. Cragg, M.R. Grever, F.J. Grifo, G. Johnson, J.A.R. Mead, S.A. Schepartz, J.M. Venditti and M. Wolpert. *Int. J. Pharmacog.* **33**, 5-16 (1995).
10. A.D. Kinghorn, N.R. Farnsworth, C.W.W. Beecher, D.D. Soejarto, G.A. Cordell, J.M. Pezzuto, M.E. Wall, M.C. Wani, D.M. Brown, M.J. O'Neill, J.A. Lewis and J.M. Besterman. *Int. J. Pharmacog.* **33**, 48-58 (1995).
11. G.A. Cordell, N.R. Farnsworth, C.W.W. Beecher, D.D. Soejarto, A.D. Kinghorn, J.M. Pezzuto, M.E. Wall, M.C. Wani, D.M. Brown, M.J. O'Neill, J.A. Lewis, R.M. Tait and T.J.R. Harris. In *Human Medicinal Agents from Plants* (A.D. Kinghorn and M.F. Balandrin, eds.), Symposium Series No. 534, American Chemical Society, Washington, D.C. 193-204 (1993).

12. F. Valeriote, T. Corbett, P. LoRusso, R.E. Moore, P. Scheuer, G. Patterson, V. Paul, G. Grindey, R. Bonjouklian, H. Pearce and M. Suffness. *Int. J. Pharmacog.* **33**, 59-66 (1995).
13. J.D. Phillipson and C.W. Wright. *Planta Med.* **57**, S53-59 (1991)
14. C. Mahidol, H. Prawat and S. Ruchirawat. In *Biocative Natural Products from Thai Medicinal Plants*, chapter "Phytochemical Diversity : A Source of New Industrial Products", published by The Royal Society of Chemistry, RSC, England, 1997 (in press)
15. R. Finlay. *Chem. Ind. (London)* **24**, 991-996 (1997).
16. K. Gerth, N. Bedorf, G. Hoffe, H. Irschik and H. Reichenbach. *J. Antibiot.* **49**, 560-563 (1996).
17. M.E. Wall, M.C. Wani, C.E. Cook, K.H. Palmer, A.T. McPhail and G.A. Sim. *J. Am. Chem. Soc.* **88**, 3888-3890 (1966).
18. M.E. Wall and M.C. Wani. In *Human Medicinal Agents from Plants* (A.D. Kinghorn and M.F. Balandrin, eds.), Symposium Series No. 534, American Chemical Society, Washington, D.C. 149-169 (1993)
19. R.G. Powell, D. Weisleder and C.R. Smith Jr. *J. Pharm. Sci.* **61**, 1227-1230 (1972).
20. E. Feldman, Z. Arlin, T. Ahmed, A. Mittelman, C. Pucchio, H. Chun, P. Cook and P. Baskind. *Leukemia* **6**, 1189-1191 (1992).
21. A. Somanabandhu, S. Nitayangkura, C. Mahidol, S. Ruchirawat, K. Likhitwitayawuid, H-L. Shieh, H. Chai, J.M. Pezzuto and G.A. Cordell. *J. Nat. Prod.* **56**, 233-239 (1993).
22. S. Engprasert. M.Sc. thesis, Mahidol University 1995; H.G. Capraro and A. Brossi. In *The Alkaloids* (A. Brossi, ed.) **23**, 1-70 (1984). Academic Press; K.W. Bentley. In *The Isoquinoline Alkaloids*, chapter 20, 389-445 (1998) Harwood Academic Publishers
23. S. Sirisinha, S. Petmir, P. Utaisincharoen and T. Tengchaisri. *Asian J. Surg.* **19**, 198-206 (1993).
24. S. Sirisinha, T. Tengchaisri, S. Boonpucknavig, N. Prempracha, S. Ratanarapee and A. Pausawasdi. *Asian Pacific J Allergy Immunol.* **9**, 153-157 (1991).
25. C. Mahidol, H. Prawat, S. Ruchirawat, K. Likhitwitayawuid, Long-ZeLin and G.A. Cordell. *Phytochemistry* **45**, 825-829 (1997).

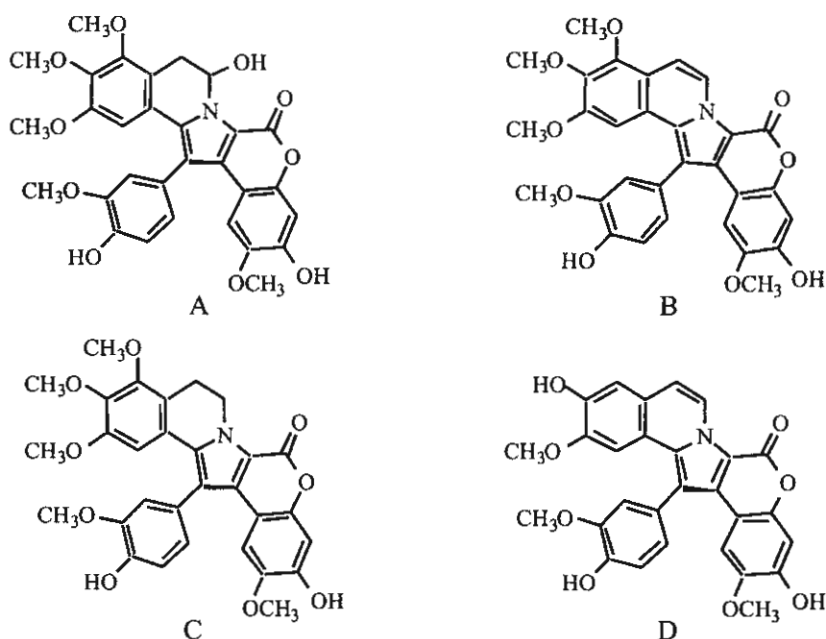
The Syntheses of Lamellarins and Isoindolobenzazepine Alkaloids

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Abstract: Two efficient synthetic routes for the syntheses of lamellarin alkaloid and isoindolobenzazepine alkaloid are described.

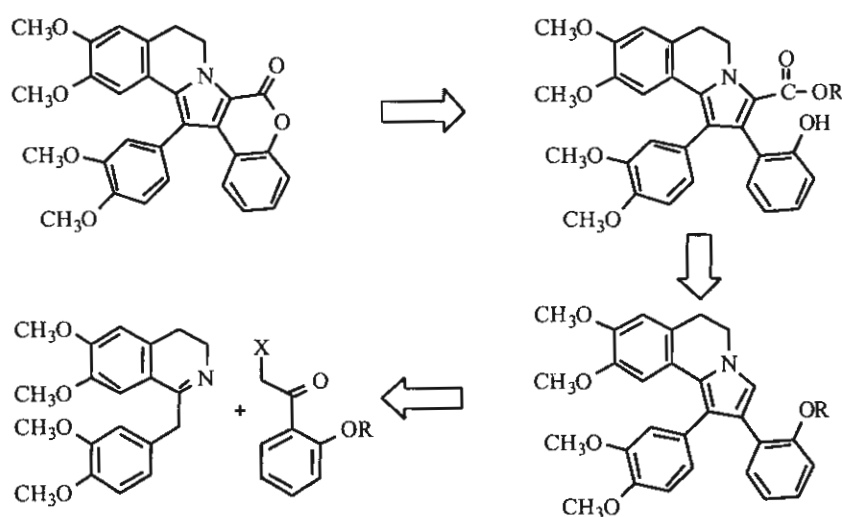
Lamellarins are a group of marine natural products which were isolated from the prosobranch mollusc *Lamellaria sp* and the ascidians.



*Invited lecture presented at the International Conference on Biodiversity and Bioresources: Conservation and Utilization, 23–27 November 1997, Phuket, Thailand. Other presentations are published in *Pure Appl. Chem.*, Vol. 70, No. 11, 1998.

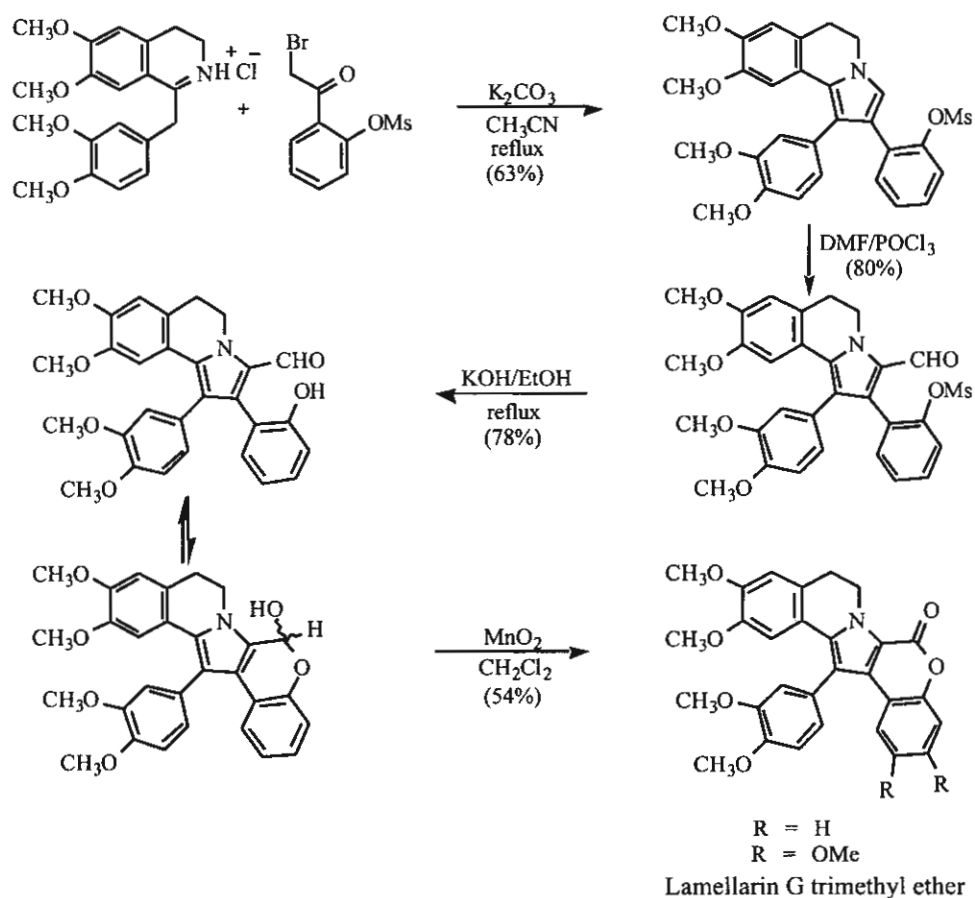
The first four lamellarins were isolated by Faulkner et al. in 1985 and named Lamellarins A, B, C, D. The structure of Lamellarin A was determined by X-ray crystallographic analysis and the structures of the remaining compounds were derived from spectroscopic data (1). More than twenty lamellarins have so far been isolated and identified (2). Some of these lamellarins exhibit interesting biological activities (3) including cell division inhibition, cytotoxicity, and immunomodulatory activity.

The core skeleton of these lamellarins can be viewed as the linking of the pyrroloisoquinoline with the lactone unit and so far three synthetic routes have been reported for the synthesis of these skeletons (4-6). Our synthetic route is based on the retrosynthetic analysis as shown.



Breaking of the lactone ring leads to carboxy phenolic compound, this carboxy phenolic compound can in turn be generated from the phenolic pyrroloisoquinoline molecule. It is envisaged that the pyrroloisoquinoline unit can be derived from the reaction of 3,4-dihydroisoquinoline derivative and the phenacyl halide molecule.

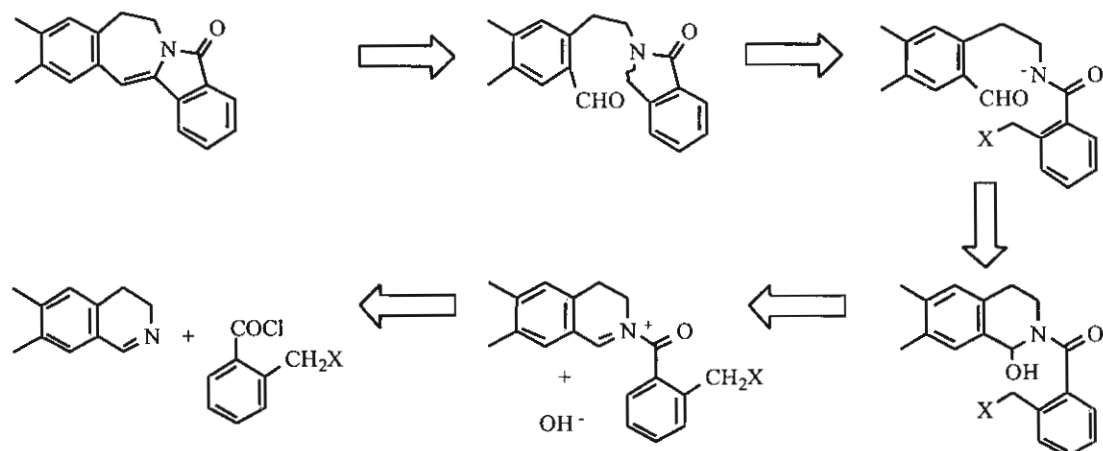
Indeed, the synthesis of the lamellarin skeleton can be accomplished via the above retrosynthetic analysis and this is shown in the scheme I.



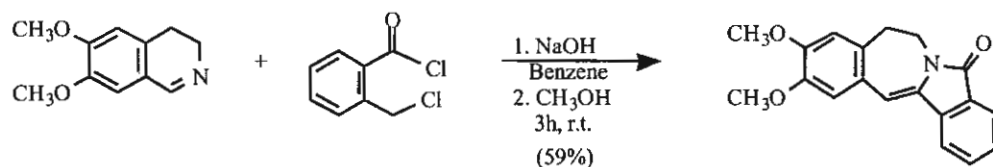
Scheme I

With the appropriate starting materials, Lamellamin G trimethyl ether was successfully synthesized.

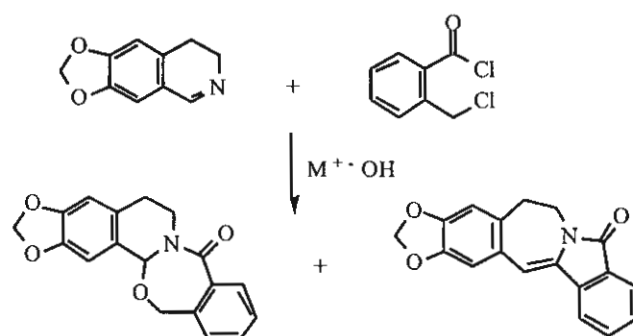
Our research group has also been interested in the synthesis of various benzylisoquinoline-derived alkaloids, including the isoindolobenzazepines (7). Our synthetic approach was based on the retrosynthetic analysis of the isoindolobenzazepine skeleton as shown.



Breaking of the carbon-carbon double bond of isoindolobenzazepine can lead to the aldehyde lactam intermediate. Disconnection of the carbon-nitrogen bond followed by condensation of amide anion with aldehyde group then gives the pseudobase. Pseudobase can be formed by the reaction of 3,4-dihydroisoquinoline with 2-halomethylbenzoyl chloride in the presence of hydroxide ion. This retrosynthetic analysis has been exploited for a one-pot synthesis of simple isoindolobenzazepine as shown.



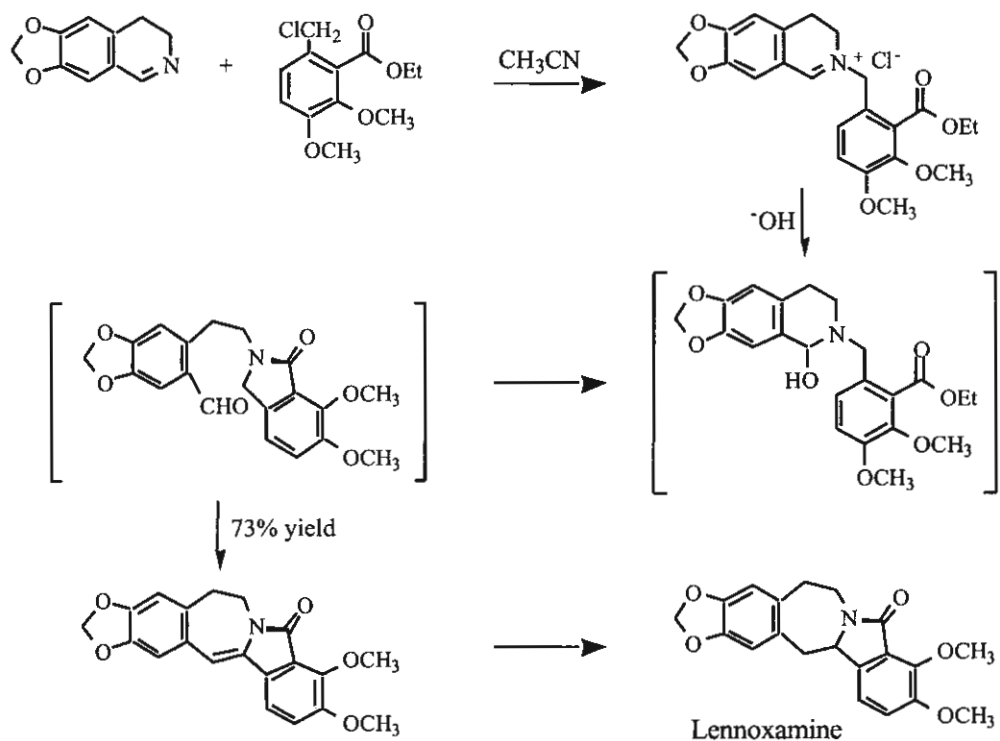
We have extended our study to the reaction of 2-chloromethylbenzoyl chloride with 3,4-dihydroisoquinoline and 3,4-dihydro-6,7-methylenedioxyisoquinoline in the presence of both sodium hydroxide and potassium hydroxide and these are shown.



	Cyclic ether		Benzazepine
For $M^+ = Na^+$	19 %	Yield	27 %
For $M^+ = K^+$	34 %	Yield	9 %

The above results suggested that while the approach is very efficient for the methoxylated isoquinoline, it cannot be efficiently utilized for the synthesis of isoindolobenzazepine containing

methylenedioxy group. However, we have successfully devised an alternative method for a very efficient synthesis of Lennoxamine, a natural product containing the methylenedioxy group, and the approach is as shown in scheme II.



Scheme II

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REFERENCES

1. R.J. Andersen, D.J. Faulkner, H. Cun-heng, G.D. Van duyne and J. Clardy. *J. Am. Chem. Soc.* **107**, 5492-5495 (1985).
2. M. Venkata Rami Reddy, D.J. Faulkner, Y. Venkateswarlu and M. Rama Rao. *Tetrahedron* **53**, 3457-3466 (1997).
3. A.R. Carroll, B.F. Bowden and J.C. Coll. *Aust. J. Chem.* **46**, 489-501 (1993).
4. A. Heim, A. Terpin and W. Steglich. *Angew. Chem.* **109**, 155-156 (1997).
5. F. Ishibashi, Y. Miyazaki and M. Iwao. *Tetrahedron* **53**, 5951-5962 (1997).

6. M.G. Banwell, B.L. Flynn and D.C.R. Hockless. *Chem. Commun.* 2259-2260 (1997).
7. C.Schöpf and M. Schweickert. *Chem. Ber.* **98**, 2566-2571 (1965); H.O. Bernhard and V. Snieckus. *Tetrahedron Lett.* **51**, 4867-4870 (1971); S. Teitel, W. Klötzer, J. Borgese and A. Brossi. *Can. J. Chem.* **50**, 2022-2024 (1972); S. Ruchirawat, W. Lertwanawatana, S. Thianpatanagul, J.L. Cashaw and V.E. Davis. *Tetrahedron Lett.* **25**, 3485-3488 (1984); P.L. Barili, R. Fiaschi, E. Napolitano, L. Pistelli, V. Scartoni and A. Marsilli. *J. Chem. Soc., Perkin Trans. I* 1654-1658 (1981); J. Chiefari, W. Janowski and R. Prager. *Tetrahedron Lett.* **27**, 6119-6122 (1986); P.H. Mazzocchi, C.R. King and H.L. Ammon. *Tetrahedron Lett.* **28**, 2473-2476 (1987); S.V. Kessar, T. Singh and R. Vohra. *Tetrahedron Lett.* **28**, 5323-5326 (1987); C.J. Moody and G.J. Warrellow. *Tetrahedron Lett.* **28**, 6089-6092 (1987); F.G. Fang and S.J. Danishefsky. *Tetrahedron Lett.* **30**, 2747-2750 (1989); S. Yasuda, Y. Sugimoto, C. Mukai and M. Hanaoka. *Heterocycles* **30**, 335-337 (1990); Y. Koseki and T. Nagasaka. *Chem. Pharm. Bull.* **43**, 1604-1606 (1995); G. Rodriguez, M. Cid Magdalena, C. Saa, L. Castedo and D. Dominguez. *J. Org. Chem.* **61**, 2780-2782 (1996); H. Ishibashi, H. Kawanami and M. Ikeda. *J. Chem. Soc., Perkin trans. I* 817-821 (1997) and references cited therein.



Pergamon

Tetrahedron Letters 41 (2000) 8007–8010

TETRAHEDRON
LETTERS

A novel synthesis of isoindolobenzazepine alkaloids: application to the synthesis of lennoxamine

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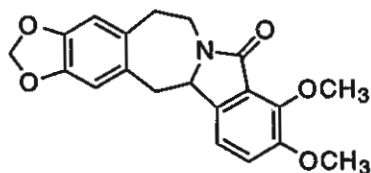
Received 5 June 2000; revised 9 August 2000; accepted 16 August 2000

Abstract

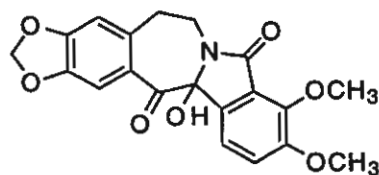
A novel, highly efficient synthesis of lennoxamine, a representative of isoindolobenzazepine alkaloid, is described. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: lennoxamine; isoindolobenzazepine.

Lennoxamine **1**¹ and chilenine **2**² are two representatives of a class of isoindolobenzazepine alkaloids.³ Both compounds were first found in the plants of the Chilean *Berberis* species, lennoxamine was isolated from *Berberis darwinii* Hook while chilenine was found in *Berberis empetrifolia* Lam. Due to their unique structural features, the isoindolobenzazepine alkaloids⁴ in general and chilenine^{5,6} and lennoxamine^{6,7} in particular, have captured the interest of many groups of synthetic chemists.



1



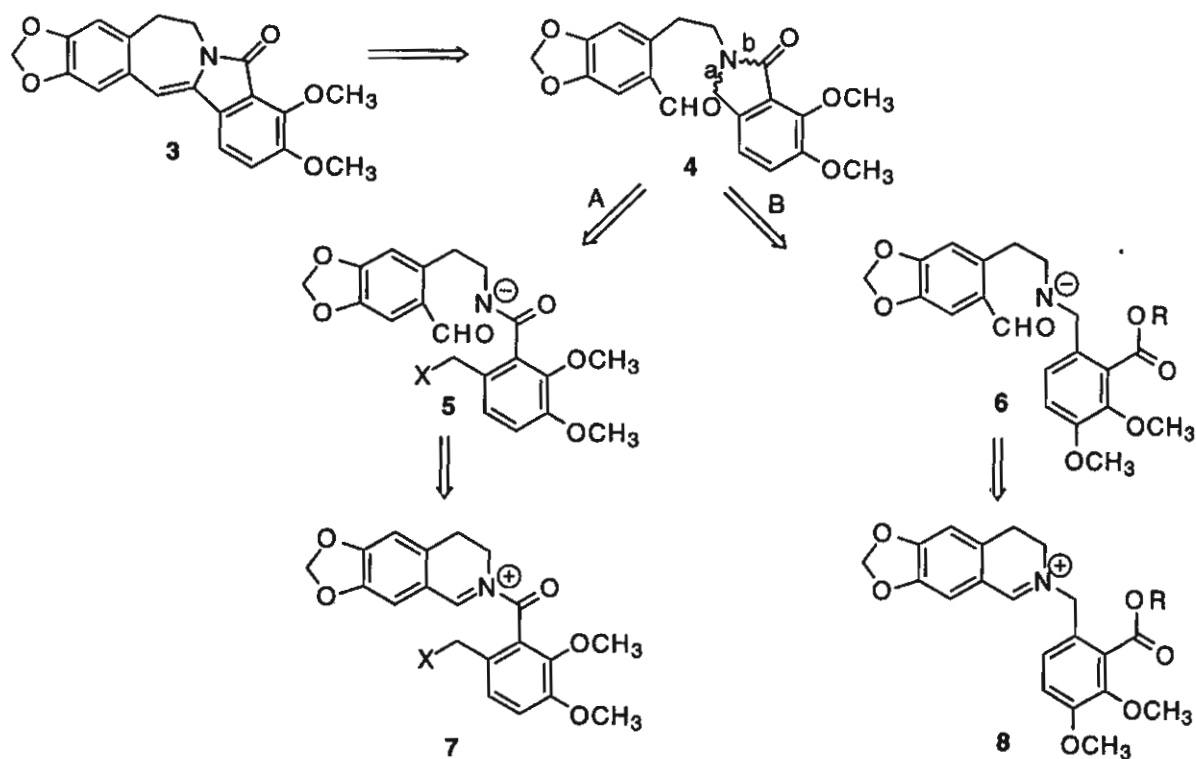
2

We have previously reported^{4b} the synthesis of the isoindolobenzazepine (aporphoadane) skeleton by using the route suggested by retrosynthetic analysis as shown in route A. However, attempts to apply this route to the synthesis of more complex oxygenated natural products

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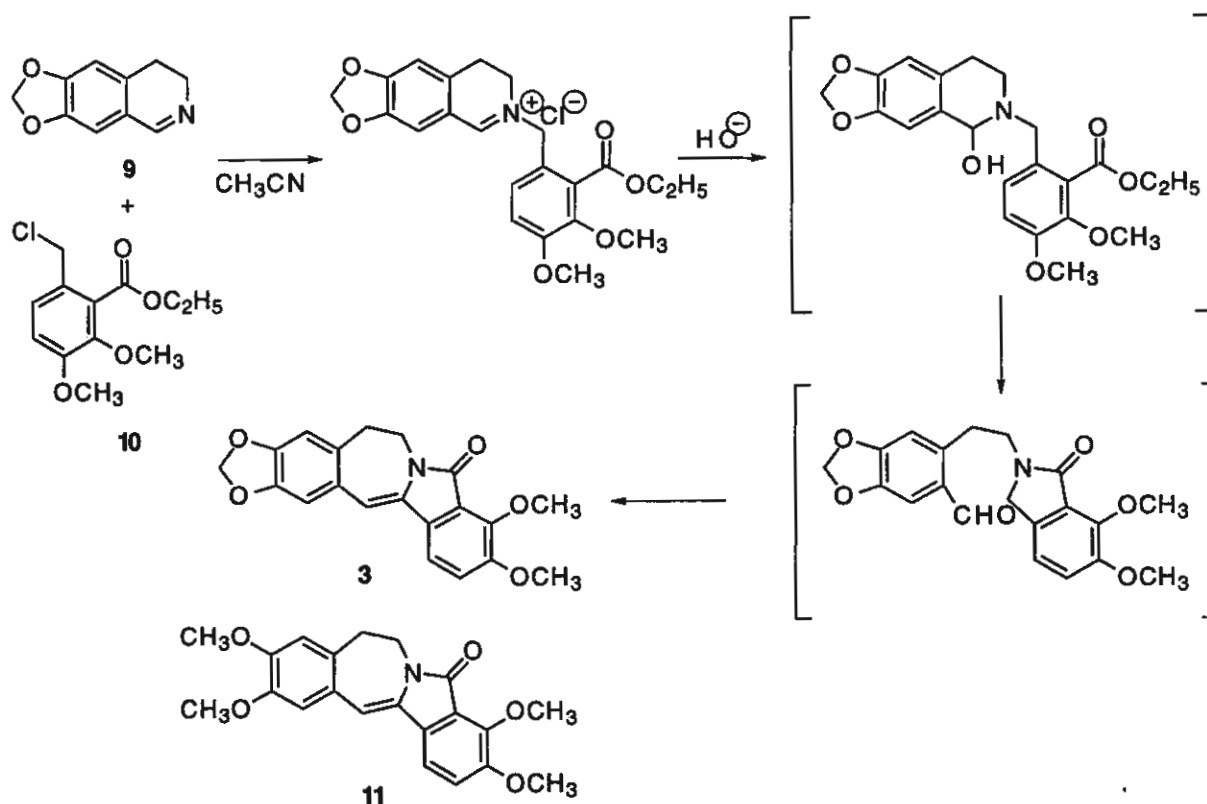
required the synthesis of not readily accessible halomethylbenzoyl chlorides. We have also found that when 6,7-methylenedioxy-3,4-dihydroisoquinoline was used as one of the components in the reaction with chloromethylbenzoyl chloride, the required lactam was obtained in disappointing yield. We have now solved the above drawbacks and successfully applied a new approach to the synthesis of lennoxamine.

Our retrosynthetic analysis is shown in Scheme 1. Breaking of the carbon-carbon double bond of the benzazepine skeleton in dehydrobenzazepine **3** leads to the lactam intermediate **4**. In our previous analysis, this lactam intermediate would be formed by the intramolecular alkylation of the amide intermediate **5** in route A. However, the alternative breaking of bond b in route B leads to the amino aldehyde intermediate **6**, i.e. the formation of the lactam would involve the reaction of an amine and an ester. It was expected that formation of the amide bond via route B would be more facile than the alkylation in route A due to the basicity of the nitrogen which is an amine in route B and an amide moiety in route A. Intermediates **5** and **6** could be obtained from acyliminium salt **7** or benzylium salt **8**, respectively.



Scheme 1.

In order to test the validity of the above mentioned idea, lennoxamine was synthesized as shown in Scheme 2. Alkylation of the 6,7-methylenedioxy-3,4-dihydroisoquinoline **9** with the readily available ethyl 6-chloromethyl-2,3-dimethoxybenzoate⁸ **10** in acetonitrile gave the required iminium chloride. The iminium chloride so obtained was not isolated but was treated with potassium hydroxide or sodium hydroxide. It was indeed gratifying to find that the iminium salt was smoothly converted directly to dehydrolennoxamine **3** in 73% yield by potassium hydroxide and in 58% yield by sodium hydroxide. The presumed pseudobase and the aldehydic lactam intermediates were not isolated in the reaction.



Scheme 2.

By replacement of 6,7-methylenedioxy-3,4-dihydroisoquinoline with 6,7-dimethoxy-3,4-dihydroisoquinoline in the above reaction, the analogue of the dehydrolennoxamine **11** was successfully synthesized in 75% overall yield. Dehydrolennoxamine and its analogue were hydrogenated with 10% palladium on carbon in ethyl acetate to give lennoxamine and its analogue⁹ in 76 and 80% yields, respectively.

Acknowledgements

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References

1. Valencia, E.; Freyer, A. J.; Shamma, M.; Fajardo, V. *Tetrahedron Lett.* **1984**, *25*, 599–602.
2. Fajardo, V.; Elango, V.; Cassels, B. K.; Shamma, M. *Tetrahedron Lett.* **1982**, *23*, 39–42.
3. Valencia, E.; Weiss, I.; Firdous, S.; Freyer, A. J.; Shamma, M. *Tetrahedron* **1984**, *40*, 3957–3962.
4. For synthetic approaches to isoindolobenzazepine alkaloids, see: (a) Bernhard, H. O.; Snieckus, V. *Tetrahedron Lett.* **1971**, *51*, 4867–4870. (b) Ruchirawat, S.; Lertwanawatana, W.; Thianpatanagul, S.; Cashaw, J. L.; Davis, V. E. *Tetrahedron Lett.* **1984**, *25*, 3485–3488. (Part of this work was presented at IUPAC conference 23–27 November 1997, Phuket, Thailand, *Pure Appl. Chem.* **1998**, *70*, 2128.) (c) Chiefari, J.; Janowski, W.; Prager, R. *Tetrahedron Lett.* **1986**, *27*, 6119–6122. (d) Mazzocchi, P. H.; King, C. R.; Ammon, H. L. *Tetrahedron Lett.* **1987**, *28*, 2473–2476. (e) Kessar, S. V.; Singh, T.; Vohra, R. *Tetrahedron Lett.* **1987**, *28*, 5323–5326. (f) Yasuda, S.; Sugimoto, Y.; Mukai,

- C.; Hanaoka, M. *Heterocycles* **1990**, *30*, 335–337. (g) Lamas, C.; Saa, C.; Castedo, L.; Dominguez, D. *Tetrahedron Lett.* **1992**, *33*, 5653–5654.
5. For total synthesis of chilenine, see: (a) Dorn, C. R.; Koszyk, F. J.; Lenz, G. R. *J. Org. Chem.* **1984**, *49*, 642–2644. (b) Fang, F. G.; Danishefsky, S. J. *Tetrahedron Lett.* **1989**, *30*, 2747–2750. (c) Kessar, S. V.; Singh, T.; Vohra, R. *Indian J. Chem.* **1991**, *30B*, 299–301. (d) Ishibashi, H.; Kawanami, H.; Iriyama, H.; Ikeda, M. *Tetrahedron Lett.* **1995**, 6733–6734.
 6. For total syntheses of lennoxamine and chilenine, see: (a) Ishibashi, H.; Kawanami, H.; Ikeda, M. *J. Chem. Soc., Perkin Trans. 1* **1997**, 817–821. (b) Koseki, Y.; Kusano, S.; Nagasaka, T. *Tetrahedron Lett.* **1999**, *40*, 2169–2172.
 7. For total synthesis of lennoxamine, see: (a) Teitel, S.; Klötzer, W.; Borgese, J.; Brossi, A. *Can. J. Chem.* **1972**, *50*, 2022–2024. (b) Napolitano, E.; Spinelli, G.; Fiaschi, R.; Marsili, A. *J. Chem. Soc., Perkin Trans. 1* **1986**, *5*, 785–787. (c) Moody, C. J.; Warrellow, G. J. *Tetrahedron Lett.* **1987**, *28*, 6089–6092. (d) Koseki, Y.; Nagasaka, T. *Chem. Pharm. Bull.* **1995**, *43*, 1604–1606. (e) Rodriguez, G.; Cid, M.; Saa, C.; Castedo, L.; Dominguez, D. *J. Org. Chem.* **1996**, *61*, 2780–2782. (f) Rodriguez, G.; Castedo, L.; Dominguez, D.; Saa, C. *Tetrahedron Lett.* **1998**, *39*, 6551–6554. (g) Couture, A.; Deniau, E.; Grandclaude, P.; Hoarau, C. *Tetrahedron* **2000**, *56*, 1491–1499.
 8. Dean, R. T.; Rapoport, H. *J. Org. Chem.* **1978**, *43*, 2115–2122.
 9. All compounds have been fully characterized. Dehydrolennoxamine **3**: m.p. 208–209°C; IR (nujol) 1690, 1642 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 3.0 (t, 2H, $J=4.9$ Hz), 3.95 (s, 3H), 4.08 (s, 3H), 6.00 (s, 2H), 6.35 (s, 1H), 6.68 (s, 1H), 6.80 (s, 1H), 7.12 (d, 1H, $J=8$ Hz), 7.48 (d, 1H, $J=8$ Hz). ^{13}C NMR (100 MHz) δ 35.39, 41.72, 56.62, 62.38, 101.19, 104.87, 110.06, 110.18, 114.28, 116.24, 120.22, 127.71, 130.99, 133.16, 133.83, 146.49, 146.72, 146.82, 152.82, 163.58. EIMS 351(100), 336(20), 322(10), 175(7). Anal. calcd for $\text{C}_{20}\text{H}_{17}\text{NO}_5$: C, 68.37; H, 4.84; N, 3.98. Found: C, 68.50; H, 4.80; N, 3.95. Dehydrolennoxamine analogue **11**: m.p. 185–189°C; IR (nujol) 1690, 1638 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 3.03 (t, 2H, $J=4.6$ Hz), 3.87 (s, 3H), 3.88 (s, 3H), 3.89 (s, 3H), 4.08 (s, 3H), 6.35 (s, 1H), 6.65 (s, 1H), 6.81 (s, 1H), 7.09 (d, 1H, $J=8.4$ Hz), 7.39 (d, 1H, $J=8.4$ Hz). ^{13}C NMR (100 MHz) δ 35.18, 41.62, 55.87, 56.62, 62.35, 104.97, 113.02, 113.70, 114.21, 116.27, 120.25, 126.44, 131.05, 132.50, 133.17, 146.70, 147.44, 148.00, 152.74, 163.64. EIMS 368(31), 367(100), 353(16), 352(65), 308(9), 184(8). Anal. calcd for $\text{C}_{21}\text{H}_{21}\text{NO}_5$: C, 68.66; H, 5.72; N, 3.81. Found: C, 68.60; H, 5.72; N, 3.83. Lennoxamine **1**: m.p. 226–227°C; Lit¹, 225°C; Lit^{7a}, 228–229°C; IR (nujol) 1688 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 2.83 (m, 2H), 2.93 (m, 2H), 3.10 (dd, 1H, $J=14.7, 1.7$ Hz), 3.92 (s, 3H), 4.10 (s, 3H), 4.30 (dd, 1H, $J=10.5, 1.3$ Hz), 4.74 (m, 1H), 5.95 (d, 1H, $J=1.47$), 5.96 (d, 1H, $J=1.47$), 6.71 (s, 1H), 6.78 (s, 1H), 7.13 (d, 1H, $J=8.2$ Hz), 7.18 (dd, 1H, $J=8.8, 0.4$ Hz). ^{13}C NMR (100 MHz) δ 35.93, 41.11, 42.71, 56.75, 60.17, 62.54, 101.04, 110.34, 110.34, 116.26, 117.05, 124.18, 130.94, 134.85, 138.22, 146.08, 146.35, 147.26, 152.63, 165.18. EIMS 354(29), 353(96), 352(20), 338(51), 335(27), 162(69), 161(100), 160(29), 149(27), 131(47). Anal. calcd for $\text{C}_{20}\text{H}_{19}\text{NO}_5$: C, 67.98; H, 5.38; N, 3.96. Found: C, 67.83; H, 5.42; N, 3.97. Lennoxamine analogue: m.p. 213–214°C; IR (nujol) 1680 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 2.84 (m, 2H), 2.95 (m, 2H), 3.13 (dd, 1H, $J=14.5, 1.6$ Hz), 3.90 (s, 3H), 3.92 (s, 3H), 3.915 (s, 3H), 4.11 (s, 3H), 4.32 (dd, 1H, $J=10.7, 0.5$ Hz), 4.76 (m, 1H), 6.74 (s, 1H), 6.82 (s, 1H), 7.14 (d, 1H, $J=8.2$ Hz), 7.21 (dd, 1H, $J=8.2, 0.5$ Hz). ^{13}C NMR (100 MHz) δ 35.80, 41.19, 42.64, 55.95, 56.06, 56.66, 62.44, 113.46, 113.68, 116.15, 117.02, 124.10, 129.72, 133.67, 138.28, 146.70, 147.14, 147.46, 152.53, 165.11. EIMS 370(20), 369(85), 353(44), 351(29), 177(100). Anal. calcd for $\text{C}_{21}\text{H}_{23}\text{NO}_5$: C, 68.29; H, 6.23; N, 3.79. Found: C, 68.38; H, 6.32; N, 3.77.

CYTOTOXIC NATURAL PRODUCTS FROM THAI PLANTS: A RECENT STUDY

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ABSTRACT

Natural products will continue to be the most prolific source of bioactive compounds. Natural products exhibiting antitumor activity continue to be the subject of extensive research aimed at the development of drugs for the treatment of different human tumors. It is generally accepted that natural products offer a diversity and complexity of structure unmatched by even the most active imaginations of synthetic organic chemists. This paper reviews the research of selected Thai plants for the discovery of therapeutic agents. Attention will be focused on our recent research on Thai plants that possess cytotoxic properties. Synthetic modification and reaction of some of these compounds aimed at enhancing their potency will also be presented.

INTRODUCTION

Due to the structural and biological diversity of their constituents, terrestrial plants offer a unique and renewable resource for the discovery of potential new drugs and biological entities (Shu, 1998). However, only approximately 5,000 of the world's estimated 250,000–400,000 flowering plants have as yet been analysed for their possible medicinal uses (Balandrin et al., 1993). Moreover, in developing countries, medi-

cal plants continue to be the main source of medication. In China alone, 7,295 plant species are utilized as medicinal agents. The World Health Organization has estimated that for some 3.4 billion people in the developing world, plants represent the primary source of medicine. This represents about 88% of the world's inhabitants who rely mainly on traditional medicine for their primary health care. Farnsworth (1988) reported that at least 119 compounds derived from 90 plant species can be considered as important drugs currently in use in one or more countries, with 77% of these being derived from plants used in traditional medicine. The importance of natural products is also evidenced by the fact that in 1991 nearly half of the best selling drugs were either natural products or their derivatives (Farnsworth & Bingel, 1977; Farnsworth et al., 1985; Farnsworth, 1988). It is thus a matter of utmost concern to public health and indeed to human life that urgent action is taken to prevent further diminution of actual and potential availability of medicinal and biological agents (O'Neill et al., 1993). Cragg et al. (1997) of the NCI, USA have reported some interesting statistics that for new drug applications, anticancer drugs and drugs for infectious diseases show a high number of compounds from natural sources indicating the increasing importance of natural products for the treatment of these two types of diseases.

Natural Products as Anticancer Agents

Apart from being an excellent source of anti-infectious drugs (Phillipson & Wright, 1991; Mahidol et al., 1997a), plants are also a good source of anticancer agents (Cordell et al., 1993; Hamburger & Hostettmann, 1991; Hostettmann et al., 1998; King-

Keywords: Anticancer, bioactive natural products, cholangiocarcinoma, colchicine analogues, *Derris dericulata*, *Gloriosa superba*.

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horn et al., 1995; Mahidol et al., 1998; Pezzuto, 1997; Suffness et al., 1995; Valeriote et al., 1995). The National Cancer Institute (NCI), USA has launched an extensive program for the development of natural products for the treatment of various forms of cancer. Many clinically useful drugs have been discovered from various plants.

Vinblastine (1) and vincristine (2) (Neuss et al., 1964; Rahman et al., 1994), derivatives of the periwinkle plant (*Catharanthus roseus*), have proved to be active against tumor growth. Vinblastine has been shown to be a very effective treatment for Hodgkin's disease, and has also been used to treat breast cancer, Kaposi's sarcoma, and other diseases. Vincristine is well known for its 90% success rate in treating different types of childhood leukemia.

Other anticancer agents include the semisynthetic epipodophyllotoxin, etoposide (3) (Issell, 1982), as well as the recently discovered taxol (4) and its semisynthetic derivative, taxotere (5) (Rowinsky, 1997; DeFuria, 1997). Taxol (paclitaxel, 4) has been isolated from the bark of the Pacific or American yew tree, *Taxus brevifolia*. The discovery of taxol has indeed heightened the interest in plant-derived anticancer drugs, and more than 350 taxane diterpenoids have now been identified (Baloglu & Kingston, 1999). Discovered in 1971 (Wani et al., 1971), taxol appears to be an exceptionally promising drug. It exhibits a very broad spectrum of activity against leukemias and solid tumors and taxol has been found to be beneficial in treatment of refractory ovarian, breast, and other cancers, and the drug was recently approved for marketing. It is estimated that four mature yew trees which take about 100 years to reach full maturity are needed to produce enough taxol to treat a single case of ovarian cancer. At present, although the synthesis of this extremely complicated taxol molecule has been accomplished, it has not yet become commercially feasible. It is likely that taxol will be produced semisynthetically for commercial production using intermediates related to baccatin III.

The addition of taxol to the list of anticancer drugs is testimony to the synergism of broadly based contributions from multidisciplinary scientific endeavour. The isolation and structure elucidation led the way to pharmacological and toxicological testing. The finding of baccatin III from other phytochemical sources coupled with synthetic organic chemistry make the drug available for human trials.

The finding that taxoids act through the stabilization of microtubules has led to the search for new agents that function by a comparable mechanism. As a result,

new compounds have been discovered. Epothilones (6 and 7) are a new class of macrocyclic natural products which were first isolated from myxobacteria (Gerth et al., 1996). Epothilones (Finlay, 1997) are more potent than taxol in some cell lines and they hold great promise for further investigation. Like taxol, epothilones have captured the interest of many synthetic organic chemists and the syntheses of these compounds have recently been accomplished (Harris & Danishefsky, 1999).

In addition to the above-mentioned clinically approved drugs and promising drug candidates, some other plant-derived compounds show a great deal of potential for future use as anticancer agents. Camptothecin (8) (Wall et al., 1966) was originally isolated from the Chinese tree *Camptotheca acuminata* and a number of camptothecin analogues (Wall & Wani, 1993) are currently being developed as anticancer agents. Camptothecin was established as having *in vivo* activity against murine leukemia and rat Walker carcinoma 256 models. While early clinical trials on the parent alkaloid and camptothecin sodium were not particularly successful due to toxicity problems, interest in camptothecin intensified once it was discovered that it exhibits a novel mechanism of action by inhibiting the enzyme DNA topoisomerase I. Accordingly, a number of camptothecin analogues have been developed in an attempt to reduce toxicity, optimize efficacy, and improve water solubility without opening the lactone ring present in the parent molecule, and topotecan (9) and irinotecan are two interesting camptothecin analogues (Henegar et al., 1997; Cao et al., 1998).

Another plant-derived alkaloid which is also under clinical trial is homoharringtonine (10), a cephalotaxine alkaloid (Powell et al., 1972; Feldman et al., 1992). The compound was originally isolated from *Cephalotaxus harringtonia*; it shows antineoplastic activity, especially against murine lymphocytic leukemias. It was found to be more active than vincristine against mouse leukemias and melanomas.

We have investigated the plant *Phyllanthus amarus* Schum. & Thonn. (Euphorbiaceae), locally known as Look Tai Bai, for cytotoxic activity. *Phyllanthus amarus* (Somanabandhu et al., 1993) has been traditionally used for the treatment of jaundice and other hepatic diseases. Although the antihepatotoxic potential of the plant has been controversial, the major chemical components were known to be phyllanthin (11) and hypophyllanthin (12), with structures as shown. Apart from the structural study, the biological activities of these compounds have also been investigated.

In addition to *Phyllanthus amarus*, *Gloriosa superba* L. was investigated for anticancer activity. *Gloriosa superba* is known in Thai as "Dong Dueng" or "Dao Dueng", a climber plant in the family Colchicaceae, which is widely distributed in the tropical parts of Asia and Africa, with many varieties present in Thailand. The active principle of *Gloriosa superba* is the alkaloid colchicine (13) which is obtained from the dried tuber of the plant. Colchicine has long been used for the treatment of arthritis. From the dried tuber of Thai *Gloriosa superba*, four tropolone alkaloids (13–16) (Engprasert, 1995; Capraro & Brossi, 1984; Bentley, 1998) were isolated and the structures are as shown.

The structures were elucidated using various spectroscopic techniques, such as UV, IR, MS, one- and two-dimensional NMR. The cytotoxicity data of various colchicines with various cell lines have also been established.

Another cancer cell line of interest to us is the cholangiocarcinoma cell line. Cholangiocarcinoma, a form of bile duct cancer, is a rare type of cancer in the Western world, but it is highly prevalent in Thailand and in many other Asian countries. The cause of the disease is believed to be associated with infestation of *Opisthorchis viverrini* or liver fluke and exposure to a chemical carcinogen in food or in the environment, presumably, dimethylnitrosamine (DMN). The effectiveness of some new anticancer agents against cholangiocarcinoma was evaluated. This process was carried out by *in vitro* testing with the cholangiocarcinoma (HuCCA-1) cell line (Sirisinha et al., 1991, 1993). Some colchicine derivatives have been subjected to cytotoxicity testing, using a microculture protein assay (Table 1).

The ED₅₀ for the cholangiocarcinoma cell line with the methanol extract of *Gloriosa superba* was found to be 2.5 µg/ml, while the ED₅₀ of 3-demethyl-*N*-formyl-*N*-deacetylcolchicine was found to be 0.0625 micrograms per ml, in contrast with the ED₅₀ of about 0.02 µg/ml for colchicine. These values were approximately two-times higher than the ED₅₀ values for the KB cell line. These results showed that the cholangiocarcinoma cell line is highly susceptible to derivatives of the tropolone alkaloids, at least when tested *in vitro*:

whether or not these agents will be effective *in vivo* remains to be determined in further experiments.

Various analogues of colchicine have been synthesized with the aim of improving the therapeutic index of the target compound by enhancing the potency of these analogues. The modification of the aromatic ring of colchicine was first studied.

Demethylation at the C-2 position of colchicine (Scheme 1) could be performed by the action of concentrated sulfuric acid at 45 °C for 7 h (Rösner et al., 1981). The 2-demethylated product was alkylated with C₁₆ alkyl iodide to give the corresponding ether (Scheme 2).

Acetylation of the C-2 demethylated product with acetic anhydride in dry pyridine gave colchicine acetate (Scheme 3). Esterification with longer chain fatty acids could also be effected, for example, acylation with palmitic acid chloride in pyridine gave the corresponding colchicine palmitate (Scheme 4). The results of the biological testing indicated that the longer chain of the alkyl or ester group attached to the aromatic ring of colchicine did not improve the biological activity in the cholangiocarcinoma cell line.

The Seitz procedure was also applied to modify the tropolone ring by using the Diels-Alder reaction of colchicine with various dienophiles (Brecht et al., 1997). Reaction of singlet oxygen with colchicine produced the cycloaddition product of colchicine peroxide (Scheme 5).

N-Phenyl-1,2,4-triazolinedione (PTAD), the highly reactive nitrogen dienophile, reacted with colchicine. When the two reactants were heated in toluene at 110 °C for half an hour, only the endo adduct was formed. Under relatively drastic conditions, colchicine gave the maleimide adduct. When colchicine was heated with *N*-methyl maleimide in mesitylene at 166 °C for 14 h, two stereoisomers of endo and exo adducts were obtained in a (1:1) ratio (Scheme 6).

The modification of the peripheral functional group of the tropolone ring was also studied according to the known procedure (Cavazza & Pietra, 1998). Reaction of colchicine with guanidine in methanol led to the guanidyl colchicine derivative (Scheme 7). Also, reac-

Table 1. ED₅₀ values against KB and HuCCA-1 cell lines.

<i>Gloriosa superba</i>	KB (µg/ml)	HuCCA-1 (µg/ml)
Methanol extract	0.5	2.5
Colchicine (13)	0.01	0.02
3-Demethyl- <i>N</i> -formyl- <i>N</i> -deacetylcolchicine (15)	0.03125	0.0625

HuCCA-1 = Human cholangiocarcinoma cell line

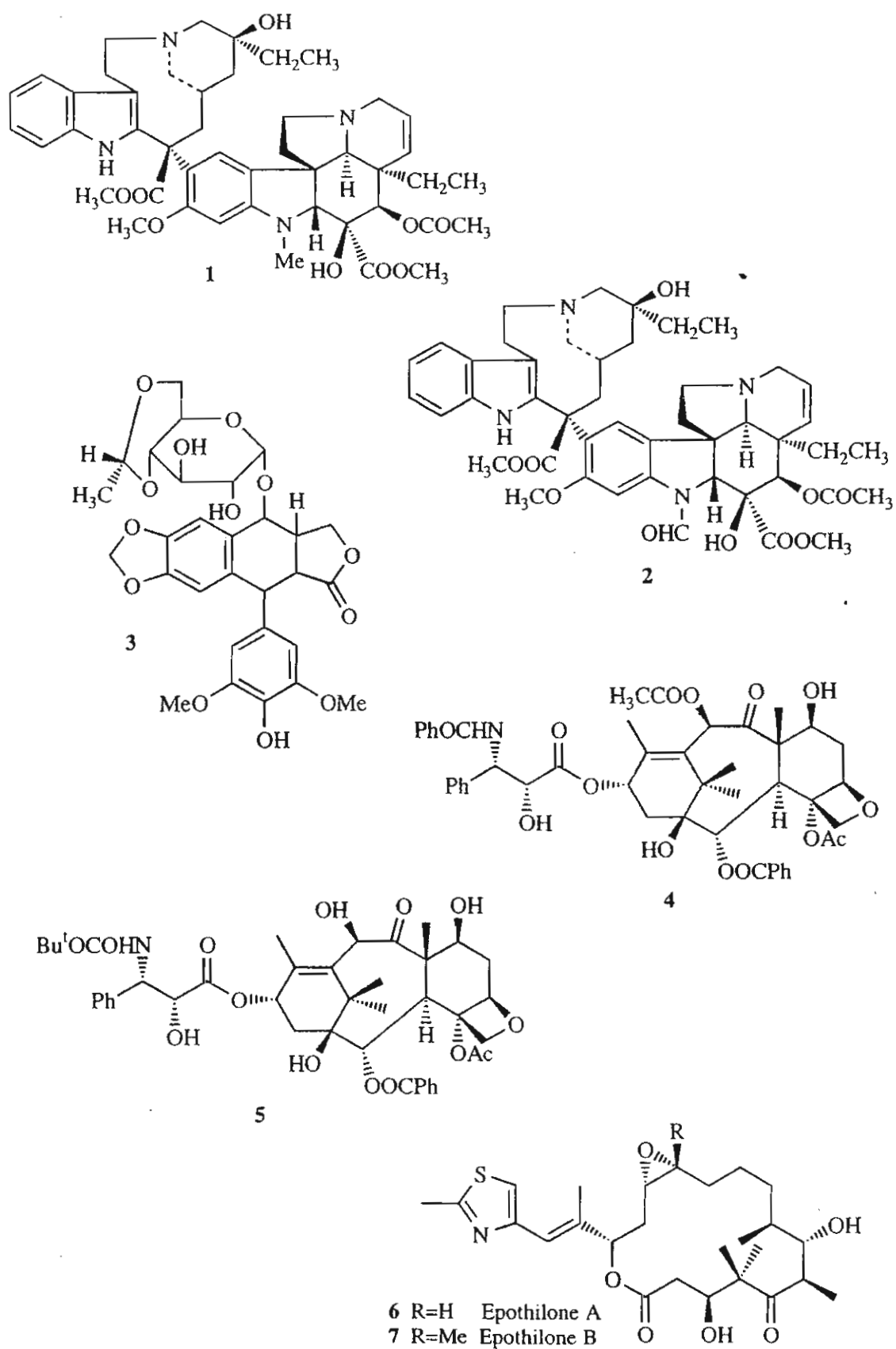
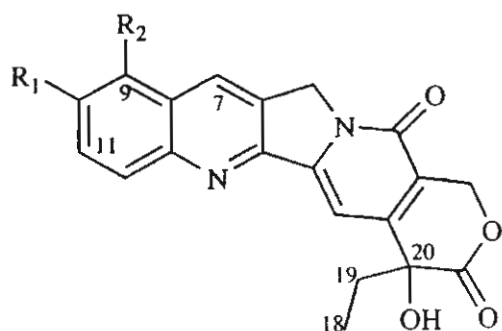
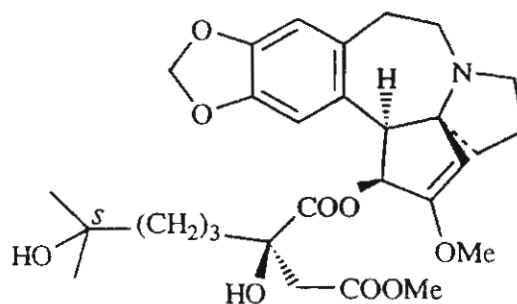


Fig. 1. Structures of compounds 1-7.

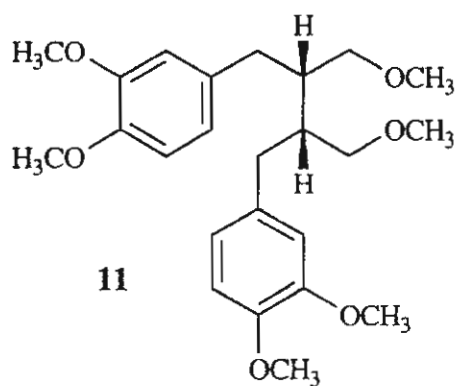


8 $R_1=R_2=H$

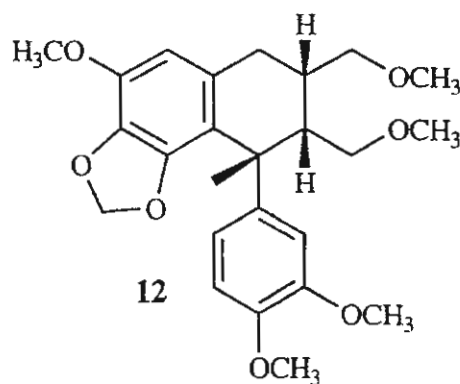
9 $R_1=OH$, $R_2=-CH_2-NMe_2$



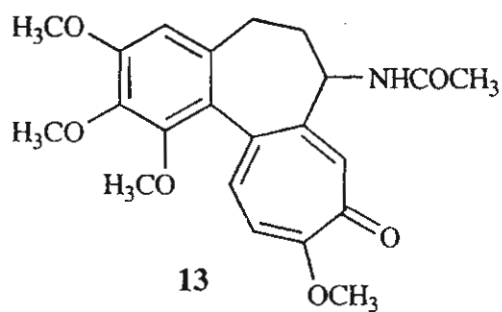
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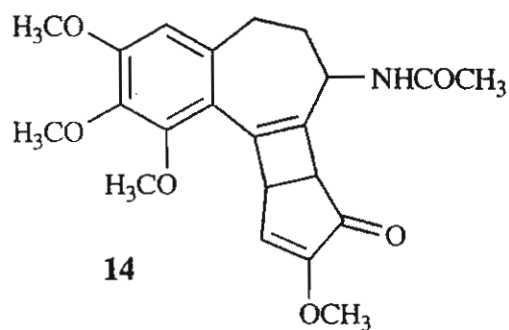
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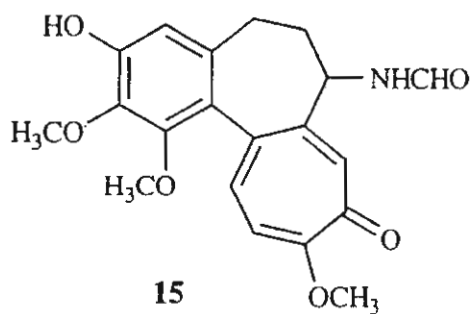
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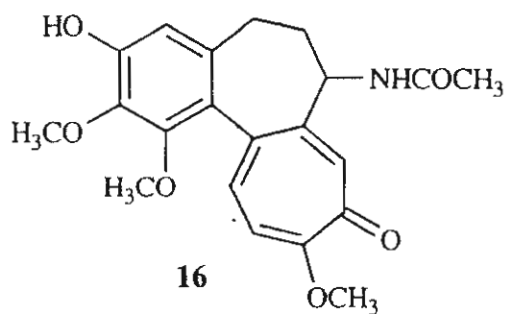
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15



16

Fig. 2. Structures of compounds 8–16.

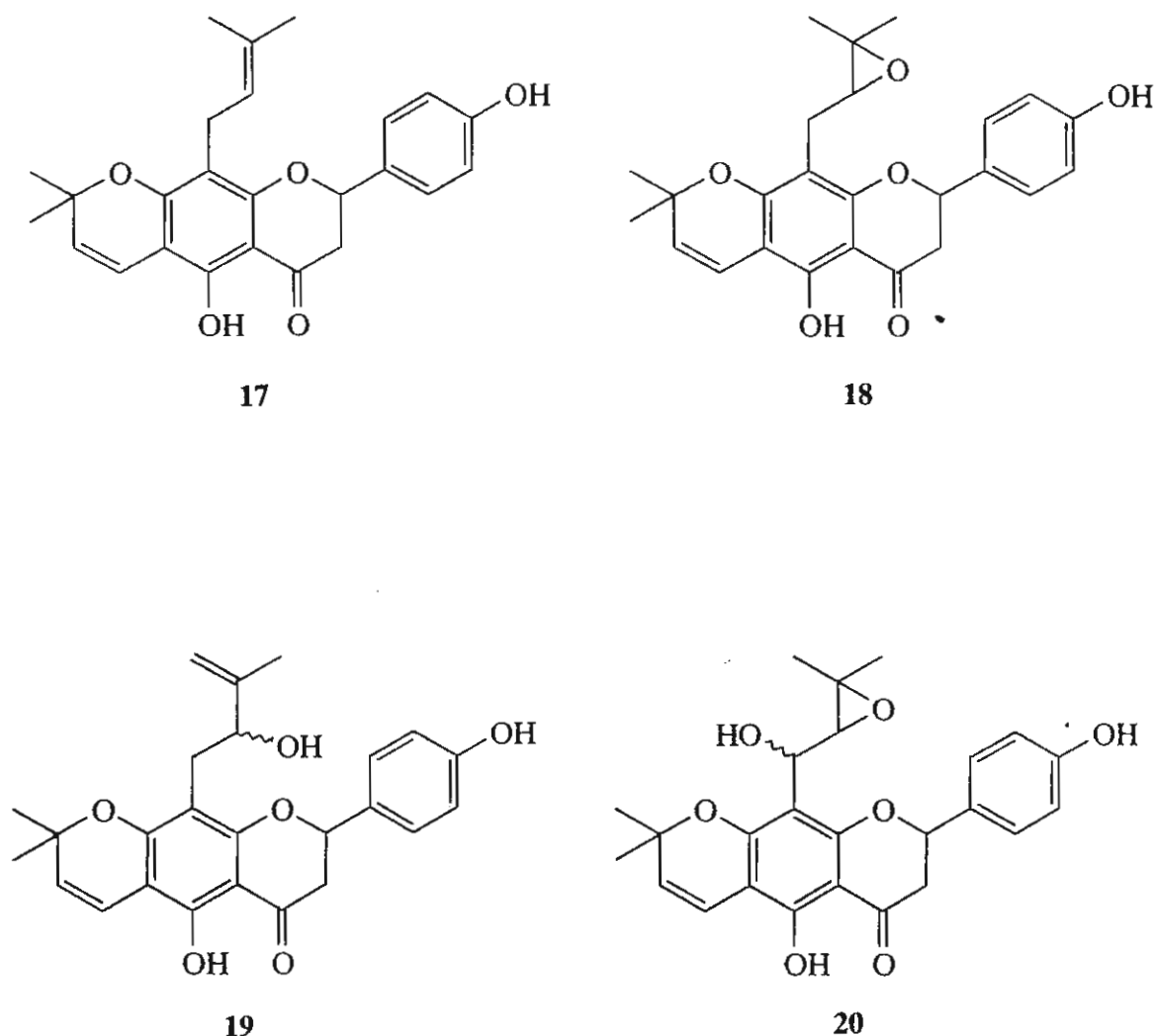


Fig. 3. Structures of compounds 17–20.

tion of colchicine with benzamidine in dry benzene gave the benzamidyl colchicine derivative (Scheme 8).

The results of biological testing of these compounds using the cholangiocarcinoma cell line showed very low biological activities as compared to colchicine; these results clearly illustrated the importance of the tropolone ring in the activity.

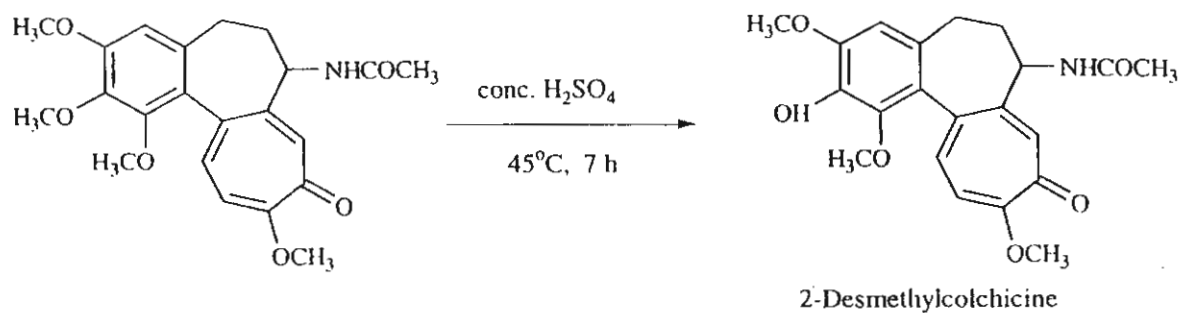
We have recently investigated another plant, *Derris reticulata*, locally known as Cha-aim thai (Mahidol et al., 1997b). Cha-aim thai is a medicinal plant of Thailand used for the relief of thirst and as an expectorant. Our studies led to the isolation of three new pyranoflavanone compounds. Lupinifolin (17), a known flavanone, was isolated as the major constituent of this plant. Its structure was confirmed by detailed analysis of NMR spectra such as COSY, NOESY, APT, HETCOR and selective INEPT.

The first unknown isolate, named epoxylupinifolin (18), was shown to be the 2'',3''-epoxide of lupinifolin by spectroscopic methods. The structure of the epoxide was further confirmed by successful epoxidation of lupinifolin with magnesium monoperoxyphthalate hexahydrate (MMPP)

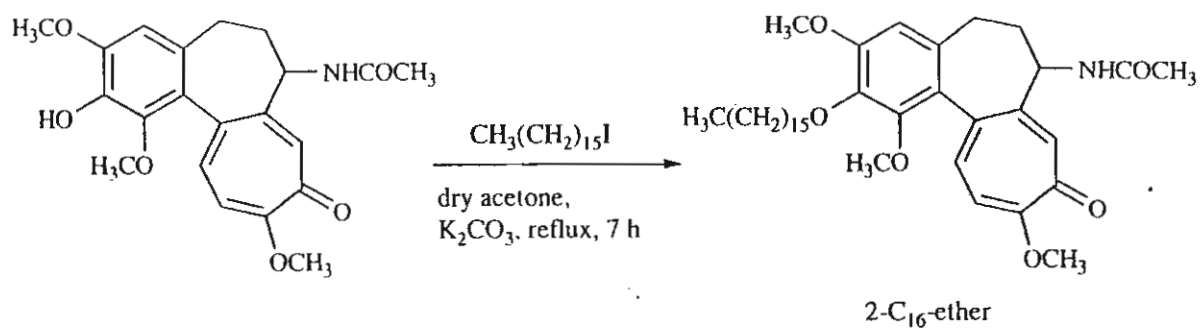
Two other new compounds isolated were named dereticulatin (19) and hydroxy-epoxylupinifolin (20) and the structures were found to be hydroxy derivatives through the analysis of the NMR spectra of these compounds and the corresponding derivatives.

The *in vitro* bioassay evaluation of lupinifolin, epoxylupinifolin and dereticulatin triacetate was also carried out. The results showed that each of them inhibited the P-388 cell line at 0.4–0.5 µg/ml, but were inactive against the KB cell line.

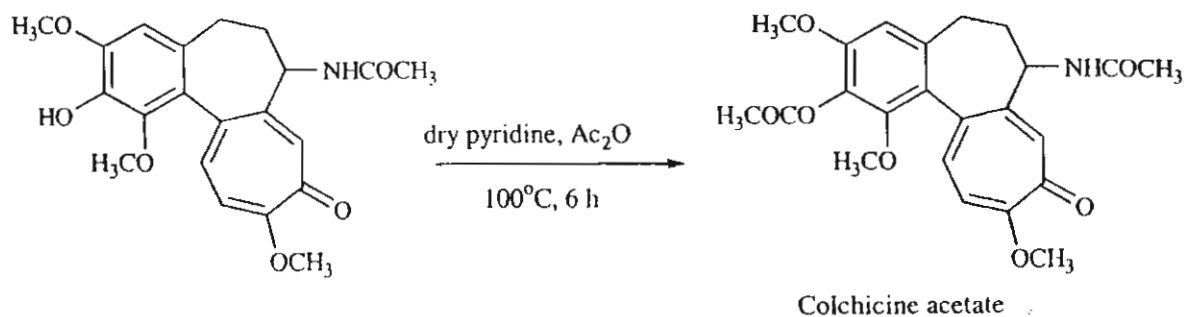
In conclusion, it is our conviction that research on



Scheme 1



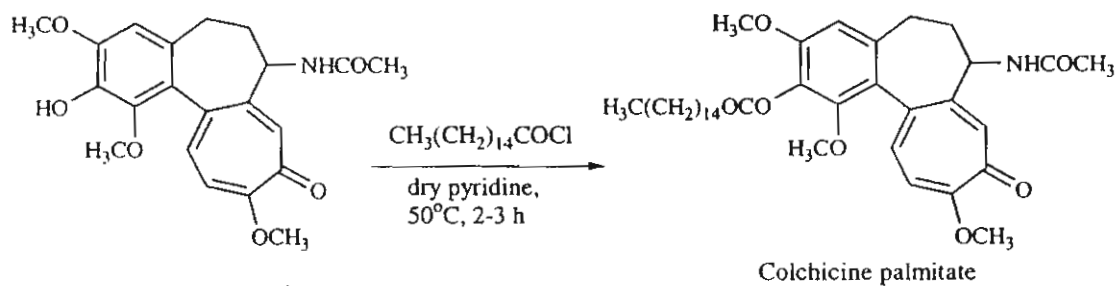
Scheme 2



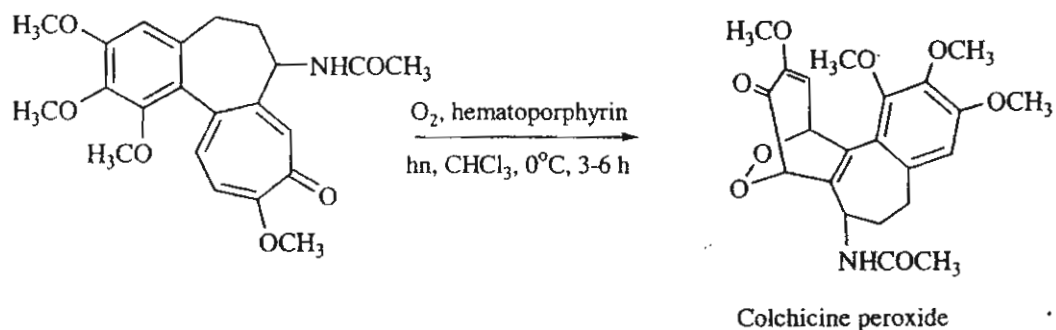
Scheme 3

natural products is most worthwhile despite some signs that the interest in natural product research is

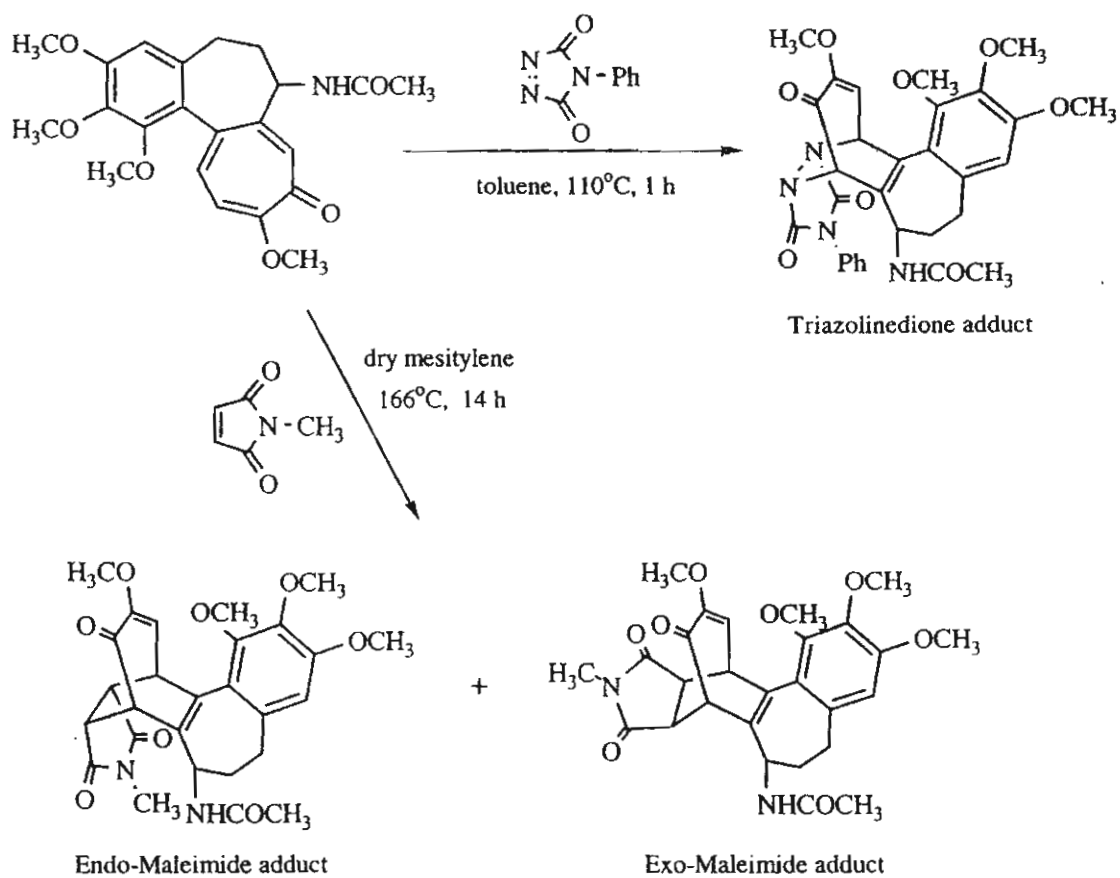
waning with the explosive growth of combinatorial chemistry.



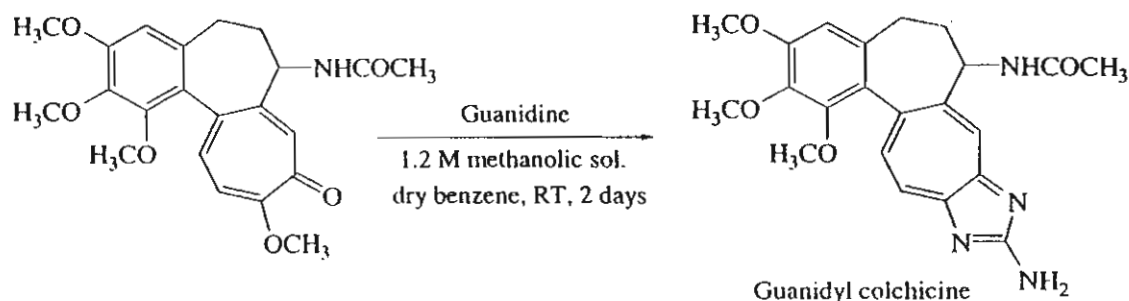
Scheme 4



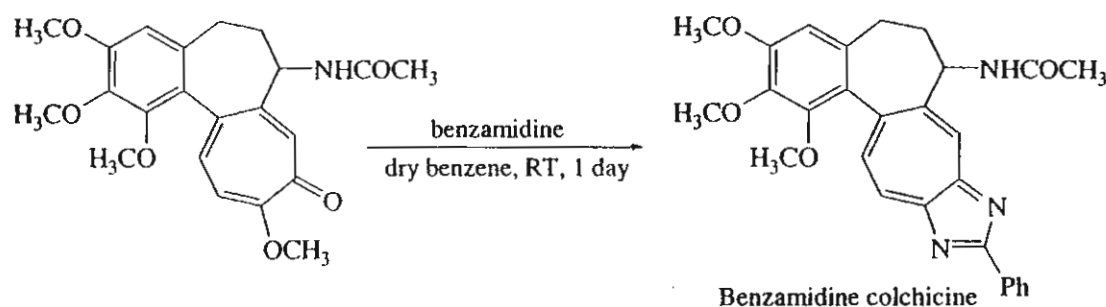
Scheme 5



Scheme 6



Scheme 7



Scheme 8

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REFERENCES

- Balandrin MF, Kinghorn AD, Farnsworth NR (1993): Plant-derived natural products in drug discovery and development. In *Human Medicinal Agents from Plants*: Kinghorn AD, Balandrin MF, eds., Symposium Series No. 534, Washington D.C., American Chemical Society, pp. 2–12.
- Baloglu E, Kingston DGI (1999): The taxane diterpenoids. *J Nat Prod* 62: 1448–1472.
- Bentley KW (1998): Colchicine and Related Alkaloids. In *The Isoquinoline Alkaloids*: Chapter 20, Amsterdam, Harwood Academic Publishers, pp. 389–445.
- Brecht R, Haenel F, Seitz G, Frenzen G, Pilz A, Massa W, Wolcadlo W (1997): Positional and facial selectivity in Diels-Alder reactions of (-)-(AS,7S)-colchicine – synthesis of novel analogues of the alkaloid. *Liebigs Ann* 851–857.
- Cao Z, Harris N, Kozielski A, Vardeman D, Stehlin JS, Giovannella B (1998): Alkyl esters of camptothecin and 9-nitrocamptothecin: synthesis, in vitro pharmacokinetics, toxicity, and antitumor activity. *J Med Chem* 41: 31–37.
- Capraro HG, Brossi A (1984): Tropolonic *Colchicum* alkaloids. In *The Alkaloids*: Brossi A, ed., Vol 23, London, Academic Press, pp. 1–70.
- Cavazza M, Pietra F (1998): Synthesis of 1,3-diazaazulene derivatives of colchicinoids and isocolchicinoids via ipso- or tele-substitution-condensation with amidines. *Tetrahedron* 54: 14059–14064.
- Cordell GA, Farnsworth NR, Beecher CWW, Soejarto DD, Kinghorn AD, Pezzuto JM, Wall ME, Wani MC, Brown DM, O'Neill MJ, Lewis JA, Tait RM, Harris TJR (1993): Novel strategies for the discovery of plant-derived anticancer agents. In *Human Medicinal Agents from Plants*: Kinghorn AD, Balandrin MF, eds., Symposium Series No. 534, Washington D.C., American Chemical Society, pp. 191–204.
- Cragg GM, Newman DJ, Snader KM (1997): Natural products in drug discovery and development. *J Nat Prod* 60: 52–60.
- DeFuria MD (1997): Paclitaxel (Taxol®): a new natural product with major anticancer activity. *Phytomedicine* 4: 273–282.
- Engprasert S (1995): *Isolation, Structure Elucidation, Assay and Cytotoxic Property of Tropolone Alkaloids from Tubers of Gloriosa superba Linn.* M.Sc. Thesis: Mahidol University, Bangkok.
- Farnsworth NR, Bingel AS (1977): Problems and prospects of discovering new drugs from higher plants by pharmacological screening. In *New Natural Products and Plant Drugs with Pharmacological, Biological or Therapeutic Activity*: Wagner H, Wolff P, eds., New York, Springer, pp. 61–73.
- Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z (1985): Medicinal plants in therapy. *Bull WHO* 63: 965–981.
- Farnsworth NR (1988): Screening plants for new medicines. In *Biodiversity*, Wilson EO, ed., Washington D.C., National Academy Press, pp. 83–97.
- Feldman E, Arlin Z, Ahmed T, Mittelman A, Pucchio C, Chun H, Cook P, Baskind P (1992): Homoharringtonine in

- combination with cytarabine for patients with acute myelogenous leukemia. *Leukemia* 6: 1189–1191.
- Finlay R (1997): Metathesis vs metastasis: the chemistry and biology of the epothilones. *Chem Ind (London)* 24: 991–996.
- Gerth K, Bedorf N, Hofle G, Irschik H, Reichenbach H (1996): Epothilons A and B: antifungal and cytotoxic compounds from *Sorangium cellulosum* (Myxobacteria). Production, physico-chemical and biological properties. *J Antibiot* 49: 560–563.
- Hamburger M, Hostettmann K (1991): Bioactivity in plants: the link between phytochemistry and medicine. *Phytochemistry* 30: 3864–3874.
- Harris CR, Danishefsky SJ (1999): Complex target-oriented synthesis in the drug discovery process: a case history in the dEpoB series. *J Org Chem* 64: 8434–8456.
- Henegar KE, Ashford SW, Baughman TA, Sih JC, Gu RL (1997): Practical asymmetric synthesis of (S)-4-ethyl-7,8-dihydro-4-hydroxy-1H-pyrano[3,4-f]indolizine-3,6,10(4H)-trione E, a key intermediate for the synthesis of irinotecan and other camptotecin analogs. *J Org Chem* 62: 6588–6597.
- Hostettmann K, Potterat O, Wolfender JL (1998): The potential of higher plants as a source of new drugs. *Chimia* 52: 10–17.
- Issell BF (1982): The podophyllotoxin derivatives VP16-213 and VM26. *Cancer Chemother Pharmacol* 7: 73–80.
- Kinghorn AD, Farnsworth NR, Beecher CWW, Soejarto DD, Cordell GA, Pezzuto JM, Wall ME, Wani MC, Brown DM, O'Neill MJ, Lewis JA, Besterman JM (1995): Novel strategies for plant-derived anticancer agents. *Int J Pharmacog* 33: 48–58.
- Mahidol C, Prawat H, Ruchirawat S (1997a): Bioactive natural products from Thai medicinal plants. In *Phytochemical Diversity: A Source of New Industrial Products*: Wrigley S, Hayes M, Thomas R, Chrystal E, eds., London, The Royal Society of Chemistry, pp. 96–105.
- Mahidol C, Prawat H, Ruchirawat S, Likhitwitayawuid K, Long-ZeLin, Cordell GA (1997b): Prenylated flavanones from *Derris reticulata*. *Phytochemistry* 45: 825–829.
- Mahidol C, Ruchirawat S, Prawat H, Pisutjaroenpong S, Engprasert S, Chumsri P, Tengchaisri T, Sirisinha S, Picha P (1998): Biodiversity and natural product discovery. *Pure Appl Chem* 70: 2065–2073.
- Neuss M, Gorman M, Hargrove W, Cove NJ, Biemann K, Büchi G, Manning RE (1964): Vinca alkaloids. XXI. The structures of the oncolytic alkaloids vinblastine (VLB) and vincristine (VCR). *J Am Chem Soc* 86: 1441.
- O'Neill MJ, Lewis JA (1993): The renaissance of plant research in the pharmaceutical industry. In *Human Medicinal Agents from Plants*: Kinghorn AD, Balandrin MF, eds., Symposium Series No. 534, Washington D.C., American Chemical Society, pp. 48–55.
- Pezzuto JM (1997): Plant-derived anticancer agents. *Biochem Pharmacol* 53: 121–133.
- Phillipson JD, Wright CW (1991): Antiprotozoal agents from plant sources. *Planta Med* 57: S53–59.
- Powell RG, Weisleder D, Smith Jr CR (1972): Antitumor alkaloids from *Cephalataxus harringtonia*: structure and activity. *J Pharm Sci* 61: 1227–1230.
- Rahman AU, Iqbal Z, Nasir H (1994): Synthetic approaches to vinblastine and vincristine – anticancer alkaloids of *Catharanthus roseus*. In *Studies in Natural Products Chemistry*: Atta-Ur-Rahman, ed., Amsterdam, Elsevier, 14, 805–884.
- Rösner M, Capraro HG, Jacobson AE, Atwell L, Brossi A, Iorio MA, Williams TH, Sik RH, Chignell CF (1981): Biological effects of modified colchicines. Improved preparation of 2-demethylcolchicine, 3-demethylcolchicine, and (+)-colchicine and reassignment of the position of the double bond in dehydro-7-deacetamidocolchicines. *J Med Chem* 24: 257–261.
- Rowinsky EK (1997): The development and clinical utility of the taxane class of antimicrotubule chemotherapy agents. *Annu Rev Med* 48: 353–374.
- Shu YZ (1998): Recent natural products based drug development: a pharmaceutical industry perspective. *J Nat Prod* 61: 1053–1071.
- Sirisinha S, Tengchaisri T, Boonpucknavig S, Prempracha N, Ratanarapee S, Pausawasdi A (1991): Establishment and characterization of a cholangiocarcinoma cell line from a Thai patient with intrahepatic bile duct cancer. *Asian Pacific J Allergy Immunol* 9: 153–157.
- Sirisinha S, Petmir S, Utaisincharoen P, Tengchaisri T (1993): Cholangiocarcinoma. *Asian J Surg* 19: 198–206.
- Somanabandhu A, Nitayangkura S, Mahidol C, Ruchirawat S, Likhitwitayawuid K, Shieh HL, Chai H, Pezzuto JM, Cordell GA (1993): ¹H- and ¹³C-nmr assignments of phyllanthin and hypophyllanthin: lignans that enhance cytotoxic responses with cultured multidrug-resistant cells. *J Nat Prod* 56: 233–239.
- Suffness M, Cragg GM, Grever MR, Grifo FJ, Johnson G, Mead JAR, Schepartz SA, Venditti JM, Wolpert M (1995): The National Cooperative Natural Products Drug Discovery Group (NCNPDDG) and International Cooperative Biodiversity Group (ICBG) programs. *Int J Pharmacog* 33: 5–16.
- Valeriote F, Corbett T, LoRusso P, Moore RE, Scheuer P, Patterson G, Paul V, Grindey G, Bonjouklian R, Pearce H, Suffness M (1995): Discovery of anticancer agents from natural products. *Int J Pharmacog* 33: 59–66.
- Wall ME, Wani MC (1993): Camptothecin and analogues. In *Human Medicinal Agents from Plants*: Kinghorn AD, Balandrin MF, eds., Symposium Series No. 534, Washington D.C., American Chemical Society, pp. 149–169.
- Wall ME, Wani MC, Cook CE, Palmer KH, McPhail AT, Sim GA (1966): Plant antitumor agents. I. The isolation and structure elucidation of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. *J Am Chem Soc* 88: 3888–3890.
- Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT (1971): Potent antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. *J Am Chem Soc* 93: 2325–2327.

REINVESTIGATION OF *DERRIS RETICULATA*

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ABSTRACT

The novel compound 1'''-hydroxy-2'''',3'''-epoxylupinifolin (1), together with three known prenylated flavanones were identified from the stem of *Derris reticulata* during our reinvestigation of the plant. The structures were determined by spectroscopic methods including detailed study of NMR spectral data (DEPT, 2D-COSY, HMQC and HMBC) as well as by chemical derivatizations.

INTRODUCTION

Derris reticulata Benth. (Leguminosae) is a Thai medicinal plant used for the relief of thirst and as an expectorant. We have recently further investigated this plant and the novel 1'''-hydroxy-2'''',3'''-epoxylupinifolin (1), along with three previously identified pyranoflavanones, lupinifolin, 2'''',3'''-epoxylupinifolin and dereticulatin (Mahidol et al., 1997) have now been isolated and identified from the dichloromethane extract of dry powdered stems of this plant. The structural determination of compound 1 is reported here.

MATERIALS AND METHODS

Melting points (uncorr.) were determined on a Buchi 535 apparatus. IR (CHCl₃ or KBr pellets) spectra were measured on a Perkin-Elmer system 2000 FT-IR

Keywords: *Derris reticulata*, Leguminosae, 1D-NMR, 2D-NMR, prenylated flavanone.

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infrared spectrometer. UV (MeOH) spectra were measured on a Shimadzu UV-2100S spectrophotometer. Mass spectra were determined on Finnigan Mat 90 or INCOS 50 mass spectrometers. NMR data were recorded on a Bruker AM 400 (400 MHz for ¹H and 100 MHz for ¹³C) spectrometer, using TMS as internal standard. Precoated Si gel 60 PF₂₅₄ plates (Merck, layer thickness 0.2 mm) were used for analytical TLC while preparative TLC was performed on Si gel PF₂₅₄ plates (Merck, thickness 1 mm). Spots and bands were revealed under UV light (254 nm). HPLC was carried out on a Thermal Separation Product, UV-VIS, λ 280 nm (UV6000LP for analytical measurements).

Plant Material

Dried stems of *Derris reticulata* were purchased from a local traditional drug store in Bangkok, Thailand. Botanical identification was achieved through comparison with the specimen provided by Prof. Nijisiri Ruan-grungsi. A herbarium voucher specimen is retained at the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Extraction and Isolation

The dried stems (2 kg) of *Derris reticulata* were exhaustively extracted with CH₂Cl₂ at room temperature. After filtration, the extract was evaporated *in vacuo* to dryness (88 g). The CH₂Cl₂ extract (13 g) was separated by vacuum liquid chromatography (VLC) over silica gel using gradient elution from hexane to hexane-EtOAc (35–65) to yield 8 fractions. VLC F₆ (429 mg) was separated by preparative TLC using a mixture of methanol: acetone: hexane (2: 3: 64) to give compound 1 (150 mg).

1'''-Hydroxy-2'''',3'''-epoxylupinifolin (1): yellow crystalline solid, mp 137–140 °C; IR(KBr) ν_{max} 3386 (OH), 2975, 2928, 1647, 1520, 1448, 1380, 1244, 1198, 1162, 1130, 1011, 895, 835, 747 cm⁻¹. UV

(MeOH) λ_{\max} 225, 273, 296, 307, 362 nm. EIMS m/z 420 ($M^+ - H_2O$, 28), 405 ($M^+ - H_2O - CH_3$, 29), 387 (11), 347 (13), 303 (3), 285 (60), 267 (34), 227 (34), 215 (5), 120 (10), 43 (100). FABMS (positive mode) m/z 439 ($[M+H]^+$, 40), 421 ($[M+H-H_2O]^+$, 100).

Acetylation of compound 1: A solution of compound 1 (12.5 mg) in pyridine (5 drops) and acetic anhydride was stirred at 15 °C for 3 h. After general work-up, the product was chromatographed on preparative TLC (silica gel) using 2% MeOH in CH_2Cl_2 as developing solvent to give triacetate 2 (3.0 mg; 31%) and methoxy-monoacetate 3 (5.5 mg; 50%). Triacetate 2: colorless solid, FABMS (positive mode) 565 ($[M+H]^+$, 8). 1H NMR ($CDCl_3$) δ : 5.69 (*m*, H-2), 2.95 (*dd*, $J = 16.7, 13.2$ Hz, H-3 α), 2.82, 2.78 (*dd*, $J = 16.7, 3.2$ Hz, H-3 β), 7.51, 7.50 (*d*, $J = 8.6$ Hz, H-2', 6'), 7.16, 7.15 (*d*, $J = 8.6$ Hz, H-3', 5'), 5.68 (*d*, $J = 10.0$ Hz, H-3''), 6.39, 6.38 (*d*, $J = 10.0$ Hz, H-4''), 1.50, 1.49, 1.49, 1.485 (*s*, CH_3 -5'', 6''), 6.09, 5.68 (*d*, $J = 8.2$ Hz, H-1'''), 3.69, 3.66 (*d*, $J = 8.2$ Hz, H-2'''), 1.28, 1.27, 1.25, 1.22 (*s*, CH_3 -4''', 5'''), 2.43 (*s*, $OCOCH_3$ -5), 2.32 (*s*, $OCOCH_3$ -4'), 2.07, 2.05 (*s*, $OCOCH_3$ -1'''). Methoxy-monoacetate 3: yellow crystalline solid, mp 183–184 °C, IR ($CHCl_3$) ν_{\max} 3009, 1754, 1645, 1628, 1579, 1509, 1447, 1371 cm^{-1} . FABMS (positive mode) 495 ($[M+H]^+$, 20). EIMS m/z 494 (M^+ , 13), 479 (18), 423 (100), 405 (5), 389 (18), 347 (2), 305 (1), 285 (3), 261 (57), 243 (19), 227 (48), 215 (4), 120 (5), 43 (88). 1H NMR ($CDCl_3$) δ : 5.42, 5.40 (*dd*, $J = 12.7, 3.2$ Hz, H-2), 3.05, 3.04 (*dd*, $J = 17.2, 12.7$ Hz, H-3 α), 2.89, 2.86 (*dd*, $J = 17.2, 3.2$ Hz, H-3 β), 7.46, 7.44 (*d*, $J = 8.2$ Hz, H-2', 6'), 7.15 (*d*, $J = 8.2$ Hz, H-3', 5'), 5.54 (*d*, $J = 10.0$ Hz, H-3''), 6.65, 6.64 (*d*, $J = 10.0$ Hz, H-4''), 1.462, 1.458, 1.45, 1.447 (*s*, CH_3 -5'', 6''), 4.52 (*d*, $J = 7.2$ Hz, H-1'''), 3.64, 3.62 (*d*, $J = 7.2$ Hz, H-2'''), 1.24, 1.19, 1.15, 1.13 (*s*, CH_3 -4''', 5'''), 12.44, 12.42 (*s*, OH-5), 2.32 (*s*, $OCOCH_3$ -4'), 3.35, 3.33 (*s*, OCH_3 -1''').

Transformation of compound 1 to chalcone derivatives 4 and 5 by pyridine: Compound 1 (25 mg) was dissolved in pyridine at room temperature for several weeks. After general work-up, the product was chromatographed by preparative TLC using Si gel and 7% MeOH in CH_2Cl_2 as developing solvents to give chalcones 4 and 5. 1H NMR ($CDCl_3$ + acetone- d_6) (4) δ : 7.81, 7.80 (*d*, $J = 15.5$ Hz, H- α), 7.98 (*d*, $J = 15.5$ Hz, H- β), 7.52 (*d*, $J = 8.6$ Hz, H-2, 6), 6.89 (*d*, $J = 8.6$ Hz, H-3, 5), 5.47, 5.469 (*d*, $J = 10.1$ Hz, H-3''), 6.65 (*d*, $J = 10.1$ Hz, H-4''), 1.49, 1.46 (*s*, CH_3 -5'', 6''), 5.51 (*brd*, H-1'''), 4.57 (*d*, $J = 3.7$ Hz, H-2'''), 1.44, 1.39 (*s*, CH_3 -4''', 5'''), 8.88 (*s*, OH-4), 14.68 (*s*, OH-6'). 1H NMR ($CDCl_3$ + acetone- d_6) (5) δ : 7.93 (*d*, $J = 15.4$ Hz, H-

α), 8.18 (*d*, $J = 15.4$ Hz, H- β), 7.66 (*d*, $J = 8.6$ Hz, H-2, 6), 6.97 (*d*, $J = 8.6$ Hz, H-3, 5), 5.58 (*d*, $J = 10.1$ Hz, H-3''), 6.77 (*d*, $J = 10.1$ Hz, H-4''), 1.51 (*s*, CH_3 -5'', 6''), 6.65 (*s*, H-1'''), 1.76 (*s*, CH_3 -4''', 5'''), 8.95 (*s*, OH-4), 14.76 (*s*, OH-6').

RESULTS AND DISCUSSION

During reinvestigation of the dichloromethane extract of the stems of *Derris reticulata*, the new 1'''-hydroxy-2'',3'''-epoxylupinifolin (1) has been isolated. Compound 1 was obtained as a yellow crystalline solid, mp 137–140 °C. The molecular formula was determined as $C_{25}H_{26}O_7$ on the basis of the ion peak at m/z 439 $[M+H]^+$ in the positive FABMS and 1H and ^{13}C NMR (Tables 1 and 2) spectral data. The IR (KBr) spectrum showed the presence of an hydroxyl group (3386 cm^{-1}). The UV absorptions at 225, 273, 296, 307, 362 nm were indicative of a pyranoflavanone chromophore (Smalberger et al., 1974). This compound showed one spot on TLC and one peak on reversed-phase HPLC (Luna C₈ and C₁₈, Hichrom C₁₈) with several solvent systems. However, 1H and ^{13}C NMR spectra (Tables 1 and 2) exhibited clearly two sets of signals with partial overlapping. The 1H and ^{13}C NMR spectra of flavanone 1 were nearly identical with those of 2'',3'''-epoxylupinifolin previously isolated from this plant (Mahidol et al., 1997). The only difference appeared in the prenyl group. Compound 1 showed the presence of an extra hydroxyl group at the benzylic position at δ 5.47, 5.46 (*d*, $J = 7.2$ Hz) in the 1H NMR and at δ 66.42, 66.37 in the ^{13}C NMR. The structure of compound 1 was further confirmed by various other 2D-NMR techniques including HMBC and COSY experiments as shown in Figure 1. Acetylation of flavanone 1 with acetic anhydride in pyridine for 3 h was attempted. The product was chromatographed on silica gel preparative TLC to give triacetate 2 (31%) and methoxy-monoacetate 3 (51%), respectively. Examination of the 1H NMR spectrum of 2 revealed the signals of H-3'' and H-4'' of the 2,2-dimethylpyran ring at δ 5.68 and 6.38, 6.39, respectively. The diamagnetic shift of the H-4'' resonance required its placement *peri* to the 5- $OCOCH_3$ group (Arnone et al., 1967), thereby locating the pyran ring between C-6 and C-7 of ring A.

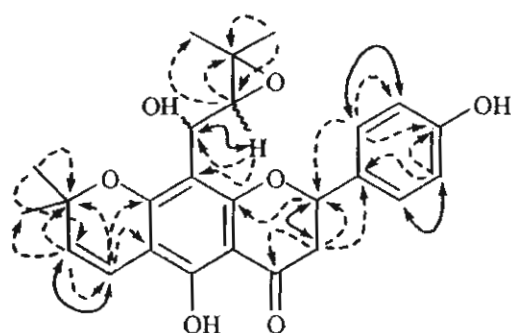
The formation of the abnormal methoxy derivative 3 under these acetylation conditions can be explained by the mechanism shown in Scheme 1. Acetylation of the hydroxyl group led to the acetoxy derivative. Loss of the acetic acid with the help of the phenolic hydroxyl

Table 1. ^1H NMR spectroscopic assignments of compound 1.

Proton	Compound 1 ($\text{CD}_3\text{OD} + \text{acetone-}d_6$)	Compound 1 (pyridine- d_5)
2	5.43, 5.41 (<i>dd</i> , $J = 13.2, 3.0$)	5.48, 5.41 (<i>dd</i> , 13.0, 2.9)
3 α	3.18, 3.15 (<i>dd</i> , 17.2, 13.2)	3.24, 3.237 (<i>dd</i> , 17.1, 13.0)
3 β	2.74, 2.72 (<i>dd</i> , 17.2, 3.0)	2.91, 2.89 (<i>dd</i> , 17.1, 2.9)
2', 6'	7.34, 7.33 (<i>d</i> , 8.6)	7.51, 7.48 (<i>d</i> , 8.6)
3', 5'	6.80 (<i>d</i> , 8.6)	7.18 (<i>d</i> , 8.6)
3''	5.61 (<i>d</i> , 10.1)	5.58 (<i>d</i> , 10.0)
4''	6.56 (<i>d</i> , 10.1)	6.85 (<i>d</i> , 10.0)
5'', 6''	1.44, 1.434, 1.427, 1.42 (<i>s</i>)	1.46, 1.43, 1.41, 1.40 (<i>s</i>)
1'''	4.75, 4.74 (<i>d</i> , 7.3)	5.47, 5.46 (<i>d</i> , 7.2)
2'''	3.50, 3.49 (<i>d</i> , 7.3)	4.16, 4.15 (<i>d</i> , 7.2)
4''', 5'''	1.14, 1.12, 1.09, 1.08 (<i>s</i>)	1.36, 1.36, 1.34, 1.32 (<i>s</i>) ^a
OH-5	—	13.06 (<i>s</i>)
OH-4'	—	11.81, 11.80 (<i>s</i>)

Table 2. ^{13}C NMR spectroscopic assignments of compound 1.

Carbon	Compound 1 (pyridine- d_5)
2	80.09, 79.98
3	43.61, 43.13
4	197.85
4a	103.47 ^a
5	158.47
6	103.32 ^a
7	160.44, 160.30 [#]
8	110.36
8a	160.82, 160.57 [#]
1'	129.51, 129.38
2', 6'	128.72, 128.60
3', 5'	116.56
4'	159.65, 159.59
2''	79.08
3''	126.79
4''	115.85
5'', 6''	28.49, 28.34, 28.26, 28.12
1'''	66.42, 66.37
2'''	68.30, 68.07
3'''	58.09, 58.02
4''', 5'''	25.36, 25.36, 20.04, 19.80

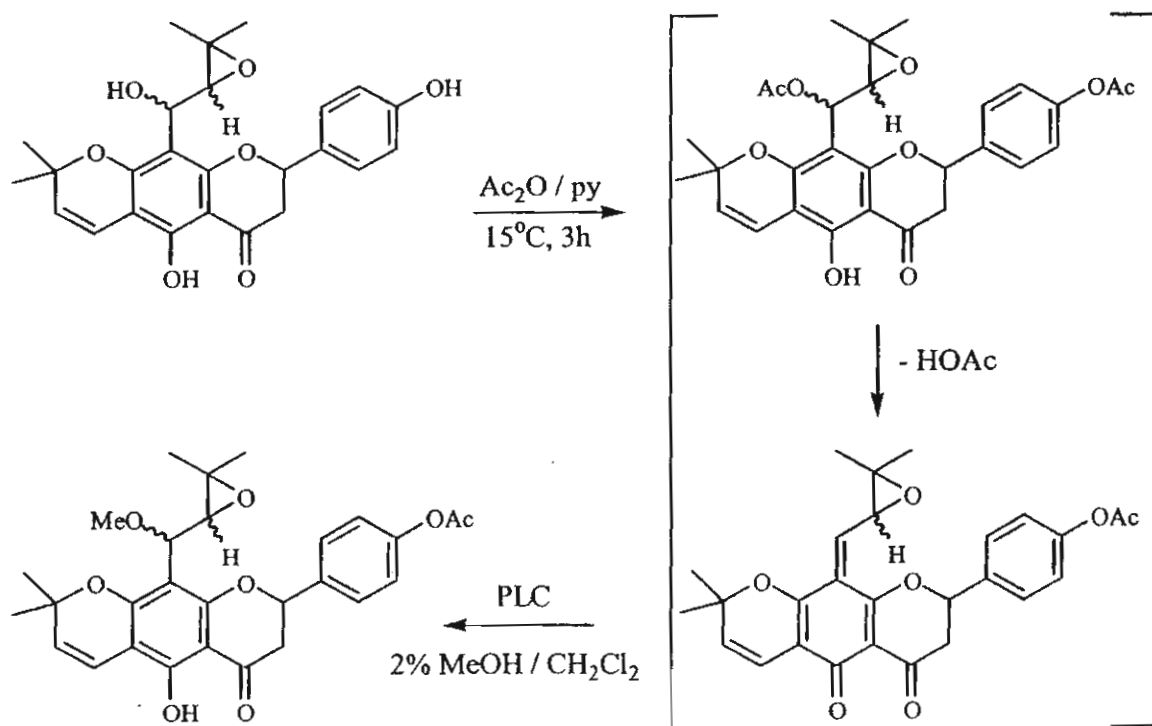
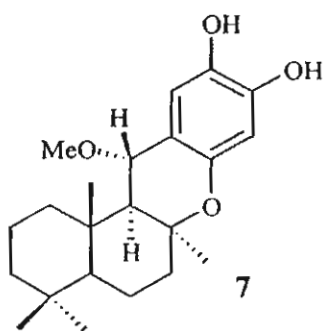
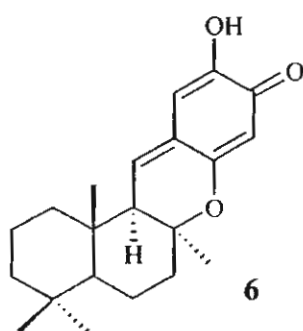
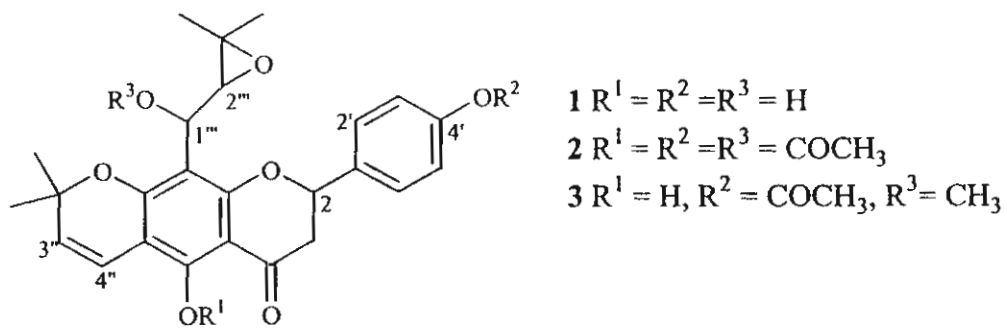
^a, [#] Interchangeable assignments.

--- HMBC ^1H - ^1H COSY

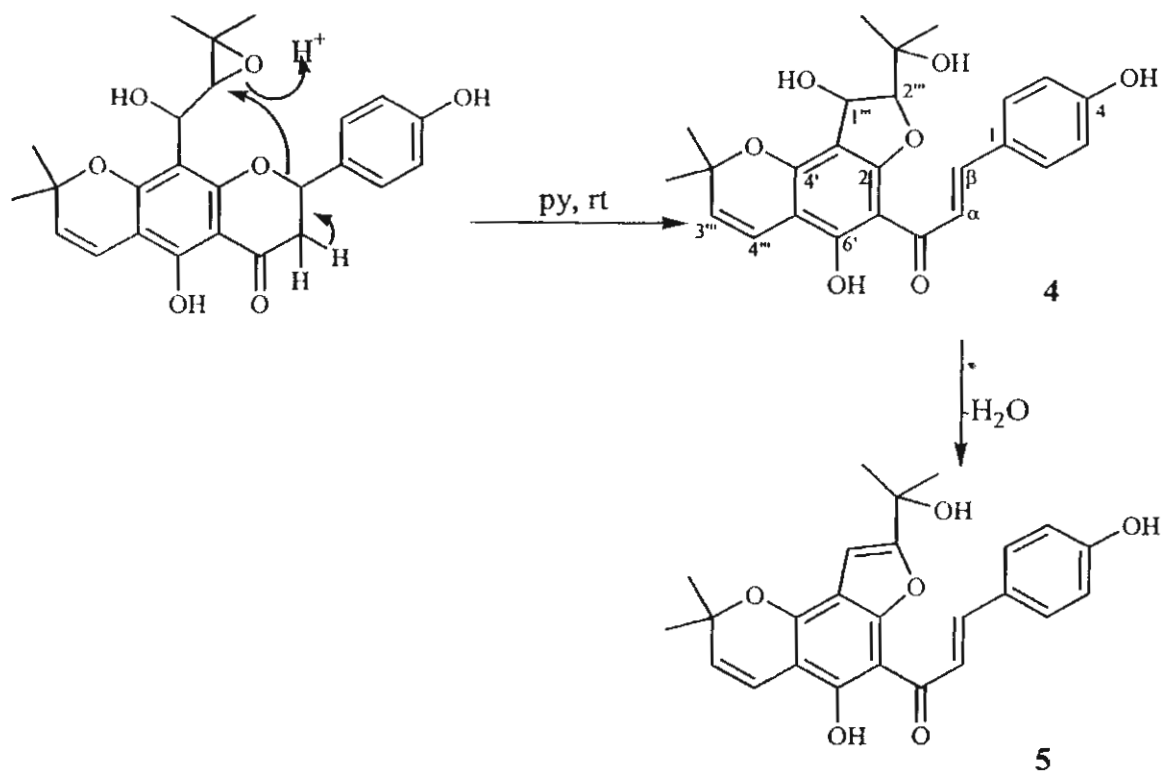
Fig. 1. Summary of important connectivities observed in compound 1 by HMBC and ^1H - ^1H COSY.

group led to the quinone methide intermediate which could then react with methanol to give the methoxy compound 3. Apparently the compound was formed during preparative TLC purification when methanol was used as developing solvent. Interestingly, a methanol adduct 6 was very recently isolated as an artefact resulting from the addition of methanol on quinone methide 7 (Rourguet-Kondracki et al., 1999).

The structure of 1 was further supported by chemical transformation (Scheme 2), revealing the labile character of the epoxide. When compound 1 was dissolved in pyridine at room temperature for several weeks, two chalcone derivatives 4 and 5 were obtained. From the above evidence, the structure of compound 1 was proposed as 1'''-hydroxy-2'',3'''-epoxylupinifolin.



Scheme 1



Scheme 2

ACKNOWLEDGMENT

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REFERENCES

- Arnone A, Cardillo G, Mertini L, Mondelli R (1967): NMR effects of acetylation and long-range coupling as a tool for structural elucidation of hydroxychromenes. *Tetrahedron Lett*: 4201-4206.
- Mahidol C, Prawat H, Ruchirawat S, Lihkitwitayawuid K, Lin LZ, Cordell GA (1997): Prenylated flavanones *Derris reticulata*. *Phytochemistry* 45: 825-829.
- Rourguet-Kondracki ML, Lacombe F, Guyot M (1999): Methanol adduct of puerphenone, a biologically active derivative from the marine sponge *Hyrtios* species. *J Nat Prod* 62: 1304-1305.
- Smalberger TM, Vlegaar R, Weber JC (1974): Flavonoids from *Tephrosia*-VII. The constitution and absolute configuration of lupinifolin and lupinifolinol, two flavanones from *Tephrosia lupinifolia* Burch (DC). *Tetrahedron* 30: 3927-3931.

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NMR STUDY OF SEVEN COUMARINS FROM *MAMMEA SIAMENSIS*

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ABSTRACT

Seven known *mammea* coumarins, *mammea* A/AA cyclo D (1), *mammea* A/AD cyclo D (2), *mammea* A/AB cyclo D (3), *mammea* A/AC cyclo F (4), *mammea* A/AB cyclo F (5), *mammea* A/AA cyclo F (6), *mammea* B/AC cyclo F (7), were isolated for the first time from the hexane extract of *Mammea siamensis*. A detailed analysis of both 1D and 2D NMR spectral data of these compounds was made.

INTRODUCTION

Mammea siamensis (Miq.) T. Anders, locally known in Thailand as 'sarapee', is a member of the tribe Calophylleae, subfamily Calophylloideae, family Guttiferae, which is distributed in Thailand, Myanmar, Laos, Cambodia and Vietnam. The flowers of this plant have been used in traditional Thai medicine as a heart tonic. Previous phytochemical investigations of the flowers, twigs and leaves of *M. siamensis* have demonstrated that it contains coumarins (Thebtaranonth et al., 1981), xanthenes (Poobrasert et al., 1998) and proanthocyanidine polymers (Balza et al., 1989). Continuing our investigation on the twigs of *M. siamensis*, we have isolated and identified coumarins in addition to other previously reported constituents.

Keywords: Coumarins, *Mammea siamensis*, Guttiferae, NMR.

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MATERIALS AND METHODS

Melting points (uncorr.) were determined on an electrothermal melting point apparatus (Electrothermal 9100). ¹H-, ¹³C- and 2D-NMR spectra were recorded on a Bruker-AM 400 MHz and a Bruker DPX-300 with TMS as the internal standard. IR spectra were obtained on a Perkin Elmer System 2000 FT-IR spectrometer. Mass spectra were determined using Finnigan INCOS 50 and MAT 90 mass spectrometers. UV spectra were measured with Milton Roy Spectronic 3000 Array and Jasco UVIDEK-650 double beam spectrophotometers. Optical rotations were measured using JASCO DIP-370 digital polarimeter in CHCl₃. Column chromatography was carried out by using silica gel 60 (70–230 mesh ASTM, ≤ 230 mesh, ASTM) and silica gel 60 PF₂₅₄ (Merck) for TLC. Semipreparative HPLC was performed using an ODS column (HICHROM Exsil 100–10 ODS, 20 mm i.d. × 250 mm; detector UV 280 nm).

Plant Material

Twigs of *Mammea siamensis* (Miq.) T. Anders. were obtained from a specimen growing in a botanical garden in Saraburi province, Thailand in November 1996.

Extraction and Isolation

The air-dried twigs (6.5 kg) of *Mammea siamensis* were ground and extracted with hexane (16 L × 7 days × 3 times). The filtrate was concentrated to give a crude extract (74 g) that was chromatographed over silica gel and eluted with solvents of increasing polarity (hexane, ethyl acetate, methanol) to give 17 fractions. Fractions 10 and 11, which were eluted by 5–15% hexane-ethyl acetate (10 g), were further separated by flash column chromatography over silica gel using a gradient mixture of hexane and ethyl acetate as eluents

to give subfractions A-G. Subfraction C was purified by preparative TLC on silica gel using hexane:EtOAc:Et₃N (30:1:3) as mobile phase to afford two isolated bands. The higher *R_f* band was purified by preparative TLC on silica gel using hexane:diisopropylamine (9:1) as an eluent to yield compound 1 (12 mg). The lower *R_f* band was separated and purified by RP-prep. HPLC (ODS 20 × 250 mm, flow rate 10 mL min⁻¹, UV detector operating at 280 nm) using MeOH-H₂O (9:1) as an eluent to give compounds 2 (5 mg, *R_t* 18.01 min) and 3 (3 mg, *R_t* 21.20 min). Subfraction G was separated using RP-prep HPLC [ODS 20 × 250 mm, flow rate 10 mL min⁻¹, UV detector operating at 280 nm] with MeOH-H₂O (8:3), yielding (in order) 4 (16.6 mg, *R_t* 51.76 min), 5 (20 mg, *R_t* 66.67 min), and 6, 7 as a mixture (82 mg, *R_t* 72.71 min). The mixture was further separated by preparative TLC, eluting with CH₂Cl₂-MeOH (99:1) to give compounds 6 (58.5 mg) and 7 (12.8 mg).

Mammea A/AA cyclo D (1): yellow needles, mp 149–150 °C; UV (EtOH) λ_{max}: 205 (4.57), 234 (4.62), 286 (4.73) nm; FTIR (KBr) ν_{max}: 3400, 2956, 2869, 1744, 1641, 1611, 1467, 1422, 1375, 1257, 1190, 1137, 1120, 850, 770, 705 cm⁻¹; ¹³C NMR (100 MHz, CDCl₃), Table 2; ¹H NMR (400 MHz, CDCl₃) δ 6.00 (1H, s, H-3), 14.81 (1H, s, 5-OH), 7.32 (2H, m, H-2', 6'), 7.41 (2H, m, H-3', 5'), 7.41 (1H, m, H-4'), 2.96 (2H, d, *J* = 6.7 Hz, H-2''), 2.23 (1H, m, H-3''), 0.96 (6H, d, *J* = 6.7 Hz, H-4''), 5.63 (1H, d, *J* = 10 Hz, H-3'''), 6.90 (1H, d, *J* = 10 Hz, H-4'''), 1.58 (6H, s, H-5''', 6'''); EIMS *m/z*: 404 (M⁺, 43), 389 (100), 371 (18), 347 (16), 115 (16), 77 (17), 43 (53), 41 (39).

Mammea A/AD cyclo D (2): yellow needles, mp 153–154 °C; UV (EtOH) λ_{max}: 205 (4.61), 234 (4.64), 286 (4.72) nm; FTIR (KBr) ν_{max}: 3350, 2925, 2852, 1735, 1611, 1645, 1582, 1464, 1379, 1257, 1193, 1134, 1115, 854, 746, 705, 693 cm⁻¹; ¹³C NMR (100 MHz, CDCl₃), Table 2; ¹H NMR (400 MHz, CDCl₃) δ 5.99 (1H, s, H-3), 14.64 (1H, s, 5-OH), 7.31 (2H, m, H-2', 6'), 7.40 (2H, m, H-3', 5'), 7.40 (1H, m, H-4'), 3.83 (1H, sept, *J* = 6.7 Hz, H-2''), 1.18 (6H, d, *J* = 6.7 Hz, H-3''), 5.63 (1H, d, *J* = 10 Hz, H-3'''), 6.90 (1H, d, *J* = 10 Hz, H-4'''), 1.57 (6H, s, H-5''', 6'''); EIMS *m/z*: 390 (M⁺, 41), 375 (82), 357 (42), 347 (100), 149 (16), 115 (15), 81 (25), 69 (52), 57 (25), 43 (54).

Mammea A/AB cyclo D (3): yellow needles, mp 98–99 °C; UV (EtOH) λ_{max}: 204 (4.39), 234 (4.41), 286 (4.52) nm; FTIR (KBr) ν_{max}: 3300, 2397, 2925, 2362, 1733, 1648, 1609, 1466, 1421, 1380, 1258, 1195, 1136, 1119, 911, 861, 766, 703, 574 cm⁻¹; ¹³C NMR (100 MHz, CDCl₃), Table 2; ¹H NMR (400 MHz, CDCl₃) δ

5.99 (1H, s, H-3), 14.69 (1H, s, 5-OH), 7.32 (2H, m, H-2', 6'), 7.40 (2H, m, H-3', 5'), 7.40 (1H, m, H-4'), 3.71 (1H, sext, *J* = 6.7 Hz, H-2''), 1.83 (1H, m, H-3a''), 1.41 (1H, m, H-3b''), 0.90 (3H, t, *J* = 7.4 Hz, H-4''), 1.16 (3H, d, *J* = 6.7 Hz, H-5''), 5.64 (1H, d, *J* = 10 Hz, H-3'''), 6.90 (1H, d, *J* = 10 Hz, H-4'''), 1.58 (6H, s, H-5''', 6'''); EIMS *m/z*: 404 (M⁺, 25), 389 (59), 371 (31), 347 (100), 115 (30), 105 (19), 77 (32), 57 (25), 43 (29), 41 (31), 29 (24); [α]_D²⁵ -8.21° (c 0.195, CHCl₃).

Mammea A/AC cyclo F (4): yellow needles, mp 176–177.5 °C; UV (EtOH) λ_{max}: 226 (4.19), 279 (4.43), 351 (4.07) nm; FTIR (KBr) ν_{max}: 3462, 2925, 1707, 1610, 1438, 1384, 1235, 1197, 1146, 1035, 927, 867, 764, 704 cm⁻¹; ¹H and ¹³C NMR data (CDCl₃), in good agreement with published data (Morel et al., 1999); EIMS *m/z*: 408 (M⁺, 96), 375 (23), 365 (25), 347 (24), 349 (77), 337 (26), 321 (55), 307 (100), 293 (89), 279 (28), 165 (31), 115 (38), 59 (92), 43 (69); [α]_D²⁵ -2.45° (c 0.245, CHCl₃).

Mammea A/AB cyclo F (5): yellow needles; FTIR (KBr) ν_{max}: 3423, 2973, 2935, 1738, 1713, 1618, 1434, 1388, 1234, 1139, 1114, 916, 850 cm⁻¹; ¹H and ¹³C NMR (CDCl₃), Table 1; EIMS *m/z*: 422 (M⁺, 10), 365 (19), 347 (17), 349 (3), 307 (29), 293 (97), 171 (40), 152 (23), 115 (57), 105 (36), 77 (34), 59 (100).

Mammea A/AA cyclo F (6): yellow needles, mp 110–112 °C; UV (EtOH) λ_{max}: 205 (4.43), 231 (4.10), 282 (4.39), 350 (3.98) nm; FTIR (KBr) ν_{max}: 3476, 3220, 2926, 1712, 1610, 1440, 1382, 1233, 1195, 1153, 1117, 1036, 926, 868, 767, 702 cm⁻¹; ¹³C NMR (75 MHz, CDCl₃), see Table 2; ¹H NMR (300 MHz, CDCl₃) δ 5.86 (1H, s, H-3), 14.47 (1H, s, 5-OH), 7.25 (2H, m, H-2', 6'), 7.38 (2H, m, H-3', 5'), 7.38 (1H, m, H-4'), 2.84 (1H, dd, *J* = 15.5, 7.0 Hz, H-2a''), 3.00 (1H, dd, *J* = 15.5, 7.0 Hz, H-2b''), 2.21 (1H, m, H-3''), 0.97 (3H, d, *J* = 6.5 Hz, H-4''), 0.96 (3H, d, *J* = 6.5 Hz, H-5''), 4.91 (1H, t, *J* = 9.0 Hz, H-2'''), 3.26 (1H, dd, *J* = 15.4, 9.4 Hz, H-3a''), 3.28 (1H, dd, *J* = 15.4, 8.3 Hz, H-3b''), 2.25 (1H, br s, OH, H-4'''), 1.32 (3H, s, H-5'''), 1.44 (3H, s, H-6'''); EIMS *m/z*: 422 (M⁺, 47), 389 (16), 365 (18), 363 (31), 349 (19), 347 (15), 335 (24), 307 (39), 293 (57), 279 (18), 165 (30), 152 (20), 115 (43), 105 (27), 69 (24), 59 (100); [α]_D²⁵ -7.32° (c 0.41, CHCl₃).

Mammea B/AC cyclo F (7): yellow needles, mp 121–122.5 °C; UV (EtOH) λ_{max}: 223 (4.18), 282 (4.41), 335 (4.00) nm; FTIR (KBr) ν_{max}: 3455, 3223, 2957, 2917, 2849, 1715, 1615, 1443, 1401, 1248, 1176, 1135, 1109, 1082, 907, 832 cm⁻¹; ¹³C NMR (100 MHz, CDCl₃), Table 2; ¹H NMR (400 MHz, CDCl₃) δ 5.87 (1H, s, H-3), 14.96 (1H, s, 5-OH), 2.88 (1H, dt, *J*

Table 1. ^{13}C (75 MHz) and ^1H NMR (300 MHz) data for compound 5^a.

Position	$^{13}\text{C}^b$	^1H (J , in Hz)	COLOC
2	159.77 (s)		
3	111.94 (d)	5.91 (s)	C-1', C-2, C-4a
4	156.65 (s)		
4a	102.48 (s)		
5	164.88/164.85 (s)	14.60/14.61 (OH)	C-5, C-4a
6	102.83/102.74 (s)		
7	163.85 (s)		
8	105.10 (s)		
8a	155.53 (s)		
1'	139.05 (s)		
2', 6'	127.16 (d)	7.28 (m)	C-4
3', 5'	127.56 (d)	7.37 (m)	C-1'
4'	128.19 (d)	7.37 (m)	
1''	209.50/209.39 (s)		
2''	45.76/45.56 (d)	3.62 (m)	
3''	26.41/26.29 (t)	1.44 (m)	
		1.80 (m)	
4''	11.60/11.80 (q)	0.91 (t, $J = 7.4$)	
5''	16.44/16.03 (t)	1.15/1.17 (d, $J = 6.6$)	C-1''
2'''	92.86/92.75 (d)	4.91/4.92 (t, $J = 9$)	
3'''	26.68/26.74 (t)	3.31/3.32 (d, $J = 9$)	C-8
4'''	71.52/71.56 (s)		
5'''	24.87/24.81 (q)	1.32 (s)	C-4''', C-6'''
6'''	26.07/26.19 (q)	1.42/1.43 (s)	C-2'''

^aData were recorded in CDCl_3 at 300 MHz for ^1H and 75 MHz for ^{13}C NMR.^bMultiplicity deduced from DEPT spectroscopy.Table 2. ^{13}C NMR data for compounds 1, 2, 3, 6, 7 in CDCl_3 (δ_{C}).

Position	1 ^a	2 ^a	3 ^a	6 ^b	7 ^a
2	159.63	159.67	159.67	159.83	160.19
3	112.70	112.67	112.72	111.68	109.55
4	156.37	156.41	156.44	156.70	159.64
4a	102.50	102.27	102.50	102.19	103.36
5	164.44	164.54	164.46	164.30*	164.91
6	107.18	106.38	107.10	103.27	103.07
7	158.11	157.79	157.85	164.21*	163.63*
8	101.51	101.44	101.56	105.15	105.12
8a	154.81	154.77	154.80	155.40	155.82*
1'	139.25	139.27	139.29	138.90	38.24
2', 6'	127.17	127.15	127.17	127.15	22.66
3', 5'	127.61	127.61	127.62	127.15	13.94*
4'	128.21	128.20	128.21	128.16	
1''	206.74	211.46	211.43	205.19	205.77
2''	53.60	39.92	46.67	51.87	45.23
3''	25.10	19.28	26.68	24.99	17.84
4''	22.65	19.28	11.84	22.54*	13.84*
5''	22.65		16.69	22.65*	
2'''	79.88	79.87	79.85	92.92	92.80
3'''	126.31	126.26	126.33	26.57	26.74
4'''	115.56	115.52	115.59	71.31	71.51
5'''	28.27	28.20	28.17	24.94	24.79
6'''	28.27	28.20	28.17	26.24	26.22

^aThe ^{13}C NMR spectra were recorded at 100 MHz in CDCl_3 . ^b75 MHz in CDCl_3 .

*Chemical shifts within a given column are interchangeable.

= 14.4, 7.5 Hz, H-1a'), 2.93 (1H, *dt*, $J = 14.4, 7.5$ Hz, H-1b'), 1.62 (2H, *sext*, $J = 7.5$ Hz, H-2'), 1.01* (3H, *t*, $J = 7.5$ Hz, H-3'), 3.02 (1H, *dt*, $J = 16.5, 7.4$ Hz, H-2a''), 3.07 (1H, *dt*, $J = 16.5, 7.4$ Hz, H-2b''), 1.76 (2H, *sext*, $J = 7.4$ Hz, H-3''), 1.02* (3H, *t*, $J = 7.4$ Hz, H-4''), 4.88 (1H, *t*, $J = 9$ Hz, H-2'''), 3.23 (1H, *dd*, $J = 15.5, 9.5$ Hz, H-3a'''), 3.28 (1H, *dd*, $J = 15.5, 8.6$ Hz, H-3b'''), 1.29 (3H, *s*, H-5'''), 1.42 (3H, *s*, H-6'''); EIMS

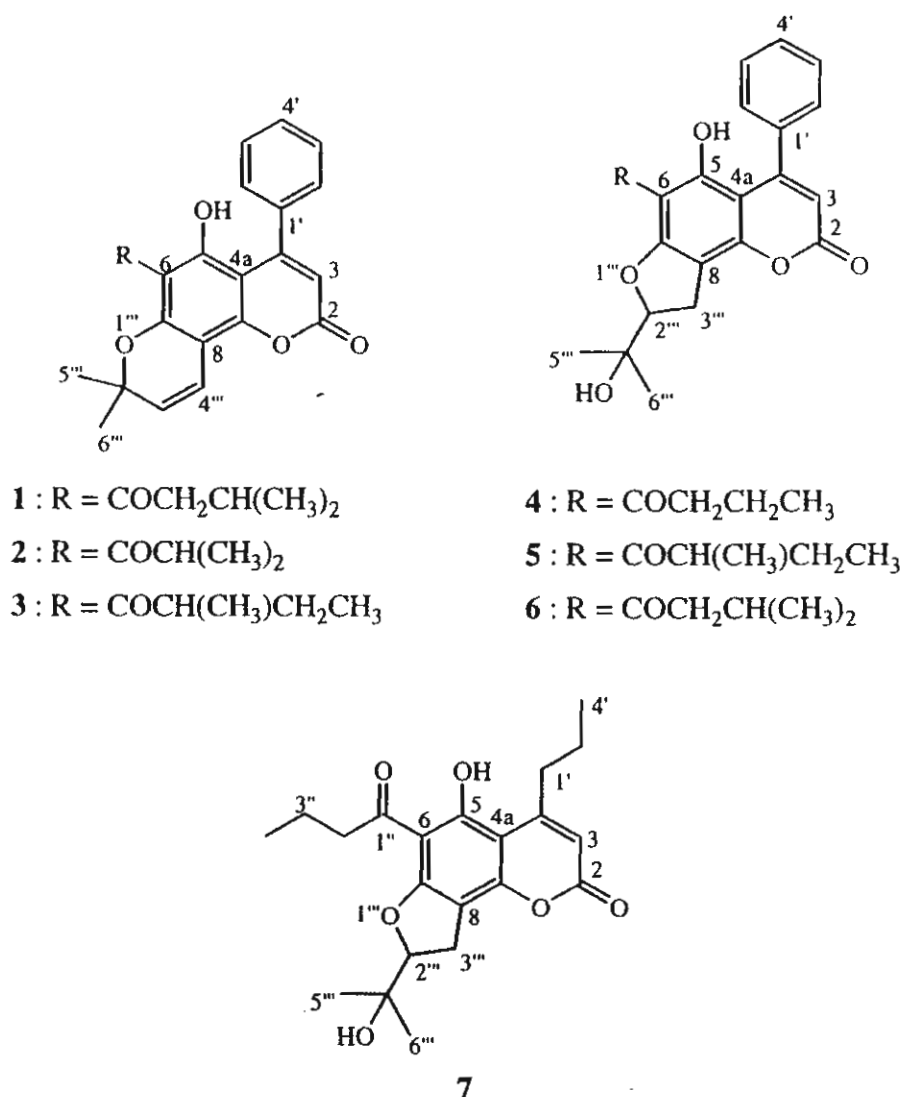


Fig. 1. Structures of compounds 1-7.

m/z : 374(M^+ , 100), 359 (7), 346 (9), 341 (25), 331 (11), 315 (20), 313 (14), 303 (59), 287 (14), 273 (28), 259 (23), 245 (13), 59 (34); $[\alpha]_D^{28} +1.90^\circ$ (c 0.42, CHCl₃).

*These values may be interchanged.

RESULTS AND DISCUSSION

Dried twigs of *M. siamensis* were extracted with hexane to give a crude extract that was separated by column chromatography, preparative TLC, semipreparative HPLC on a reverse-phase column to afford seven coumarins (1-7) (Fig. 1).

Compounds 1-7, which have never been previously isolated from *M. siamensis*, were identified as mammea A/AA cyclo D (1) (Chakraborty et al., 1969; Crombie

et al., 1967), mammea A/AD cyclo D (2) (Chakraborty et al., 1969), mammea A/AB cyclo D (3) (Carpenter et al., 1971), mammea A/AC cyclo F (4) (Morel et al., 1999), mammea A/AB cyclo F (5) (Crombie et al., 1972), mammea A/AA cyclo F (6) (Crombie et al., 1972), and mammea B/AC cyclo F (7) (Crombie et al., 1987). The detailed assignments of the NMR spectral data of all of these coumarins, except for compound 4, have not been reported. Using ¹H and ¹³C NMR spectra with the aid of ¹H-¹H COSY, ¹H-¹³C COSY, ¹H-¹³C COLOC and by comparison with existing data from the literature, we have confirmed the structures of these coumarins. Among them, mammea A/AB cyclo F (5), was isolated as an inseparable mixture. Both ¹H and ¹³C NMR (Table 1) showed two sets of resonances of nearly equal intensity and virtually identical chemical

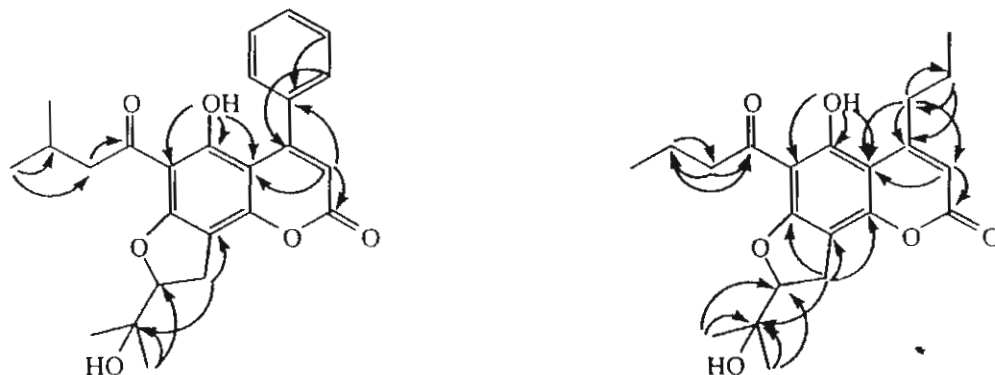


Fig. 2. COLOC correlations of compounds 6 and 7.

shifts due to the presence of a diastereomeric mixture. Furthermore, the position of the chelated OH group was confirmed by COLOC experiment. In the COLOC spectrum, the chelated OH showed cross peaks with the carbons C-4a and C-5, demonstrating that the coumarin nucleus was substituted by the acyl chain at C-6. The COLOC correlations of compounds 6 and 7 are illustrated in Figure 2. Interpretations of their ^{13}C NMR spectra, except for mammea A/AC cyclo F (4), are given in Table 2, and the assignments were based on the analysis of HETCOR, DEPT and COLOC spectra.

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REFERENCES

- Balza F, Abramowski Z, Towers GHN, Wiriyaichitra P (1989): Identification of proanthocyanidin polymers as the piscicidal constituents of *Mammea siamensis*, *Polygonum stagnium* and *Diospyros diepenhorstii*. *Phytochemistry* 28: 1827–1830.
- Carpenter I, McGarry EJ, Scheinmann F. Extractives from Guttiferae. Part XXI (1971): The isolation and structure of nine coumarins from the bark of *Mammea africana* G. Don. *J Chem Soc (C)*: 3783–3790.
- Chakraborty DP, Chatterji D (1969): Structure of mesuagin. A new 4-phenylcoumarin. *J Org Chem* 34: 3784–3788.
- Crombie L, Games DE, McCormick A (1967): Extractives of *Mammea americana* L. Part II. The 4-phenylcoumarins. Isolation and structure of mammea A/AA, A/A cyclo D, A/BA, A/AB and A/BB. *J Chem Soc (C)*: 2553–2558.
- Crombie L, Games DE, Haskins NJ, Reed GF (1972): Extractives of *Mammea americana* L. Part IV. Identification of new 7,8-annulated relatives of the coumarins mammea A/AA, A/AB, B/AA, and B/AB, and new members of the 6-acyl family B/AA, B/AB, and B/AC. *J Chem Soc Perkin Trans I*: 2248–2253.
- Crombie L, Jones RCF, Palmer CJ (1987): Synthesis of the *Mammea* coumarins. Part 1. The coumarins of the mammea A, B, and C series. *J Chem Soc Perkin Trans I*: 317–331.
- Morel C, Guilet D, Oger JM, Séraphin D, et al. (1999): 6-Acylcoumarins from *Mesua racemosa*. *Phytochemistry* 50: 1243–1247.
- Poobrasert O, Constant HL, Beecher CWW, Farnsworth NR, et al. (1998): Xanthones from the twigs of *Manneia siamensis*. *Phytochemistry* 47: 1661–1663.
- Thebtaranonth C, Imraporn S, Padungkul N (1981): Phenylcoumarins from *Ochrocarpus siamensis*. *Phytochemistry* 20: 2305–2306.

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CHEMICAL INVESTIGATION OF *MAMMEA SIAMENSIS*

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ABSTRACT

A new 4-alkylcoumarin, *mammea B/AC cyclo D* (1), together with a 4-phenylcoumarin, *mammea A/AC cyclo D* (2), were isolated from the hexane extract of the dried flower of *Mammea siamensis*. Their structures were determined on the basis of spectroscopic evidence.

INTRODUCTION

Mammea siamensis T. Anders. belongs to the Guttiferae and the tribe Calophylleae of the subfamily Calophylloideae. The plant was previously known as *Ochrocarpus siamensis* and is widely distributed in Thailand, Myanmar, Laos, Cambodia and Vietnam. Plants of this subfamily are known to be rich sources of xanthenes (Bandaranayake et al., 1980), triterpenes (Bandaranayake et al., 1980), flavonoids (Tosa et al., 1997) and coumarins (Games et al., 1972). The Thai name for the plant is 'sarapee' and its flower is a well known ingredient in traditional Thai medicine, especially used as a cardiac stimulant.

MATERIALS AND METHODS

Melting points were determined on an electrothermal melting point apparatus (Electrothermal 9100) and

reported without correction. ¹H NMR spectra were recorded on a Bruker AM 400 in deuterochloroform using tetramethylsilane as an internal standard. Infrared spectra (IR) were obtained on Perkin Elmer System 2000 FT-IR and JASCO A-302 spectrometers. Mass spectra were determined using GC-MS Finnigan INCOS 50 and GC-MS MAT 90 instruments. UV spectra were measured with a Shimadzu UV-VIS 2001S spectrophotometer. Column chromatography was carried out using silica gel 60 (0.063–0.200 mm) and silica gel 60 (particle size less than 0.063 mm). Thin-layer chromatography (TLC) and preparative thin-layer chromatography (preparative TLC) were carried out on silica gel 60 PF₂₅₄ (cat. No. 7747E, Merck).

Plant Material

Dried flowers of *Mammea siamensis* T. Anders. were purchased from a local traditional drug store in Bangkok, Thailand.

Extraction and Isolation

The air-dried flowers (8.5 kg) of *Mammea siamensis* were ground and extracted with hexane (12 L × 2 days × 7 times) at room temperature for 14 days, followed by filtration. The filtrates were combined and evaporated under reduced pressure to give a dark brown gum (428 g). A portion of this gum (300 g) was first subjected to coarse separation by column chromatography over silica gel using gradient elution of ethyl acetate in hexane with increasing polarity of ethyl acetate. Successive fractions were combined on the basis of their behavior on TLC and GC-MS and evaporated to give six fractions. Fraction 5 (62.5 mg) was purified further by preparative TLC on silica gel using 7% ethyl acetate-hexane to give a new *mammea B/AC cyclo D*, 1 (20 mg), together with the known *mammea A/AC cyclo D* or 6-butyryl-5-hydroxy-4-phenylseselin, 2 (27 mg).

Keywords: *Mammea siamensis*, Guttiferae, 4-substituted coumarins.

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Table 1. NMR spectral data of mammea B/AC cyclo D, 1.

¹ H and ¹³ C No. (group)*	¹³ C δ ppm	HETCOR correlates with ¹ H-No. δ ppm	Multiplicity J in Hz	COSY correlates with	COLOC correlates with
2(C)	160.06	—	—	—	—
3(CH)	110.32	H-3, 5.93	s	—	C-4a
4(C)	159.49	—	—	—	—
4a(C)	103.22	—	—	—	—
5(C)	165.11	OH-5, 15.34	s	—	C-4a, C-6
6(C)	106.99	—	—	—	—
7(C)	157.67	—	—	—	—
8(C)	101.50	—	—	—	—
8a(C)	155.09	—	—	—	—
1'(CH ₂)	38.45	H-1', 2.92	dd, 7.6, 7.5	H-2'	C-3, C-4, C-4a
2'(CH ₂)	22.74	H-2', 1.63	br sextet	H-1', H-3'	—
3'(CH ₂)	13.98	H-3', 0.99	t, 7.3	H-2'	—
1''(C)	207.47	—	—	—	—
2''(CH ₂)	46.88	H-2'', 3.06	t, 7.4	H-3''	C-1''
3''(CH ₂)	18.28	H-3'', 1.72	sextet, 7.4	H-2'', H-4''	—
4''(CH ₂)	13.90	H-4'', 1.00	t, 7.4	H-3''	—
2'''(C)	79.65	—	—	—	—
3'''(CH)	126.20	H-3''', 5.57	d, 10.0	H-4'''	C-2''', C-8
4'''(CH)	115.67	H-4''', 6.81	d, 10.0	H-3'''	—
5'''(CH ₂)	28.69	H-5''', 1.52	s	—	C-2''', C-3'''
6'''(CH ₂)	29.69	H-6''', 1.52	s	—	C-2''', C-3'''

*Determined from DEPT spectra

Mammea B/AC cyclo D (1) was recrystallized from dichloromethane-hexane to give yellow needles, m.p. 105–106 °C. IR ν_{\max} CHCl₃: 3028, 2928, 2855, 1731, 1643, 1615, 1583, 1465, 1426, 1384, 1267, 1189, 1152, 1115 cm⁻¹. UV (MeOH) λ_{\max} nm (log ϵ): 227 (4.245), 285 (4.446), 335 (3.732), 375 (3.586). High resolution FABMS (positive mode) obs. 357.1697 calcd. for C₂₁H₂₄O₅ + H 357.1701. EIMS m/z (rel. int.): 356 (M⁺, 32), 341 ([M-CH₃]⁺, 100), 269 ([341-OCCH₂CH₂CH₃]⁺, 2), 227 ([269-CH₂CH₂CH₃]⁺).

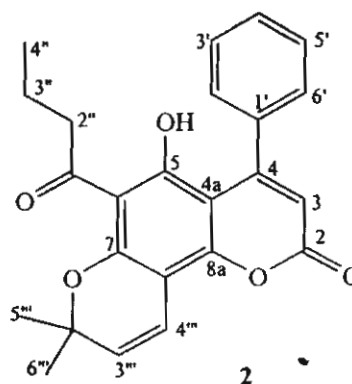
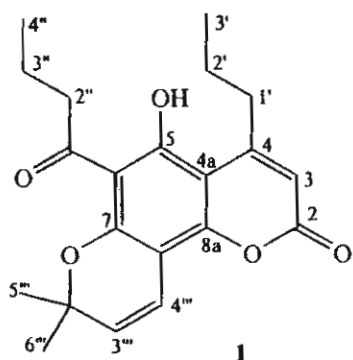
Mammea A/AC cyclo D or 6-butyryl-5-hydroxy-4-phenylseselin (2) was recrystallized from ethyl acetate-hexane to give yellow crystals, m.p. 133–134.7 °C. (138–139 °C; Thebtaranonth et al., 1981). IR ν_{\max} CHCl₃: 3500, 2969, 1725, 1642, 1610, 1582, 1465, 1380, 1140, 1118, 860, 700 cm⁻¹. UV (EtOH) λ_{\max} nm (log ϵ): 234 (4.75), 285 (4.66). FABMS m/z (rel. int.): 391 ([M+H]⁺, 100). EIMS m/z (rel. int.): 390 (M⁺, 30), 375 ([M-CH₃]⁺, 100), 357 (20), 347 (14). ¹H NMR (400 MHz, CDCl₃) δ : 5.96 (s, H-3), 14.73 (s, OH-5), 7.29 (m, H-2', H-6'), 7.38 (m, H-3', H-4', H-5'), 3.02 (t, J = 7.3 Hz, H-2''), 1.67 (sextet, J = 7.3 Hz, H-3''), 0.97 (t, J = 7.3 Hz, H-4''), 5.60 (d, J = 10.0 Hz, H-3'''), 6.86 (d, J = 10.0 Hz, H-4'''), 1.55 (s, H-5''', H-6'''). ¹³C NMR (100 MHz, CDCl₃) δ : 159.63 (C-2), 112.66 (C-3), 156.38 (C-4), 102.15 (C-4a), 164.37 (C-5), 106.97 (C-6), 158.20 (C-7), 101.48 (C-8), 154.79 (C-8a), 139.21 (C-1'), 127.15 (C-2', C-6'), 127.60 (C-3', C-5'), 128.21 (C-4'), 207.20 (C-1''), 46.79 (C-2''), 18.19

(C-3''), 13.07 (C-4''), 79.84 (C-2'''), 126.31 (C-3'''), 115.51 (C-4'''), 28.26 (C-5''', C-6''').

RESULTS AND DISCUSSION

Dried flowers of *Mammea siamensis* were extracted with hexane and chromatographic separation on silica gel led to the isolation of the new mammea B/AC cyclo D (1) and known mammea A/AC cyclo D (2).

Mammea B/AC cyclo D, 1, was obtained as yellow needles, m.p. 105–106 °C. The IR spectrum of 1 showed two carbonyl group absorptions at 1731 (unsaturated δ -lactone) cm⁻¹ and at 1643 (aryl ketone) cm⁻¹. The UV spectrum of compound 1 exhibited the absorption maxima at 227, 285, 335, 375 nm. The molecular formula of 1 was determined to be C₂₁H₂₄O₅ from (M⁺ + H) at m/z 357.1701 in the high resolution FABMS (positive mode). Its EI mass spectrum displayed the molecular ion peak at m/z 356 (M⁺) and the base peak for (M⁺ - CH₃) at m/z 341 (100%). Mammea 1 has been detected by GC-MS in *Mammea americana*, and tentatively assigned the structure without ¹H and ¹³C spectral data (Games et al., 1972). We have carried out the detailed assignment of the chemical shifts of protons and carbons in 1 using COSY, HETCOR and COLOC experiments as shown in Table 1. The ¹H NMR spectrum of compound 1 showed the presence of a 2,2-dimethylchromene ring system [δ



5.57 (H-3''), 6.81 (H-4''), 2H, AB system $J_{AB} = 10.0$ Hz; 1.52 (H-5'', H-6'', 6H, s), hydroxyl group at C-5 (δ 15.34, 1H, s), propyl group [δ 2.92 (H-1'), 2H, dd, $J = 7.6, 7.5$ Hz; 1.63 (H-2'), 2H, br sextet; and 0.99 (H-3'), 3H, t, $J = 7.3$ Hz]. Substitution at C-4 of the coumarin was apparent from the C-3 proton singlet at δ 5.93, and the nature of the substituent at C-6 was deduced as a butyryl chain from the signals at δ 1.0 (H-4''), 3H, dd, $J = 7.4$ Hz; 1.72 (H-3'', 2H, sextet, $J = 7.4$ Hz) and 3.06 (H-2'', 2H, t, $J = 7.4$ Hz). The ^{13}C proton decoupling NMR spectrum of **1** (Table 1) showed 21 signals. Analysis of the DEPT spectra of this compound suggested the presence of ten quaternary carbon atoms at δ 160.06 (C-2), 159.49 (C-4), 103.22 (C-4a), 165.11 (C-5), 106.99 (C-6), 157.67 (C-7), 101.50 (C-8), 155.09 (C-8a), 79.65 (C-2''), 207.47 (C=O of butyryl group), three olefinic methine carbon atoms at δ 110.32 (C-3), 126.20 (C-3''), 115.67 (C-4''), four methyl carbon atoms at δ 13.98 (Me-3'), 13.90 (Me-4''), 28.69 (Me-5''), 29.69 (Me-6''). All the connectivities were supported by the COLOC spectrum (Table 1). The proton signal of OH-5 at δ 15.34 ppm showed cross peaks with the carbon signals of C-4a (δ 103.22) and C-6 (δ 106.99), the proton signal of H-3 at δ 5.93 ppm showed a cross peak with the carbon signal C-4a (δ 103.22) and the proton signal of H-1' at δ 2.92 showed cross peaks with the carbon signals of C-3 (δ 110.32), C-4 (δ 159.49) and C-4a (δ 103.22).

The known mammea A/AC cycloD **2** or 6-butyryl-5-hydroxy-4-phenylseselin was identified by comparing its physical and spectroscopic data with literature values (Morel et al., 1999; Thebtaranonth et al., 1981).

ACKNOWLEDGEMENT

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REFERENCES

- Bandaranayake WM, Karunanayake S, Sotheeswaran S, Sultanbawa MUS (1980): Xanthones & triterpenes of *Mammea acuminata* (Guttiferae). *Indian J Chem* 19B: 463-467.
- Games DE (1972): Identification of 4-phenyl and 4-alkyl-coumarins in *Mammea americana* L., *Mammea africana* G. Don and *Calophyllum inophyllum* by gas chromatography-mass spectrometry. *Tetrahedron Lett* 31: 3187-3190.
- Morel C, Guilet D, Oger JM, Séraphin D, Sévenet T, Wiart C, Hadi A Hamid A, Richchomme P, Bruneton J (1999): 6-Acylcoumarins from *Mesua racemosa*. *Phytochemistry* 50: 1243-1247.
- Thebtaranonth C, Imraporn S, Padungkul N (1981): Phenyl-coumarins from *Ochrocarpus siamensis*. *Phytochemistry* 20: 2305-2306.
- Tosa H, Iinuma M, Murakami K, Ito T, Tanaka T, Chelladurai V, Riswan S (1997): Three xanthones from *Poe-ciloneuron pauciflorum* and *Mammea acuminata*. *Phytochemistry* 45: 133-136.

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THE FIRST SYNTHESIS OF WRIGHTIADIONE

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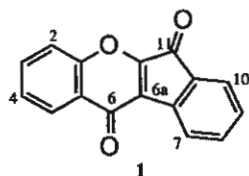
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ABSTRACT

The first synthesis of tetracyclic isoflavone wrightiadione 1 was achieved through the benzylic oxidation of the key intermediate isoflavone 2 which in turn could be obtained by condensation of 2-indanone with methyl salicylate and LDA.

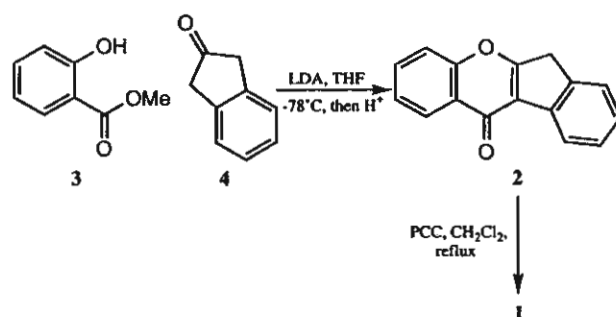
Wrightiadione 1, a novel isoflavone, was isolated from the bark of *Wrightia tomentosa* which has been used as a medicinal plant in Thailand.¹ This compound contains a unique tetracyclic ring system which represents the first natural tetracyclic isoflavone.

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The retrosynthetic analysis of compound **1** suggested isoflavone **2** as the key intermediate as methylene position of this compound **2** could be oxidised to the target molecule **1**. Compound **2** could conceivably be prepared from the condensation of methyl salicylate **3** with 2-indanone **4**.

It was found that isoflavone **2** could be synthesised from the reaction of 2-indanone **4** and the unprotected methyl salicylate **3** using 2.2 equivalents of LDA as the base in THF at -78°C in moderate yield (49 %) as shown in the scheme. The condensation of the 2-indanone **4** with O-benzyl methyl salicylate failed to give the required product. The formation of phenolate apparently activates methoxide as a leaving group. The use of unprotected methyl salicylate in the condensation with the enolate has recently been reported.²



Scheme

In order to obtain the final product, the key benzylic oxidation had to be performed. The benzylic oxidation to the corresponding carbonyl group is a well known process. We have investigated many oxidising agents for the above transformation and the results are shown in the table. We found that oxidation with PCC in methylene chloride gave the best result yielding wrightiadione **1** in 68% yield.

There are some discrepancies between the NMR spectral data of our synthetic compound and those reported for the natural product.¹⁴ In the ^{13}C NMR spectrum of the natural product the carbonyl group absorption at

Table. Yield of Wrightiadione **1** from Various Oxidation Methods

Entry	Conditions	time(h)	% yield 1
1	PDC, celite 535, benzene, reflux ³	8	39
2	PDC, celite 535, benzene, reflux ³	18	44
3	SeO ₂ ·EtOH, H ₂ O, reflux ^{4,5}	7	8
4	CuBr ₂ , EtOAc, reflux ^{6,7}	4	33
5	50 % HBr, DMSO, reflux ⁸	8	31
6	CAN, MeOH, RT ⁹	15	17
7	CAN, AcOH, reflux ¹⁰	2	22
8	PCC, CH ₂ Cl ₂ , reflux ^{11,12}	20	68
9	NHPI*, CH ₃ CN, reflux ¹³	20	25

*NHPI=N-Hydroxyphthalimide

C-11 was not observed, while in the ¹³C NMR of the synthetic compound the C-6a absorption was not detected. We have confirmed the structure of compounds **1** and **2** by 1D and 2D NMR techniques. The structure of our synthetic compound was confirmed by X-ray crystallography and found to be identical to that reported for natural wrightiadione.

EXPERIMENTAL

Melting points are uncorrected. Nuclear magnetic resonance (NMR) data for ¹H NMR were taken at 400 MHz and ¹³C NMR at 100 MHz. Tetramethylsilane was used as the internal standard, and chemical shifts are reported as δ_H (ppm) or δ_C (ppm). Mass spectra (MS) were obtained by electron impact technique (EI). Elemental analyses were performed at the Faculty of Science, Mahidol University.

Benz[b]indeno[1,2-e]pyran-6-one (**2**): Reaction of 2-Indanone **4** with Methyl Salicylate **3**

A solution of LDA in dry THF (150 mL) was prepared by adding diisopropylamine (16.8 mL, 0.12 mol) dropwise into 1.29 M of *n*-BuLi (85 mL, 0.11 mol) in hexane under nitrogen atmosphere at 0 °C. The ice-water bath was replaced by a dry ice/acetone bath. The stirring was continued for 30 min at -78 °C, then 2-indanone **4** (6.60 g, 0.05 mol) in dry THF (50 mL) was added. The pale yellow solution turned deep red, indicating anion formation. The reaction mixture was allowed to warm to 0 °C by

ice-water bath, stirred for 10 min and the ice-water bath was replaced by a dry ice/acetone bath. The solution of methyl salicylate **3** (7.60 g, 0.05 mol) in dry THF (50 mL) was added slowly by syringe to the above mixture. The stirring at this temperature was continued for 2 h and then the reaction mixture was warmed to room temperature, then 2 N HCl was added and stirred for 1 h. Removal of the solvent under vacuum gave a residue which was extracted with methylene chloride. The combined organic extracts were washed with aqueous sodium carbonate solution, water and brine solution, and dried over anhydrous sodium sulfate. After removal of the solvent, the residue was chromatographed on silica gel column to provide starting material, methyl salicylate **3** and benz[b]indeno[1,2-e]pyran-6-one **2** as adduct, respectively. After recrystallization of the adduct from ethanol benz[b]indeno[1,2-e]pyran-6-one **2** (5.71 g, 49 %) was obtained as a colorless crystal: mp 188–189 °C (lit.¹⁵ 176–177 °C); IR (Nujol) 1640 (C=O), 1615 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 3.91 (s, 2H, *H* 11), 7.26 (ddd, 1H, *J* = 8.0 Hz, 1.1, *H* 7.6, 9), 7.38 (t, 1H, *J* = 7.6, 7.6 Hz, *H* 8), 7.42 (d, 1H, *J* = 8.0 Hz, *H* 10), 7.45 (ddd, 1H, *J* = 0.8, 7.7, 7.9 Hz, *H* 4), 7.52 (dd, 1H, *J* = 0.8, 8.4 Hz, *H* 2), 7.66 (ddd, 1H, *J* = 1.6, 7.7, 8.4 Hz, *H* 3), 8.23 (dd, 1H, *J* = 1.1, 7.6 Hz, *H* 7), 8.34 (dd, 1H, *J* = 1.6, 7.9 Hz, *H* 5); ¹³C NMR (100 MHz, CDCl₃) ppm 36.7 (C-11), 118.0 (C-2), 121.1 (C-6a), 122.6 (C-7), 123.7 (C-10), 124.8 (C-5a), 125.1 (C-4), 125.8 (C-9), 126.1 (C-5), 127.4 (C-8), 132.9 (C-3), 134.1 (C-10a), 138.2 (C-6b), 156.3 (C-1a), 171.8 (C-11a), 174.2 (C-6); MS (EI) *m/z* 234 (M⁺, 100), 205 (29), 176 (15), 76 (20). Anal. calcd for C₁₆H₁₀O₂: C, 82.04; H, 4.30. Found: C, 82.17; H, 4.07.

Wrightiadione (1): Oxidation of Benz[b]indeno[1,2-e]pyran-6-one **2 with PCC in Methylene Chloride**

The mixture of benz[b]indeno[1,2-e]pyran-6-one **2** (0.24 g, 1.0 mmol) and pyridinium chlorochromate (0.2 g) was heated under reflux with stirring in methylene chloride (20 mL) for 3 h. After the precipitate was filtered and washed thoroughly with methylene chloride, the residue was obtained after evaporation of the solvent under reduced pressure. The orange residue was recrystallized from ethanol to give orange crystals of **1** (0.18 g, 68%): mp 244–246 °C (lit.¹ mp 228–230 °C); IR (Nujol) 1727 (C=O), 1641 (C=O), 1614, 1605, 1483, 1459, 1371 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.30 (ddd, 1H, *J* = 0.9, 7.3, 7.6 Hz, *H* 9), 7.53 (ddd, 1H, *J* = 1.1, 7.3, 7.6 Hz, *H* 8), 7.54 (ddd, 1H, *J* = 1.1, 7.1, 8.0 Hz, *H* 4), 7.61 (bd, 1H, *J* = 7.3 Hz, *H* 7), 7.69 (dd, 1H, *J* = 1.1, 8.5 Hz, *H* 2), 7.80 (ddd, 1H, *J* = 1.6, 7.1, 8.5 Hz, *H* 3), 7.96 (dd, 1H, *J* = 0.9, 7.3 Hz, *H* 10), 8.30 (dd, 1H, *J* = 1.6, 8.0 Hz, *H* 5) and ¹³C NMR (100 MHz, CDCl₃) ppm 120.7 (C-2), 125.1 (C-10), 126.1 (C-7), 127.4 (C-5),

127.4 (C-5a), 128.3 (C-11a), 130.1 (C-9), 136.1 (C-3), 137.4 (C-8), 141.4 (C-10a), 157.3 (C-6b), 157.6 (C-1a), 176.7 (C-6), 189.67 (C-11), C-6a absorption was not observed; MS(EI) m/z 248 (M^+ , 100), 220(24), 164(12).

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REFERENCES AND NOTES

1. Lin, L.-J.; Topcu, G.; Lotter, H.; Ruangrunsi, N.; Wagner, H.; Pezzuto, J.M.; Cordell, G.A. *Phytochemistry* **1992**, *81*, 4333.
2. Morris, J.; Wishka, D.G.; Fang, Y. *Synth. Commun.* **1994**, *24*, 849.
3. Kodukulla, R.P.K.; Trivedi, G.K.; Vora, J.D.; Mathur, H.H. *Synth. Commun.* **1994**, *24*, 819.
4. Corey, E.J.; Schaefer, J.P. *J. Amer. Chem. Soc.* **1960**, *82*, 918.
5. Bockstahler, E.R.; Wright, D.L. *J. Amer. Chem. Soc.* **1949**, *71*, 3760.
6. King, L.C.; Ostrum, G.K. *J. Org. Chem.* **1964**, *29*, 3459.
7. Doifode, K.B.; Marathe, M.G. *J. Org. Chem.* **1964**, *29*, 2025.
8. Floyd, M.B.; Du, M.T.; Fabio, P.F.; Jacob, L.A.; Johnson, B.D. *J. Org. Chem.* **1985**, *50*, 5022.
9. Badea, I.; Cotellet, P.; Catteau, J.-P. *Synth. Commun.* **1994**, *24*, 2011.
10. Trahanovsky, W.; Young, L.B. *J. Org. Chem.* **1966**, *31*, 2033.
11. Parish, E.J.; Chitrakorn, S.; Wei, T.-Y. *Synth. Commun.* **1986**, *16*, 1371.
12. Dauben, W.G.; Lorber, M.; Fullerton, D.F. *J. Org. Chem.* **1969**, *34*, 3587.
13. Ishii, Y.; Nakayama, K.; Takeno, M.; Sakaguchi, S.; Iwahama, T.; Nishiyama, Y. *J. Org. Chem.* **1995**, *60*, 3934.
14. Direct comparison of the synthetic compound and the natural product was not possible due to the unavailability of the natural wrightiadiene (Professors G.A. Cordell and N. Ruangrunsi, personal communications).
15. Boyd, G.V.; Hewson, D. *Chem. Commun.* **1965**, 536. We are grateful to a referee for bringing our attention to this reference which reports a less direct route to compound 2.

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Labdane and pimarane diterpenes from *Croton joufra*^a

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Abstract

From the chloroform extract of the leaves of *Croton joufra* two novel diterpenes $2\alpha,3\alpha$ -dihydroxy-labda-8(17),12(13),14(15)-triene and 3β -hydroxy-19-*O*-acetyl-pimara-8(9),15-dien-7-one were isolated. Their

structures were established by spectroscopic methods. One of the compounds showed weak lethality to brine shrimp.

Keywords: *Croton joufra*; Euphorbiaceae; Labdan; 2,3-Dihydroxy-labda-8(17),12(13),14(15)-triene; Pimarane; 3-Hydroxy-19-*O*-acetyl-pimara-8(9),15-dien-7-one; Brine shrimp lethality test.

1. Introduction

Croton joufra Roxb. (Euphorbiaceae) is a medium size shrub commonly named in Thai as "Plau noi", the same name as that used for *Croton sublyratus*.

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A decoction of the leaves and bark have been used as an antidyentery and peptic promotor and a decoction of the flowers as an anthelmintic (Phupattanapong & Wongprasart, 1987). The heartwood and stems have been used as a blood tonic and antipyretic (Mokkhasmit, Ngarmwathana, Sawasdimongkol & Permhiphat, 1971). In previous studies, two furanoditerpenes, plaunol A and plaunol C, were reported from the methanolic extract of the stems by TLC (Ogiso, Kitazawa, Mikuriya & Promdej, 1981). Subsequently, Roengsumran and coworkers (1982) isolated another furanoditerpene swassin from the stems of this plant. We report here on the isolation and structural characterization of a labdane and a pimarane diterpene from the leaves of this plant.

2. Results and discussion

In our search for bioactive compounds from plants of the Euphorbiaceae, we used the *Artemia salina* (brine shrimp) toxicity test as an in-house bioassay method (Meyer *et al.*, 1982). Among the hexane, chloroform and methanol extracts, the chloroform extract, which showed $LC_{50} = 62.8 \mu\text{g/ml}$, was chosen for further purification. Two novel compounds were obtained

from the chloroform extract after several chromatographic separations. Identification of these compounds was based on spectral data.

Compound **1** was obtained as a pale yellow powder, mp 72-74°. The EIMS gave a molecular ion at m/z 304, corresponding to the molecular formula $C_{20}H_{32}O_2$. The FT-IR spectrum indicated secondary alcohol absorptions (ν_{\max} 3405 and 1054 cm^{-1}) and an olefinic double bond (ν_{\max} 1645 and 891 cm^{-1}). The ^1H NMR spectrum showed three methyl group singlets at δ 0.78, 0.80 and 1.02, in addition to a downfield methyl group signal at δ 1.76 H(s) assignable to $\text{CH}_3\text{-C}=\text{C}$. The spectrum also exhibited olefinic protons at δ 5.08 (1H, *d*, $J=10.8$ Hz), 5.17 (1H, *d*, $J=17.2$ Hz), 5.26 (1H, *t*, $J=6.4$ Hz) and 6.73 (1H, *dd*, $J=10.8, 17.3$ Hz), together with additional exocyclic methylene group signals as two broad one proton singlets at δ 4.50 and 4.86. These signals, particularly the olefinic proton signals, resemble those reported in 12,13*E*-biformen (Bohlmann & Czerson, 1979). Two sets of additional one-proton signals at δ 3.02 (*d*, $J=9.6$ Hz) and 3.70 (*ddd*, $J=4.1, 9.8$ and 11.2 Hz) were characterized as two vicinal oxymethine protons of which the former carbinolic carbon was bonded to a tertiary carbon; these two protons were assigned to H-3 and H-2, respectively. The ^1H - ^1H COSY spectrum further indicated that the one-proton signal at δ 3.70 (H-2) was coupled to the one-proton doublet signal at

δ 3.02 (H-3) and also to two other signals at δ 1.21 (1H, *dd*, $J=4.3$ and 12.4 Hz, H-1e) and 2.11 (1H, *dd*, $J=3.0$ and 3.6 Hz, H-1a). The ^{13}C -NMR spectrum showed 20 carbons comprising four quaternary (of which two were olefinic), and six methine (including two oxymethine carbons at δ 69.1(*d*) and 83.4 (*d*)). The compound was proposed as a 2,3-dihydroxy-labda-8(17),12(13),14(15)-triene (**1**). Extensive use of NMR techniques, including ^1H - ^1H COSY, HETCOR, COLOC, led to the complete assignments of the ^1H and ^{13}C shift values, as shown in Table 1. The important long-range- ^1H - ^{13}C (COLOC) correlations are shown in Fig. 1.

The relative stereochemistry of **1** was deduced from the NOESY spectrum. The key NOE effects observed between H-2/H-3, H-2/C-19-Me, H-2/C-20-Me, H-3/C-18-Me and H-5/C-18-Me gave an indication that the two hydroxyl groups at C-2 and C-3 are both α oriented (Fig. 1).

Compound **2** was obtained as a colorless gum. The HR-FABMS (glycerol matrix) gave an $[\text{M}+\text{H}]^+$ ion at m/z 361.23804 corresponding to the molecular formula $\text{C}_{22}\text{H}_{32}\text{O}_4+\text{H}$. The FT-IR spectrum indicated a hydroxyl group (ν_{max} 3460 cm^{-1}), an ester group (ν_{max} 1730 cm^{-1}), an α,β -unsaturated ketone (ν_{max} 1650 cm^{-1}), and a vinylidene group (ν_{max} 3090 and 908 cm^{-1}). The ^1H NMR spectrum showed three methyl group singlets at δ 1.02, 1.11 and 1.15, together with a low field acetate methyl group signal at δ 2.08 (*s*).

Three sets of one proton signals δ 4.83 (1H, *dd*, $J=1.2$ and 17.5 Hz), 4.93 (1H, *dd*, $J=1.2$ and 10.8 Hz) and 5.66 (1H, *dd*, $J=10.8$ and 17.5 Hz), in addition to the ^{13}C NMR signals at δ 111.7 (*t*) and 144.9 (*d*), indicated a vinylidene group. The key ^1H - ^{13}C long range correlation (HMBC) between a methyl proton signal at δ 1.02 and the ^{13}C signal at δ 144.9 (*d*), in addition to correlations of the two vinylidene proton signals at either δ 4.83 or 4.93 to the ^{13}C signal at δ 34.4 (*s*), indicated a pimarane diterpene with a vinyl group bonded to C-13 (Rao, Sachdev, Seshadri & Singh, 1968). The one-proton signals at δ 4.23 (*d*, $J=11.8$ Hz) and 4.41 (*d*, $J=11.8$ Hz) and the ^{13}C signal at δ 65.0 (*t*) implied an oxymethylene moiety bonded to a COCH_3 group. The signals of two non-equivalent protons at δ 2.53 (*dd*, $J=14.0$ and 17.6 Hz) and 2.63 (*dd*, $J=3.9$ and 17.6 Hz), which were coupled to a methine proton at δ 1.73 (*dd*, $J=3.9$ and 14.0 Hz) indicated a $\text{CH-CH}_2\text{-CO}$ moiety. The absence of either an α or β -proton signal of an α,β -unsaturated carbonyl group, in addition to the ^{13}C signals at δ 129.0 (*s*), 164.3 (*s*), and 199.0 (*s*), indicated a fully substituted olefinic double bond at C-8(9) and a keto group at C-7. The 3J coupling between a one proton signal at δ 3.35 (*dd*, $J=4.8$ and 11.5 Hz) and the ^{13}C signals at δ 21.9 (*q*), 65.0 (*t*) and 34.0 (*t*) indicated the location of a hydroxyl group at C-3. Another 3J correlation between the

oxymethylene proton signals at δ 4.23 and 4.41 and the ^{13}C signal at δ 49.4 (*d*, assigned to C-5) implied the link of an OCOCH_3 group at either C-18 or C-19. The NOEs obtainable from the NOESY spectrum indicated the bonding between the OCOCH_3 group and C-19 from the interactions between C-20-Me/C-19- $\text{CH}_2\text{OCOCH}_3$ and H-2a/C-19- $\text{CH}_2\text{OCOCH}_3$. Furthermore, the NOE correlations between H-3/H-5, H-3/C-18-Me and H-3/H-2e also indicated the stereochemistry of C-3-OH as β (equatorial). Compound **2** was therefore proposed to be 3 β -hydroxy-19-*O*-acetyl-pimara-8(9),15-dien-7-one. Complete ^1H and ^{13}C chemical shifts (Table 1) were obtained from ^1H - ^1H COSY, HMQC and HMBC spectra. The key HMBC and NOESY correlations are illustrated in Fig.1.

The brine shrimp assay of **1** and **2** indicated weak toxicity in comparison to colchicine. The percent deaths at 204.2, 204.2 and 1.0 $\mu\text{g/ml}$ of **1**, **2** and colchicine after 24 h were 53.3, 20.0 and 50, respectively. The LC_{50} were found to be 197.1, >204.0 and 1.0 $\mu\text{g/ml}$, respectively.

3. Experimental

3.1 General

Mps uncorrected; ^1H and ^{13}C NMR spectra were acquired at 400 and 100 MHz, respectively. The solvent signals were taken as the reference.

3.2 Plant Material. The leaves of *Croton joufra* were collected from Kalasin Province, in the Northeast of Thailand, during December, 1995. The plant was kindly identified by N.R. The voucher specimens (SSCJ/1995) are deposited at the Department of Chemistry, Faculty of Science, Ramkhamhaeng University and the Herbarium, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

3.3 Extraction and isolation.

The dried leaves of *Croton joufra* were milled to obtain a fine powder (624 g) which were extracted successively with *n*-hexane, chloroform and methanol in a Soxhlet extraction apparatus. After evaporation of the solvents under red. pres., dark green gums of *n*-hexane (21.52 g), chloroform (31.70 g) and methanol (91.80 g) extracts were obtained.

The chloroform extract was fractionated on silica gel column using a gradient of *n*-hexane-chloroform (1:1 to 0:1) followed by chloroform-MeOH (10:0 to 1:1) to yield 8 major frs. after combination of similar frs. as judged by TLC. Fr. 6 was subjected to additional silica gel cc (CHCl_3 -

MeOH (1:0 to 1:1) to give 8 subfrs. (subfrs. 6.1-6.8). Subfr. 6.4 was subjected to chromatography (2x, silica gel cc, CH₂Cl₂-MeOH 19:1 to 1:1 and n-hexane-EtOAc 17:3) to yield **2** (5.5 mg). Subfr. 6.5 was subjected to chromatography (2x, silica gel cc, CHCl₃-MeOH 10:0 to 4:1 and n-hexane-CHCl₃ 3:7 to 1:4) to yield **1** (103.6 mg).

3.4 Compound **1** (*2 α , 3 α -Dihydroxy-labda-8(17),12(13),14(15)-triene*)

Pale yellow powder, mp 72-74°; $[\alpha]_D^{25}$ -18.24° (CHCl₃, c 0.34); HR-EIMS *m/z*: 304.24150, $[M]^+$, C₂₀H₃₂O₂ requires 304.24023; EI-MS 70 eV *m/z* (rel. int.): 304 ($[M]^+$, 14.5), 248 (50.5), 187 (25.0), 135 (100), 93 (98.5), 81 (36.5), 55 (99.5), 43 (67); IR (film, cm⁻¹): 3405, 2941, 1715, 1645, 1456, 1385, 1054, 954, 891; ¹H and ¹³C NMR spectral data are shown in Table 1.

3.5 Compound **2** (*3 β -Hydroxy-19-O-acetyl-pimara-8(9),15-dien-7-one*).

Colorless gum; $[\alpha]_D^{25}$ -127.62° (CHCl₃, c 0.105); HR-FABMS (glycerol) *m/z*: 361.23804, $[M+H]^+$, C₂₂H₃₂O₄+H requires 361.23788; IR (film, cm⁻¹): 3460, 3090, 2931, 2863, 1731, 1648, 1373, 1241, 1094, 1036, 908, 756; ¹H and ¹³C NMR spectral data are shown in Table 1.

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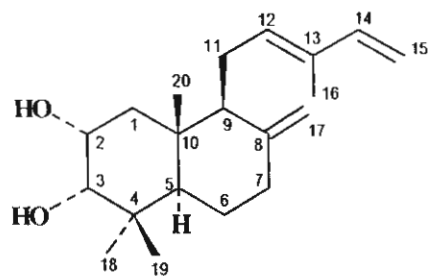
Table 1 ^1H and ^{13}C NMR spectral data of **1** and **2** (CDCl_3).

Position	1		2	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1	1.21 (<i>m</i>)	45.0 (<i>t</i>)	1.92 (<i>m</i>)	34.0 (<i>t</i>)
1'	2.11 (<i>dd</i> , 4.3, 12.4)			
2	3.70 (<i>ddd</i> , 4.2, 9.8, 11.3)	69.1 (<i>d</i>)	1.36 (<i>m</i>) ^a	27.2 (<i>t</i>)
2'			1.85 (<i>m</i>)	
3	3.02 (<i>d</i> , 9.6)	83.4 (<i>d</i>)	3.35 (<i>dd</i> , 4.8, 11.5)	78.0 (<i>d</i>)
4		39.4 (<i>s</i>)		41.8 (<i>s</i>)
5	1.19 (<i>br d</i> , 12.3)	54.5 (<i>d</i>)	1.73 (<i>dd</i> , 3.9, 14.0)	49.4 (<i>d</i>)
6	1.38 (<i>dddd</i> , 4.1, 12.8, 12.8, 12.9)	23.6 (<i>t</i>)	2.53 (<i>dd</i> , 14.0, 17.6)	35.6 (<i>t</i>)
6'	1.69 (<i>m</i>)		2.63(<i>dd</i> , 3.9, 17.6)	
7	1.99 (<i>ddd</i> , 4.7, 12.8, 12.9)	37.7 (<i>t</i>)		199.5 (<i>s</i>)
7'	2.39 (<i>m</i>)			
8		147.2 (<i>s</i>)		129.0 (<i>s</i>)
9	1.73 (<i>m</i>)	56.9 (<i>d</i>)		164.3 (<i>s</i>)
10		40.2 (<i>s</i>)		39.3 (<i>s</i>)
11	2.19 (<i>dd</i> , 7.0, 10.9)	22.3 (<i>t</i>)	2.00 (<i>m</i>) ^a	23.1 (<i>t</i>)
11'	2.37 (<i>m</i>)		2.18 (<i>m</i>)	
12	5.26 (<i>t</i> , 6.4)	130.9 (<i>d</i>)	1.28 (<i>m</i>)	33.5 (<i>t</i>)
12'			1.62 (<i>m</i>)	
13		131.9 (<i>s</i>)		34.4 (<i>s</i>)
14	6.73 (<i>dd</i> , 10.8, 17.3)	133.7 (<i>d</i>)	2.00 (<i>m</i>) ^a	33.2 (<i>t</i>)
14'			2.38 (<i>dd</i> , 1.5, 17.8)	
15	5.08 (<i>d</i> , 10.8)	113.5 (<i>t</i>)	5.66 (<i>dd</i> , 10.8, 17.5)	144.9 (<i>d</i>)
15'	5.17 (<i>d</i> , 17.3)			
16	1.76 (<i>s</i>)	19.7 (<i>q</i>)	4.83 (<i>dd</i> , 1.2, 17.5)	111.7 (<i>t</i>)
16'			4.93 (<i>dd</i> , 1.2, 10.8)	
17	4.50 (<i>br s</i>)	108.7 (<i>t</i>)	1.02 (<i>s</i>)	28.2 (<i>q</i>)
17'	4.86 (<i>br s</i>)			
18	1.02 (<i>s</i>)	28.8 (<i>q</i>)	1.15 (<i>s</i>)	21.9 (<i>q</i>)
19	0.80 (<i>s</i>)	16.6 (<i>q</i>)	4.23 (<i>d</i> , 11.8)	65.0 (<i>t</i>)
19'			4.41 (<i>d</i> , 11.8)	
20	0.78 (<i>s</i>)	15.5 (<i>q</i>)	1.11 (<i>s</i>)	17.5 (<i>q</i>)
CO				171.1 (<i>s</i>)
CH ₃			2.08 (<i>s</i>)	21.0 (<i>q</i>)

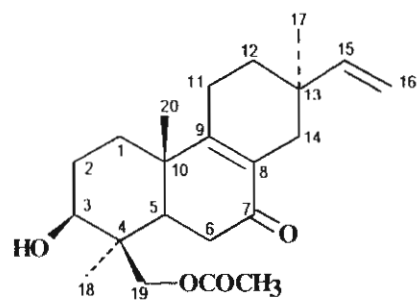
 δ in ppm and *J* (parentheses) in Hz., ^a Signal with the same superscript overlapped.

References

- Bohlmann, F. & Czerson, H. (1979). *Phytochemistry*, 18, 115-118.
- Meyer, B. N., Ferrigni, Putnam, J. E., Jacobsen, L. B., Nichols, D. E. and
McLaughlin, J. L. (1982). *Planta Medica*, 45, 31.
- Mokkhasmit, M., Ngarmwathana, W., Sawasdimongkol, K. &
Permphiphat, U. (1971). *Journal of Medical Association of Thailand*,
54, 490.
- Ogiso, A., Kitazawa, E, Mikuriya, I. & Promdej, C. (1981). *Shoyakugaku
Zasshi*, 35, 287.
- Phupattanapong, L. & Wongprasart, T. (1987). In *Thai Medicinal Plants*,
Part 5 (p. 659) Bangkok, Chutima Printing in Thai
- Rao, P. S., Sachdev, G. P., Seshadri, T. R. and Singh, H. B. (1968).
Tetrahedron Letters, 4685.
- Roengsumran, S., Luangdilok, W., Petsom, A., Praruggamo, S. &
Pengprecha, S. (1982). *Journal of Natural Products*, 45, 772.



1



2

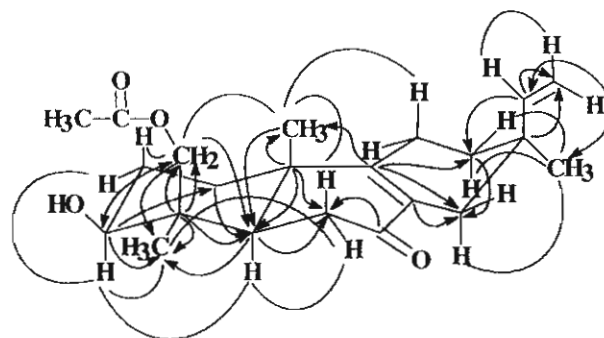
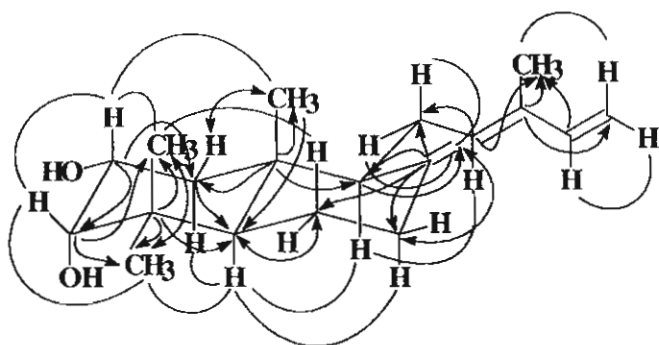


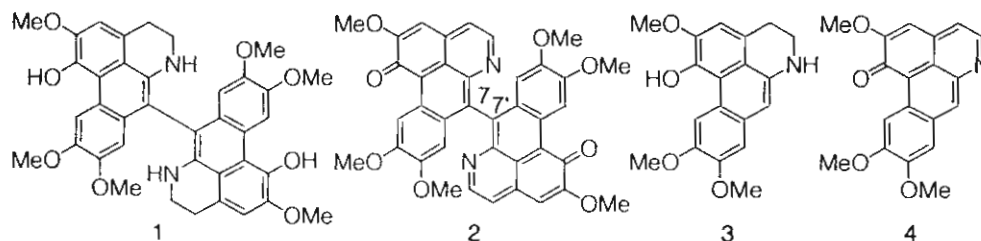
Fig. 1 Structures, key long-range ^1H - ^{13}C (C \rightarrow H) and NOESY (H \rightarrow H) correlations of **1** and **2**

A SYNTHESIS OF BIPOWINE AND BIPOWINONE

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Dimeric aporphine alkaloids¹⁻⁵ are a small group of natural alkaloids isolated from plants of the family Annonaceae. There are two types of linkages between the two aporphine units i.e. C4-C7 and C7-C7'. Bipowine (1) and bipowinone (2) are the two representative of the symmetrical 7,7'-bisaporphine alkaloids isolated from the Indonesian annonaceous plant *Popowia pisocarpa*.²

The dimerization of aporphine and dehydroaporphine alkaloids to the corresponding 7,7'-bisaporphines has been achieved. Various reagents utilized to effect such dimerization include I_2/C_2H_5OH ,⁶ $Hg(NO_3)_2/CH_3CN$,⁷ $Hg(OAc)_2/CH_3OH$,⁷ NCS/base and air.^{2,8} Bipowinone (2) has been previously obtained from the oxidation of wilsonirine (dihydro derivative of 3) with NCS followed by reaction with sodium ethoxide, but in a very low yield (1.6%)² the main product was later found to be pancoridine (4).⁸ However, no satisfactory methods for the synthesis of 2 have so far been reported. In this study various oxidizing agents were investigated for the oxidative dimerization of dehydroaporphine alkaloid. In this paper we report the synthesis of bipowine (1) and bipowinone (2).



6a,7-Dehydronorthaliporphine (3) was used in our study of the oxidative coupling reaction. This dehydroaporphine alkaloid was obtained⁹ from hydrogenation of pancoridine (4)^{9,10} using PtO_2 as catalyst.

The reaction of dehydroaporphine (**3**) with various oxidizing agents is shown in Table I.

Table I "The reaction of **3** with various oxidizing agents^{a)}"

Entry	Oxidizing agents (equivalent)	Reaction Time	Products (%)	
			1	2
1	Hg(OAc) ₂ (0.6)	15 min	60	trace
2	Hg(OAc) ₂ (2.7)	3 h	-	68
3	PhI(OAc) ₂ (0.6)	5 min	53	trace
4	PhI(OAc) ₂ (2.6)	5 min	-	74
5	air	48 h	58	-

^{a)} The reaction was carried out in dichloromethane at rt"

Hg(OAc)₂ could be used to oxidise **3** to the dimeric bipowine (**1**) and bipowinone (**2**) depending on the ratio of the oxidizing agent and the duration of the reaction. More significantly, we have found that the above dimerization process can be conveniently effected by (diacetoxyiodo)benzene [PhI(OAc)₂]. Hypervalent iodine compounds¹¹⁻¹³ have recently been utilized in many organic functional group transformations. The relatively low toxicity of hypervalent iodine compounds¹¹ as compared to the mercury compounds makes the above finding very attractive.

Table II "Formation of **2** by the oxidation of **1**^{a)}"

Entry	Oxidizing agent (equivalent)	Reaction time	Product (2) (%)
1	Hg(OAc) ₂ (4.2)	3 h	quantitative yield
2	PhI(OAc) ₂ (4.1)	5 min	73
3	Ag ₂ O (4.3)	10 min	90

^{a)} The reaction was carried out in dichloromethane at rt"

Furthermore, we have also found that bipowine (**1**) was easily oxidized to bipowinone (**2**) by excess amount of Hg(OAc)₂, PhI(OAc)₂, and Ag₂O. The results are summarized in Table II. Reaction of **3** with Ag₂O¹⁴ gave pancoridine (**4**) in 53% yield and bipowinone (**2**) in 28% yield. In addition, when [bis (trifluoroacetoxy)iodo]benzene was used as oxidizing agent, only 12% of **2** was obtained together with 12% of pancoridine (**4**).

In conclusion, the formation of bipowine and bipowinone could be adjusted according to the experimental procedure and we have introduced the use of PhI(OAc)₂ in the oxidative dimerization of the dehydroaporphine alkaloid to the corresponding bisaporphine alkaloid.

EXPERIMENTAL

Melting points are uncorrected. ¹H NMR spectra were taken at 300 or 400 MHz as specified and ¹³C NMR at 100 MHz. Tetramethylsilane was used as the internal standard, and chemical shifts are reported as δ_H (ppm) or δ_C (ppm). MS spectra were obtained by electron impact technique (EI).

6a,7-Dehydronorthaliporphine (3) was prepared according to Cava's procedure⁹ and exhibited the

following data. mp 197°C (decomp) (ether) (lit.,⁹ mp 198°-199°C); IR (KBr): ν_{\max} 3460 (NH), 3327 cm^{-1} (OH); ^1H NMR (300 MHz, acetone d_6): δ (ppm) 3.13-3.17 (m, 2H, C-4, $\text{CH}_2\text{-CH}_2\text{-NH}$), 3.38-3.42 (m, 2H, C-5, $\text{CH}_2\text{-CH}_2\text{-NH}$), 3.88, 3.89, 4.01 (3s, 9H, $3\times\text{OCH}_3$), 6.63 (s, 1H, C-8-ArH), 7.01 (s, 1H, C-7= CH), 7.11 (t, 1H, $J = 0.9$ Hz, C-3-ArH), 9.27 (s, 1H, C-11-ArH); MS: m/z 325 (M^+ , 100), 310 (54).

Formation of bipowine (1) by oxidation of 6a,7-dehydronorthaliporphine (3)

a) By air oxidation:

A solution of **3** (140 mg, 0.43 mmol) in dichloromethane-methanol (9:1, 150 mL) was oxidized by bubbling air at rt until starting materials were consumed (2 days, monitored by TLC). The solution was evaporated to dryness and the crude product so obtained was purified by column chromatography (Al_2O_3 , neutral) using dichloromethane as eluting solvent to give bipowine as a pale yellow solid, which was recrystallized from chloroform-acetone to yield **1** (80 mg, 58%). mp 250-252°C (decomp) (lit.,² mp > 249°C); IR (KBr): ν_{\max} 3467 (NH), 3388 cm^{-1} (OH); UV: λ_{\max} MeOH (log ϵ) 207 (4.40), 266 (4.82), 335 (4.16), 389 (3.90) nm, λ_{\max} MeOH+NaOH 216, 263, 355, 398, 496 nm, λ_{\max} MeOH+HCl 205, 263, 293, 338, 356, 374 nm; ^1H NMR (400 MHz, CDCl_3): δ 3.09-3.34 (m, 4H, $\text{CH}_2\text{-CH}_2\text{NH}$), 3.49, 4.05, 4.07 (3s, 9H, $3\times\text{OCH}_3$), 6.65 (s, 1H, C-8-ArH), 6.86 (s, 1H, OH), 7.03 (s, 1H, C-3-ArH), 9.33 (s, 1H, C-11-ArH), ^{13}C NMR (100 MHz, $\text{CDCl}_3\text{-CD}_3\text{OD}$): δ 143.6 (C-1), 119.8 (C-1a), 117.8 (C-1b), 148.9 (C-2), 110.6 (C-3), 125.1 (C-3a), 30.7 (C-4), 49.2 (C-5), 141.1 (C-6a), 128.4 (C-7), 138.7 (C-7a), 104.1 (C-8), 145.4 (C-9), 143.6 (C-10), 109.2 (C-11), 119.5 (C-11a), 55.3, 55.8, 56.9 (OCH_3 -2, 3, 10); MS: m/z 648 (M^+ , 89), 633 (33), 325 (92), 324 (100), 310 (68), 292 (39), 290 (69).

b) By oxidation with mercuric acetate:

$\text{Hg}(\text{OAc})_2$ (16.0 mg, 0.05 mmol) was added to a solution of **3** (28.9 mg, 0.09 mmol) in dichloromethane (3 mL). The mixture was allowed to stir at rt for 15 min, then filtered. Removal of dichloromethane gave a residue, which was chromatographed on aluminum oxide (neutral) and eluted with dichloromethane to give **1** (17.2 mg, 60%).

c) By oxidation with diacetoxyiodobenzene:

Diacetoxyiodobenzene (17.7 mg, 0.05 mmol) was added to a solution of **3** (29.3 mg, 0.09 mmol) in dichloromethane (3 mL). The mixture was stirred for 5 min at rt, then water was added and the mixture was extracted with dichloromethane (2x20 mL). The dichloromethane extract was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The crude product was purified by column chromatography (Al_2O_3 , neutral) using dichloromethane as eluting solvent to give **1** (15.5 mg, 53%).

Bipowinone (2) from 6a,7-dehydronorthaliporphine (3)

a) By oxidation with mercuric acetate:

Hg(OAc)₂ (70.0 mg, 0.22 mmol) was added to a solution of **3** (26.8 mg, 0.08 mmol) in dichloromethane (3 mL). The mixture was allowed to stir for 3 h at rt. Removal of dichloromethane gave the residue which was chromatographed on aluminum oxide (neutral) and eluted with dichloromethane to afford an orange-red solid (18 mg, 68%), which was crystallized from dichloromethane-acetone to give **2**. mp 292 °C (decomp) (lit.,² mp > 295 °C); IR (KBr): ν_{\max} 1626 cm⁻¹ (C=O); UV: λ_{\max} MeOH (log ϵ) 237 (4.72), 278 (4.42), 288 (4.37), 300 (4.29), 411 (4.26), 476 (4.23), 502 (4.20) nm, λ_{\max} MeOH+HCl 204, 250, 432, 496 nm; ¹H NMR (400 MHz, CDCl₃): δ 3.36, 4.05, 4.20 (3s, 9H, 3xOCH₃-9, 2, 10) 6.44 (s, 1H, C-8-ArH), 6.89 (s, 1H, C-3-ArH), 7.45, 8.66 (AB, J_{AB} = 4.4 Hz, 2H, CH=CHN), 9.91 (s, 1H, C-11-ArH), ¹³C NMR (CDCl₃-CD₃OD): δ 180.8 (C-1), 131.8 (C-1a), 120.5 (C-1b), 156.1 (C-2), 105.4 (C-3), 141.8 (C-3a), 121.1 (C-4), 149.7 (C-5), 156.3 (C-6a), 131.6 (C-7), 135.1 (C-7a), 105.3 (C-8), 150.5 (C-9), 144.4 (C-10), 107.5 (C-11), 120.3 (C-11a), 55.4, 56.0, 56.4 (OCH₃-2, 3, 10); MS: m/z 640 (M⁺, 17), 625 (25), 609 (15), 321 (57), 320 (18), 307 (23), 290 (100).

b) By oxidation with diacetoxyiodobenzene:

Diacetoxyiodobenzene (69.8 mg, 0.22 mmol) was added to a solution of **3** (27.4 mg, 0.08 mmol) in dichloromethane (3 mL). The mixture was stirred for 5 min at rt. Water was added and the mixture was extracted with dichloromethane (2x20 mL). The dichloromethane extract was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The crude product was purified by column chromatography (Al₂O₃; neutral) using dichloromethane as eluting solvent to give **2** as an orange-red solid (20.1 mg, 74%).

Oxidation of bipowine (**1**) to bipowinone (**2**)

a) By oxidation with mercuric acetate:

Hg(OAc)₂ (36.7 mg, 0.12 mmol) was added to a solution of **1** (17.7 mg, 0.03 mmol) in dichloromethane (3 mL). The mixture was allowed to stir for 3 h at rt, then filtered. Removal of dichloromethane gave a residue which was chromatographed on aluminum oxide (neutral) and eluted with 1% methanol-dichloromethane to give **2** (quantitative yield).

b) By oxidation with diacetoxyiodobenzene:

Diacetoxyiodobenzene (36.1 mg, 0.11 mmol) was added to a solution of **1** (17.4 mg, 0.03 mmol) in dichloromethane (3 mL). The mixture was stirred for 5 min at rt. Water was added and the mixture was extracted with dichloromethane (2x20 mL). The dichloromethane extract was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The crude product was purified by column chromatography (Al₂O₃; neutral) using 1% methanol-dichloromethane as eluting solvent to give **2** (12.5 mg, 73%).

c) By oxidation with silver oxide:

Silver(I) oxide (27.1 mg, 0.12 mmol) was added to a solution of **1** (17.7 mg, 0.03 mmol) in dichloromethane (3 mL). The solution was allowed to stir for 10 min at rt, then filtered. Removal of

dichloromethane gave a residue which was chromatographed on aluminum oxide (neutral) and eluted with 1% methanol-dichloromethane to give **2** (15.8 mg, 90%).

Pancoridine (**4**)

Silver(I) oxide (53.3 mg, 0.23 mmol) was added to a solution of **3** (28.8 mg, 0.09 mmol) in dichloromethane (3 mL). The mixture was allowed to stir for 30 min at rt, then filtered. Removal of dichloromethane gave a residue which was chromatographed on aluminum oxide (neutral) and eluted with dichloromethane to give **4** (15.2 mg, 53%) and **2** (8.0 mg, 28%). Pancoridine (**4**); mp (dichloromethane-hexane) 233-234 °C (decomp)(lit.,⁹ 214-215°C, lit.,¹⁰ 236-238 °C); FTIR (KBr) : ν_{\max} 1626 cm^{-1} (C=O); UV: λ_{\max} MeOH (log ϵ), 203(4.09), 232(4.59), 243(4.52), 285(4.15), 402(4.02), 466(3.92) nm, λ_{\max} MeOH+HCl, 203, 243, 277, 295, 419, 485 nm; ¹H NMR (400 MHz, CDCl₃) : δ 4.03, 4.09, 4.15 (3s, 9H, 3xOCH₃), 6.78 (s, 1H, C-3-ArH), 6.89 (s, 1H, C-8-ArH), 7.45, 8.66 (AB, J_{ab} = 4.3 Hz, 2H, CH=CH N), 8.70 (s, 1H, C-7-ArH), 9.51 (s, 1H, C-11-ArH), ¹³C NMR (CDCl₃-CD₃OD), δ 180.4(C-1), 132.0(C-1a), 135.1(C-1b), 156.3(C-2), 106.7(C-3), 141.4(C-3a), 121.0(C-4), 150.0(C-5), 156.5(C-6a), 104.8(C-7), 135.1(C-7a), 107.1(C-8), 150.3(C-9), 144.6(C-10), 106.7(C-11), 131.7(C-11a), 56.4, 55.9, 55.86. (OCH₃-2,3,10); MS: m/z 321(M⁺, 73.80), 290(100.00).

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REFERENCES

1. A. Jossang, M. Leboeuf, A. Cavé, T. Sévenet, and K. Padmawinata, *J. Nat. Prod.*, 1984, **47**, 504.
2. A. Jossang, M. Leboeuf, A. Cavé, and T. Sévenet, *J. Nat. Prod.*, 1986, **49**, 1028.
3. G. Arango, D. Cortes, and A. Cavé, *Phytochemistry*, 1987, **26**, 1227.
4. O. Laprévote, F. Roblot, R. Hocquemiller, and A. Cavé, *J. Nat. Prod.*, 1987, **50**, 984.
5. D. Cortes, D. Davoust, A. H. A. Hadi, S. H. Myint, R. Hocquemiller, and A. Cavé, *J. Nat. Prod.*, 1990, **53**, 862.
6. M. Gerecke, R. Borer, and A. Brossi, *Hel. Chim. Acta*, 1975, **58**, 185.
7. L. Castedo, R. Riguera, J. M. Sáa, and R. Suau, *Heterocycles*, 1977, **6**, 677.
8. A. Jossang, M. Leboeuf, and A. Cavé, *Heterocycles*, 1987, **26**, 2191.
9. M. P. Cava, I. Noguchi, and K. T. Buck, *J. Org. Chem.*, 1973, **38**, 2394.
10. S. M. Kupchan and A. J. Liepa, *J. Am. Chem. Soc.*, 1973, **95**, 4062.
11. For some reviews, see: a) T. Wirth and U. H. Hirt, *Synthesis*, 1999, 1271. b) P. J. Stang and V. V. Zhdankin, *Chem. Rev.*, 1996, **96**, 1123. c) V. V. Zhdankin and P. J. Stang, *Tetrahedron*, 1998, **54**, 10927. d) T. Umemoto, *Chem. Rev.*, 1996, **96**, 1757. e) A. Varvoglis, *Tetrahedron*, 1997, **53**, 1179. f) A. Varvoglis and S. Spyroudis, *Synlett*, 1998, 221. g) D. F. Banks, *Chem. Rev.*, 1966, **66**, 242. For some books, see: h) A. Varvoglis, 'The Organic Chemistry of Polycoordinated Iodine,' VCH, New

- York, 1992. i) A. Varvoglis, '*Hypervalent Iodine in Organic Synthesis*,' Academic Press, London, 1997. j) J. P. Fine, '*Ligand Coupling Reactions with Heteroatomic Compounds*,' Pergamon Press, Oxford, 1998.
12. S. V. Ley, O. Schucht, A. W. Thomas, and P. J. Murray, *J. Chem. Soc., Perkin Trans. 1*, 1999, 1251.
 13. S. V. Ley, A. W. Thomas, and H. Finch, *J. Chem. Soc., Perkin Trans. 1*, 1999, 669.
 14. S. R. Angle, D. O. Arnaiz, J. P. Boyce, R. P. Fruos, M. S. Louie, H. L. Mattson-Arnaiz, J. D. Rainier, K. D. Turnbull, and W. Yang, *J. Org. Chem.*, 1994, **59**, 6322.

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An efficient synthesis of argemonine, a pavine alkaloid[†]

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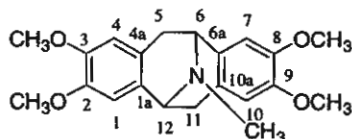
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Abstract—A method for the synthesis of 1,2-dihydroisoquinoline derivatives is described and the conversion of the 1,2-dihydroisoquinoline intermediate to a pavine alkaloid via palladium-induced intramolecular hydroarylation reaction and radical cyclization is presented. © 2001 Elsevier Science Ltd. All rights reserved.

Argemonine is a prototypical member of the pavine alkaloids, a small group of tetracyclic natural products, and contains the tetrahydroisoquinoline core embedded in its skeleton.¹ Recent findings of biological activities of the pavine alkaloids include inhibition of *herpes simplex* virus type 1² and inhibitory activity against tumor necrosis factor- α (TNF- α) production.³



Argemonine

There are a number of syntheses^{1,4} of pavine alkaloids reported, but most involve an acid-catalyzed intramolecular cyclization of the activated aromatic ring with an iminium salt as in the Pictet–Spengler reaction. The drawback of such a procedure is the failure with nonactivated aromatic compounds and the lack of chemoselectivity in the cyclization of unsymmetrical compounds.

We have developed a new synthesis of pavine alkaloids based on the retrosynthetic analysis shown in Scheme 1.

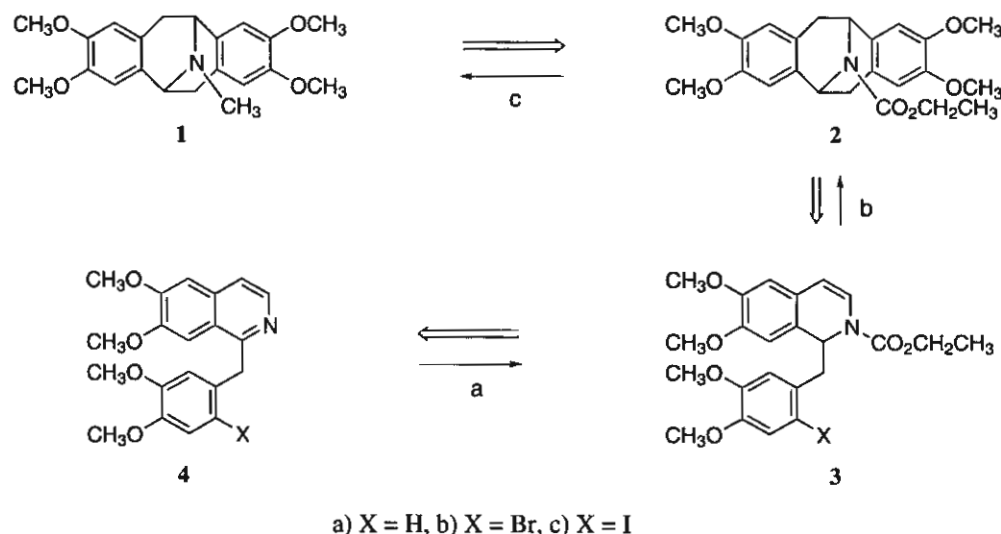
Keywords: pavine alkaloid; intramolecular hydroarylation reaction; radical cyclization reaction.

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The key steps involve a palladium-induced intramolecular hydroarylation⁵ and a radical cyclization⁶ of the key halo derivatives of 1-benzyl-*N*-carboethoxy-1,2-dihydroisoquinolines. The intramolecular hydroarylation involves the reduction of the organopalladium complex formed during the carbon–carbon bond formation in the much exploited Heck reaction.⁷ We have also developed a new method for the synthesis of the key 1,2-dihydroisoquinoline derivatives. During our investigation a method for the synthesis⁸ of this type of compound was reported involving the addition of a benzylstannane to an isoquinoline in the presence of methyl chloroformate in dichloromethane. We found that 1-benzyl-*N*-carboethoxy-1,2-dihydroisoquinolines could be conveniently obtained by the reaction of 1-benzylisoquinoline derivatives with tributyltin hydride. For example, treatment of papaverine **4a** with tributyltin hydride in dichloromethane at room temperature under a nitrogen atmosphere, followed by addition of ethyl chloroformate at -78°C and warming to room temperature gave a white solid which, after recrystallization from methanol–dichloromethane, provided compound **3a** in 79% yield. Having found a method for the synthesis of 1-benzyl-*N*-carboethoxy-1,2-dihydroisoquinolines, we then began the synthesis of our key intermediate. The synthesis started with bromination of commercially available papaverine **4a** with a solution of bromine in acetic acid at room temperature for 2 h to give 2'-bromopapaverine⁹ **4b** in 75% yield.

Application of the tin hydride reduction gave the required product which was recrystallized from methanol–dichloromethane to give compound **3b** as a white solid in 85% yield. It is interesting to note the



Scheme 1. Reagents and conditions: (a) i. $\text{Bu}_3\text{SnH}/\text{CH}_2\text{Cl}_2$, ii. $\text{EtOCOCl}/\text{CH}_2\text{Cl}_2$, $-78^\circ\text{C} \rightarrow \text{rt}$ (**3a**, 79%; **3b**, 85%; **3c**, 85%); (b) See Table 1; (c) LAH/THF, reflux 4 h (**1**, 87%).

chemoselective reduction of the iminium intermediate with tributyltin hydride in the presence of the aryl bromide group. Once the key compound **3b** was obtained, we applied the intramolecular hydroarylation cyclization to effect pavine ring formation. The cyclization of **3b** was accomplished using 10–20 mol% of a reactive catalyst formed from $\text{Pd}(\text{PPh}_3)_4$ in DMF at $80\text{--}90^\circ\text{C}$ in the presence of sodium formate as a reducing agent. After purification by PLC, *N*-ethoxycarbonylpavine **2**¹⁰ was obtained in 44% yield together with the corresponding reduction product **3a** in 34% yield as shown in entry 1 of Table 1. The yield of the *N*-ethoxycarbonylpavine **2** was increased to 56% when the starting iodo compound **3c** was used instead of the bromo compound **3b** under similar conditions (entry 3, Table 1). The 2'-iodopapaverine **4c** could be conveniently obtained by the reaction of papaverine with iodine and silver trifluoroacetate.¹¹ The appearance of four aromatic protons as singlets in the NMR spectrum indicated that 2'-iodopapaverine was obtained.

In addition, the radical cyclization using tributyltin hydride and AIBN of compounds **3b** and **3c** was investigated. It was found that the yield of *N*-ethoxycarbonylpavine **2** was only 30% from the cyclization of the bromo compound **3b** under the tributyltin hydride/AIBN conditions. The corresponding reduction product

3a was obtained from the reaction in 5% yield. Similarly, the iodo compound **3b** underwent cyclization with tributyltin hydride to afford the pavine **2** in 42% yield together with 10% of the reduction product **6**. The *N*-ethoxycarbonylpavine **2** was reduced by LAH to form the *N*-methylpavine in 87% yield. The physical and spectroscopic data of our synthetic compound are in full agreement with those of argemonine **1**.^{4b}

In conclusion, we have devised a new method for the synthesis of pavine alkaloids as illustrated in the synthesis of natural (\pm)-argemonine. With appropriate introduction of the iodo group, the approach could be used to synthesize both symmetrical and unsymmetrical pavine alkaloids. We found that the palladium-catalyzed reductive intramolecular arylation reaction is very useful and gives better yields than radical cyclization.

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Table 1. Intramolecular arylation of 3,4-dihydroisoquinoline derivatives **3a** and **3b** to pavine (**2**)

Entry	Starting material	Conditions	Yield%	Yield%
			Comp. 2	Comp. 3a
1	Compound 3b	$\text{Pd}(\text{PPh}_3)_4/\text{DMF}/\text{HCO}_2\text{Na}/\text{reflux}$ 24 h	44	34
2	Compound 3b	$\text{Bu}_3\text{SnH}/\text{AIBN}$, benzene, reflux 10 h	30	5
3	Compound 3c	$\text{Pd}(\text{PPh}_3)_4/\text{DMF}/\text{HCO}_2\text{Na}/\text{reflux}$ 24 h	56	15
4	Compound 3c	$\text{Bu}_3\text{SnH}/\text{AIBN}$, benzene reflux 10 h	42	10

References

1. Gozler, B. In *The Alkaloids*; Brossi, A., Ed.; Academic Press: Orlando, 1987; Vol. 31, pp. 317–389.
2. Varadinova, T. L.; Shishkov, S. A.; Ivanovska, N. D.; Velcheva, M. P.; Danghaaghin, S.; Samadanghiin, Z.; Yansanghiin, Z. *Phytotherapy Res.* **1996**, *10*, 414–417.
3. Fujiwara, N.; Ueda, Y.; Ohashi, N. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 743–748.
4. (a) Munchhoh, M. J.; Meyers, A. I. *J. Org. Chem.* **1996**, *61*, 4607–4610; (b) Johnson, A. P.; Luke, R. W. A.; Singh, G.; Boa, A. N. *J. Chem. Soc., Perkin Trans. 1* **1996**, 907–913; (c) Pabuccuoglu, V.; Hesse, M. *Heterocycles* **1997**, *45*, 1751–1758.
5. (a) Wolff, S.; Hofmann, M. R. H. *Synthesis* **1988**, 760–763; (b) Hoffmann, M. H. R.; Schmidt, B.; Wolff, S. *Tetrahedron* **1989**, *45*, 6113–6126.
6. For reviews see: (a) Jasperse, C. P.; Curran, D. P.; Fevig, T. L. *Chem. Rev.* **1991**, *91*, 1237; (b) Bowman, W. R.; Bridge, C. F.; Brookes, P. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1–14 and references cited therein.
7. For recent reviews see: (a) Ikeda, M.; El Bialy, S. A. A.; Yakura, T. *Heterocycles* **1999**, *51*, 1957–1970; (b) Amatore, C.; Jutand, A. *Acc. Chem. Res.* **2000**, *33*, 314–321; (c) Beletskaya, I. P.; Cheprakov, A. V. *Chem. Rev.* **2000**, *100*, 3009–3066; (d) Poli, G.; Giambastiani, G.; Heumann, A. *Tetrahedron* **2000**, *56*, 5959–5989.
8. Deline, J. E.; Miller, R. B. *Tetrahedron Lett.* **1998**, *39*, 1721–1724.
9. (a) Hegedus, L. H.; Stiverson, R. K. *J. Am. Chem. Soc.* **1974**, *96*, 3250–3254; (b) Spath, E.; Lang, N. *Chem. Ber.* **1921**, *54*, 3064–3071.
10. Barker, A. C.; Battersby, A. R. *J. Chem. Soc. C* **1967**, *1*, 1317–1323.
11. Iida, H.; Takarai, T.; Kibayashi, C. *J. Chem. Soc., Chem. Commun.* **1977**, 644–645.

All compounds have been fully characterized. Spectroscopic data of some selected compounds, 1-(2-iodo-4,5-dimethoxybenzyl)-2-ethoxycarbonyl-6,7-dimethoxy-1,2-dihydroisoquinoline **3c**: mp 154–156°C; FT-IR (Nujol) 2926, 1708, 1633, 1227 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.15 (t, 3H, $J=7.1$ Hz), 1.25 (t, 3H, $J=7.1$ Hz), 2.82–3.06 (m, 4H), 3.66 (s, 3H), 3.72 (s, 3H), 3.73 (s, 3H), 3.77 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 3.87 (s, 3H), 3.89 (s, 3H), 4.02 (m, 2H), 4.17 (q, 2H, $J=7.1$ Hz), 5.45 (dd, 1H, $J=8.4, 5.6$ Hz), 5.55 (t, 1H, $J=7.1$ Hz), 5.79, 6.79 (AB q, 2H, $J_{ab}=7.1$ Hz), 5.94, 6.96 (AB q, 2H, $J_{ab}=7.1$ Hz), 6.23 (s, 1H), 6.34 (s, 1H), 6.40 (s, 1H), 6.48 (s, 1H), 6.60 (s, 1H), 6.64 (s, 1H), 7.17 (s, 1H), 7.23 (s, 1H). ¹³C NMR (100 MHz) δ 14.28, 14.53, 43.78, 44.23, 55.20, 55.71, 55.82, 55.92, 55.97, 56.05, 56.24, 61.99, 62.19, 88.89, 89.60, 107.90, 108.03, 108.39, 108.95, 109.85, 110.23, 113.42, 113.69, 121.24, 121.34, 122.70, 123.15, 123.53, 123.56, 123.75, 124.30, 132.51, 132.61, 147.70, 147.78, 148.07, 148.52, 148.75, 148.96, 152.81, 153.54. FABMS 540 (M⁺+1, 2.24), 413 (8.16), 262 (100.00). Anal. calcd for C₂₃H₂₆NO₆I: C, 51.19; H, 4.86; N, 2.63; Found: C, 50.99; H, 4.82; N, 2.41. *N*-Ethoxycarbonylpavine **2**: mp 190–192°C (lit.¹⁰ mp 183–184°C); FT-IR (KBr) 1686, 1519, 1463, 1254 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.28 (t, 3H, $J=7.1$ Hz), 2.76 (d, 2H, $J=15.9$ Hz), 3.38 (dd, 1H, $J=15.9, 5.6$ Hz), 3.42 (dd, 1H, $J=15.9, 5.6$ Hz), 3.77 (s, 3H), 3.78 (s, 3H), 3.85 (s, 3H), 3.87 (s, 3H), 4.11–4.25 (m, 2H), 5.42 (d, 1H, $J=5.6$ Hz), 5.52 (d, 1H, $J=5.6$ Hz), 6.45 (s, 1H), 6.48 (s, 1H), 6.66 (s, 1H), 6.67 (s, 1H). ¹³C NMR (100 MHz) δ 14.65, 35.78, 36.04, 48.97, 49.70, 55.64, 55.89, 61.38, 108.97, 109.20, 111.47, 111.65, 123.95, 124.48, 128.78, 129.08, 147.45, 147.96, 148.05, 154.21. EI-MS 413 (M⁺, 17.50), 412 (4.89), 340 (8.25), 278 (6.84), 262 (52.36), 28 (100.00). Anal. calcd for C₂₆H₂₂N₂O₃: C, 66.40; H, 6.59; N, 3.48. Found: C, 66.24; H, 6.46; N, 3.73.



An efficient synthesis of argemonine, a pavine alkaloid[†]

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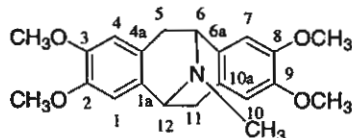
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Abstract—A method for the synthesis of 1,2-dihydroisoquinoline derivatives is described and the conversion of the 1,2-dihydroisoquinoline intermediate to a pavine alkaloid via palladium-induced intramolecular hydroarylation reaction and radical cyclization is presented. © 2001 Elsevier Science Ltd. All rights reserved.

Argemonine is a prototypical member of the pavine alkaloids, a small group of tetracyclic natural products, and contains the tetrahydroisoquinoline core embedded in its skeleton.¹ Recent findings of biological activities of the pavine alkaloids include inhibition of *herpes simplex* virus type 1² and inhibitory activity against tumor necrosis factor- α (TNF- α) production.³



Argemonine

There are a number of syntheses^{1,4} of pavine alkaloids reported, but most involve an acid-catalyzed intramolecular cyclization of the activated aromatic ring with an iminium salt as in the Pictet–Spengler reaction. The drawback of such a procedure is the failure with nonactivated aromatic compounds and the lack of chemoselectivity in the cyclization of unsymmetrical compounds.

We have developed a new synthesis of pavine alkaloids based on the retrosynthetic analysis shown in Scheme 1.

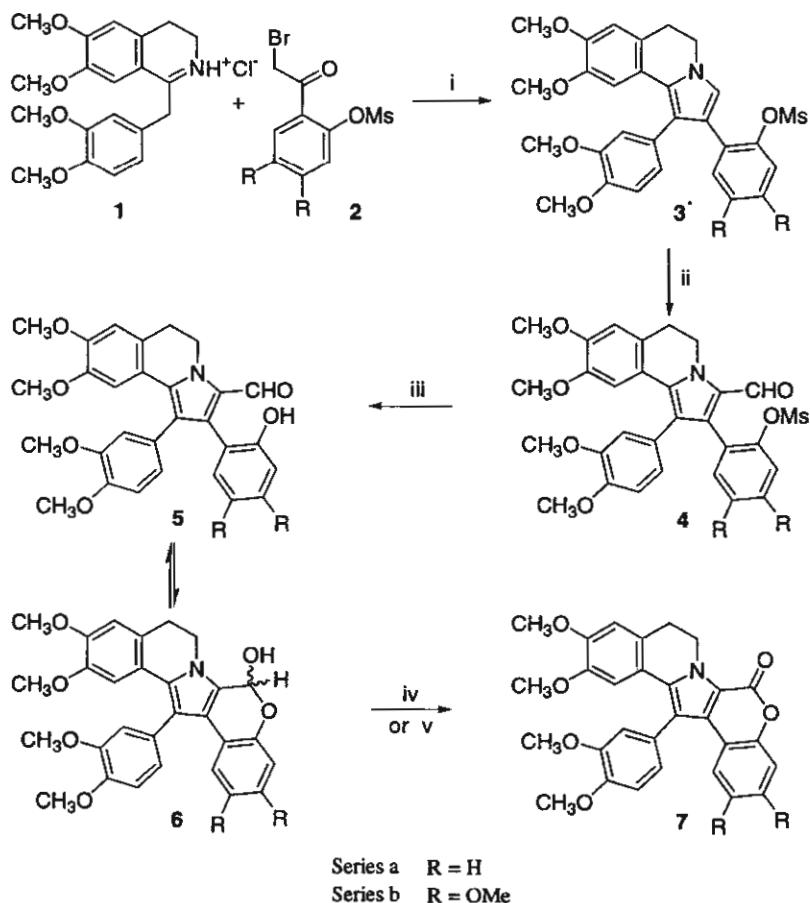
Keywords: pavine alkaloid; intramolecular hydroarylation reaction; radical cyclization reaction.

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[†] This work has been taken in part from Namsa-aid, A., M.Sc. Thesis, Mahidol University 1997, and was presented at the 8th Belgian Organic Synthesis Symposium, July 10–14, 2000, Gent, Belgium.

The key steps involve a palladium-induced intramolecular hydroarylation⁵ and a radical cyclization⁶ of the key halo derivatives of 1-benzyl-*N*-carboethoxy-1,2-dihydroisoquinolines. The intramolecular hydroarylation involves the reduction of the organopalladium complex formed during the carbon–carbon bond formation in the much exploited Heck reaction.⁷ We have also developed a new method for the synthesis of the key 1,2-dihydroisoquinoline derivatives. During our investigation a method for the synthesis⁸ of this type of compound was reported involving the addition of a benzylstannane to an isoquinoline in the presence of methyl chloroformate in dichloromethane. We found that 1-benzyl-*N*-carboethoxy-1,2-dihydroisoquinolines could be conveniently obtained by the reaction of 1-benzylisoquinoline derivatives with tributyltin hydride. For example, treatment of papaverine **4a** with tributyltin hydride in dichloromethane at room temperature under a nitrogen atmosphere, followed by addition of ethyl chloroformate at -78°C and warming to room temperature gave a white solid which, after recrystallization from methanol–dichloromethane, provided compound **3a** in 79% yield. Having found a method for the synthesis of 1-benzyl-*N*-carboethoxy-1,2-dihydroisoquinolines, we then began the synthesis of our key intermediate. The synthesis started with bromination of commercially available papaverine **4a** with a solution of bromine in acetic acid at room temperature for 2 h to give 2'-bromopapaverine⁹ **4b** in 75% yield.

Application of the tin hydride reduction gave the required product which was recrystallized from methanol–dichloromethane to give compound **3b** as a white solid in 85% yield. It is interesting to note the



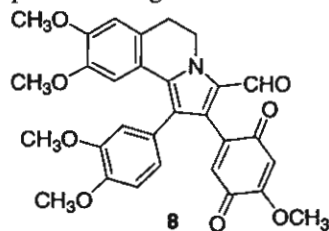
Scheme 1. Reagents and conditions: (i) K_2CO_3 , CH_3CN , reflux (**3a**, 63%; **3b**, 63%); (ii) DMF, $POCl_3$, rt (**4a**, 80%; **4b**, 82%); (iii) KOH, EtOH, reflux (**5a**, 77%; **5b**, 81%); (iv) MnO_2 , CH_2Cl_2 , rt (**7a**, 54%; **7b**, 20% and **8**, 37%); (v) $Pd(OAc)_2$, PPh_3 , K_2CO_3 , DMF, PhBr, $120^\circ C$, 12 h (**7a**, 80%; **7b**, 80%).

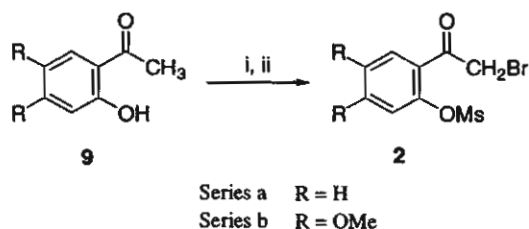
have been developed for the synthesis of lamellarins⁷ and related 3,4-diaryl pyrrole derivatives,⁸ notably by Steglich and Banwell. The core skeleton of these lamellarins A can be viewed as the fusion of the pyrrolo[2,1-*a*]isoquinoline with the lactone unit. Our retrosynthetic analysis as shown in Fig. 1 involves the lactonization of the appropriate pyrrolo[2,1-*a*]isoquinoline derivative B. Pyrrolo[2,1-*a*]isoquinoline C can be synthesized from the reaction of 3,4-dihydroisoquinoline with a phenacyl bromide derivative.

In practice, the condensation of 3,4-dihydropapaverine hydrochloride **1** with *o*-mesyloxyphenacyl bromide **2a**⁹ in the presence of potassium carbonate in acetonitrile gave the expected mesyloxy pyrrolo[2,1-*a*]isoquinoline analogue **3a** in 63% yield. The reaction presumably involves the intramolecular reaction of the derived enamine from the isoquinolinium salt and the ketone as found in the Knorr pyrrole synthesis.¹⁰ The introduction of the formyl group on the pyrrole ring was accomplished by the Vilsmeier reaction.¹¹ The reaction was carried out using dimethylformamide in phosphorus oxychloride as a formylating agent at room temperature. The expected product **4a** was obtained in 85% yield after purification by preparative thin layer chromatography. The mesyl protecting group in the derived aldehyde intermediate was easily removed by heating with potassium hydroxide in ethanol. The phenol **5a** was produced in 77% yield after

purification by preparative thin layer chromatography. We found that manganese dioxide in dichloromethane could be used to oxidize the phenolic aldehyde **5a** to the corresponding lamellarin derivative **7a** in 54% yield, presumably via the hemiacetal intermediate **6a**.

The above approach has also been applied to the synthesis of lamellarin G trimethyl ether as shown in series b of Scheme 1. The first three steps used in the synthesis proceeded well as planned. The condensation of 3,4-dihydroisoquinoline **1** with the phenacyl bromide derivative **2b**⁹ gave the corresponding pyrrolo[2,1-*a*]isoquinoline **3b** in 63% yield. The introduction of the formyl group and the removal of mesyloxy protecting group could be accomplished in 82 and 81% yield, respectively. However, the oxidation of compound **5b** with manganese dioxide gave lamellarin G trimethyl ether **7b** in disappointing yield (20%). The byproduct was found to be the quinone derivative **8** formed by the preferred oxidation of the electron rich phenolic ring.





Scheme 2. Reagents and conditions: Series a. (i) MeSO_2Cl , Et_3N , CH_2Cl_2 (97%); (ii) $\text{BnN}^+\text{Me}_3\text{Br}^-$, CH_2Cl_2 (**2a**, 81%); Series b. (i) MeSO_2Cl , Et_3N , CH_2Cl_2 (97%); (ii) $\text{BnN}^+\text{Me}_3\text{Br}^-$, CH_2Cl_2 (**2b**, 83%).

After some experimentation, we found that the above conversion could be conveniently carried out by oxidation with bromobenzene, palladium acetate and triphenylphosphine using DMF as the solvent and potassium carbonate as the base in the reaction.¹² The product **7b** was formed in 80% yield. The physical and spectroscopic data of the product **7b** are in good agreement with that reported for lamellarin G trimethyl ether.⁷ Tetrakis(triphenylphosphine)palladium(0) could be used in place of palladium acetate and the reaction proceeded in the same yield. The oxidation of unsubstituted analogue **5a** with the above system also gave the required lactone **7a** in 80% yield.

Acknowledgements

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References

- Davidson, B. S. *Chem. Rev.* **1993**, *93*, 1771–1791.
- Lamellarins A–D: Anderson, R. J.; Faulkner, D. J.; Cun-Heng, H.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1985**, *107*, 5492–5495.
- Davis, R. H.; Carroll, A. R.; Pierens, G. K.; Quinn, R. J. *J. Nat. Prod.* **1999**, *62*, 419–424 and references cited therein.
- Biological activities: Lamellarins I–N: Carroll, A. R.; Bowden, B. F.; Coll, J. C. *Aust. J. Chem.* **1993**, *46*, 489–501. Lamellarins T–X: Reddy, R. M. V.; Faulkner, D. J.; Venkateswarlu, Y.; Rao, M. R. *Tetrahedron* **1997**, *53*, 3457–3466.
- Reddy, R. M. V.; Rao, M. R.; Rhodes, D.; Hansen, M. S. T.; Rubins, K.; Bushmen, F. D.; Venkateswarlu, Y.; Faulkner, D. J. *J. Med. Chem.* **1999**, *42*, 1901–1907.
- (a) Quesada, A. R.; Garcia Gravalos, M. D.; Fernandez Puentes, J. L. *Br. J. Cancer* **1996**, *74*, 677–682; PCT int. Appl., WO 9701336 A1 970116 (*Chem. Abstr.* **1996**, *126*, 166474); (b) Boger, D. L.; Boyce, C. W.; Labroli, M. A.; Schon, C. A.; Jin, Q. *J. Am. Chem. Soc.* **1999**, *121*, 54–62;
- (c) Boger, D. L.; Soenen, D. R.; Boyce, C. W.; Hedrick, M. P.; Jin, Q. *J. Org. Chem.* **2000**, *65*, 2479–2483.
- Lamellarin G trimethyl ether: Heim, A.; Terpin, A.; Steglich, W. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 155–156. Lamellarin K: Banwell, M. G.; Flynn, B. L.; Hockless, D. C. R. *Chem. Commun.* **1997**, 2259–2260. Lamellarin D and H: Ishibashi, F.; Miyazaki, Y.; Iwao, M. *Tetrahedron* **1997**, *53*, 5951–5962. Banwell, M. G.; Flynn, B. L.; Hockless, D. C. R.; Longmore, R. W.; Rae, A. D. *Aust. J. Chem.* **1998**, *52*, 755–765. Lamellarin L: Peschko, C.; Winklhofer, C.; Steglich, W. *Chem. Eur. J.* **2000**, *6*, 1147–1152.
- Lukianol A and lamellarin O dimethyl ether: Fürstner, A.; Weintritt, H.; Hupperts, A. *J. Org. Chem.* **1995**, *60*, 6637–6641. Lamellarin O and Q, lukianol A: Banwell, M. G.; Flynn, B. L.; Hamel, E.; Hockless, D. C. R. *Chem. Commun.* **1997**, 207–208. Storniamide A nonamethyl ether: Ebel, H.; Terpin, A.; Steglich, W. *Tetrahedron Lett.* **1998**, *39*, 9165–9166. Polycitrin A: Terpin, A.; Polborn, K.; Steglich, W. *Tetrahedron* **1995**, *51*, 9941–9946. Lukianol A: Gupton, J. T.; Krumpe, K. E.; Burnham, B. S.; Webb, T. M.; Shuford, J. S.; Sikorski, J. A. *Tetrahedron* **1999**, *55*, 14515–14522. Liu, J.-H.; Yang, Q.-C.; Mak, T. C. W.; Wong, H. N. C. *J. Org. Chem.* **2000**, *65*, 3587–3595. Liu, J.-H.; Chan, H.-W.; Wong, H. N. C. *J. Org. Chem.* **2000**, *65*, 3274–3283. Polycitrin B: Rudi, A.; Evan, T.; Aknin, M.; Kashman, Y. *J. Nat. Prod.* **2000**, *63*, 832–833. Polycitrin B: Beccalli, E. M.; Clerici, F.; Marchesini, A. *Tetrahedron* **2000**, *56*, 2699–2702.
- The starting phenacyl bromides **2a** and **2b** could be prepared from the acetophenone derivatives **9a** and **9b** as shown in Scheme 2. The mesylate protecting group could be introduced by the reaction of the phenolic compounds with methanesulfonyl chloride using triethylamine as a base.¹³ The bromination of the acetophenone could be carried out by using an equimolar quantity of benzyltrimethylammonium tribromide.¹⁴
- (a) Casagrande, C.; Invernizzi, A.; Ferrini, R.; Ferrari, G. *J. Med. Chem.* **1968**, *11*, 765–770; (b) Alberola, A.; Ortega, A. G.; Sadaba, M. L.; Sanudo, C. *Tetrahedron* **1999**, *55*, 6555–6566.
- (a) Silverstein, R. M.; Ryskiewicz, E. E.; Willard, C. *Organic Synthesis Coll. Vol. IV*, pp. 831–833; (b) Majo, V. J.; Perumal, P. T. *J. Org. Chem.* **1996**, *61*, 6523–6525.
- Tamaru, Y.; Yamada, Y.; Inoue, K.; Yamamoto, Y.; Yoshida, Z. *J. Org. Chem.* **1983**, *48*, 1286–1292.
- Bates, R. W.; Rama-Davi, T. *Synlett* **1995**, 1151–1152.
- Kajigaeshi, S.; Kakinami, T.; Okamoto, T.; Fujisaki, S. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 1159–1160. All compounds have been fully characterized. Spectroscopic data of some selected compounds. 2-(3',4'-Dimethoxy-2'-methoxyphenyl)-1-(3',4'-dimethoxyphenyl)-8,9-dimethoxy-5,6-dihydropyrrolo[2,1-a]isoquinoline (**3b**) mp (MeOH): 186–187°C; FTIR (CHCl_3): ν_{max} 3027, 2938, 2839, 1539, 1465, 1365, 1259, 1205 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 2.86 (s, 3H, OSO_2CH_3), 3.08 (t, 2H, $J=6.5$ Hz, $\text{CH}_2\text{CH}_2\text{N}$), 4.13 (t, 2H, $J=6.5$ Hz, $\text{CH}_2\text{CH}_2\text{N}$), 3.41, 3.48, 3.72, 3.88, 3.89, 3.90 (6s, 18H, C-9, C-21, C-8, C-13, C-20 and C-14, $6\times\text{OCH}_3$), 6.53 (s, 1H, C-10ArH), 6.72 (s, 1H, C-7ArH), 6.74 (s, 1H, C-19ArH), 6.82 (dd, 1H, $J=8.0$ and 1.6 Hz, C-16ArH), 6.84 (d, 1H, $J=8.0$ Hz, C-15ArH), 6.87 (d, 1H, $J=1.6$ Hz, C-12ArH), 6.89

(s, 1H, C-22ArH), 6.99 (s, 1H, C-3ArH). ^{13}C NMR (75 MHz, CDCl_3): δ 148.9, 147.8, 147.4, 147.2, 147.1, 147.0, 139.9, 128.8, 125.9, 124.0, 123.0, 121.8, 121.0, 120.0, 119.2, 117.8, 114.1, 113.7, 112.3, 111.1, 107.4, 106.9, 56.1, 56.0, 55.9 (2 \times C), 55.6, 55.2, 44.8, 38.0, 29.4. MS: 595 (M^+ , 44.78), 516 (100.0), 499 (11.61), 485 (29.25), 470 (10.22), 442 (6.65), 426 (5.39), 410 (5.53), 243 (28.77). Anal. calcd for $\text{C}_{31}\text{H}_{33}\text{NO}_9\text{S}$: C, 62.49; H, 5.59; N, 2.35. Found: C, 62.46; H, 5.71; N, 2.07. 3-Formyl-2-(3',4'-dimethoxy-2''-mesyloxyphenyl)-1-(3',4'-dimethoxyphenyl)-8,9-dimethoxy-5,6-dihydropyrrolo[2,1-*a*]isoquinoline (**4b**) mp (MeOH): 192–193°C; FTIR (CHCl_3): ν_{max} 3026, 2939, 2839, 1643, 1531, 1483, 1465, 1426, 1263 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 2.89 (s, 3H, OSO_2CH_3), 3.00–3.20

(m, 2H, $\text{CH}_2\text{CH}_2\text{N}$), 4.51 (m, 1H, CH_2CHHN), 4.95 (m, 1H, CH_2CHHN), 3.35, 3.60, 3.68, 3.85, 3.89, 3.90 (6s, 18H, C-9, C-21, C-8, C-20, C-13 and C-14, 6 \times OCH_3), 6.57 (s, 1H, C-10ArH), 6.76 (s, 2H, C-19, C-22ArH), 6.82 (m, 3H, C-12, C-15, C-16ArH), 6.87 (s, 1H, C-7ArH), 9.45 (s, 1H, C-23CHO). ^{13}C NMR (75 MHz, CDCl_3): δ 180.2, 149.0 (2 \times C), 148.9, 148.0, 147.3, 147.2, 140.2, 133.8, 132.4, 126.8, 126.7, 125.7, 122.9, 122.3, 119.6, 118.0, 114.7, 114.0, 111.0, 110.7, 109.1, 106.5, 56.1, 55.9 (3 \times C), 55.8, 55.2, 42.3, 38.4, 28.7. MS: 623 (M^+ , 20.55), 528 (100.0), 516 (18.48), 485 (9.73), 470 (6.23), 454 (3.81), 440 (3.73), 410 (3.57), 272 (35.41), 264 (61.22), 243 (38.99). Anal. calcd for $\text{C}_{32}\text{H}_{33}\text{NO}_{10}\text{S}$: C, 61.61; H, 5.34; N, 2.25. Found: C, 61.56; H, 5.28; N, 2.01.

SYNTHESIS OF 4-ARYLTETRAHYDROISOQUINOLINES: APPLICATION TO THE SYNTHESIS OF CHERYLLINE

Somsak Ruchirawat^{1-3*}, Sopchok Tontoolarug², and Poolsak Sahakitpichan¹

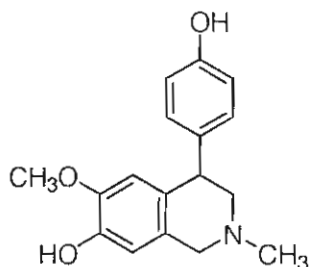
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Abstract- A concise route for the synthesis of 4-aryltetrahydroisoquinolines was developed using the addition of Grignard reagents to nitrostyrene derivatives as the key step. The application to the synthesis of cherylline was described.

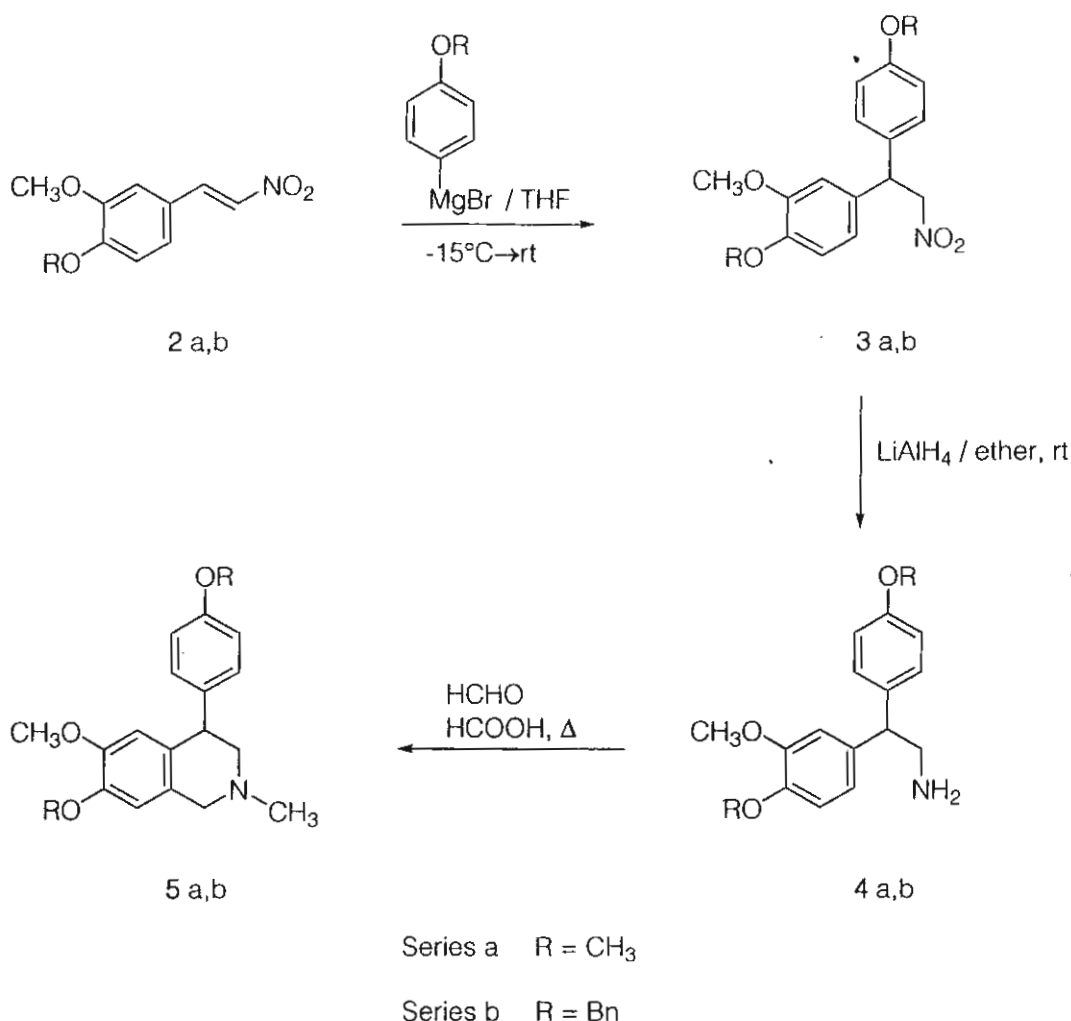
Cherylline has been isolated from *Crinum powellii* var. *alba* and other *Crinum* species.¹ It is a representative of the very rare natural 4-aryl-1,2,3,4-tetrahydroisoquinoline alkaloids. Due to the uniqueness of the structure and potential medicinal properties of the 4-arylisoquinoline derivatives,² many synthetic routes for these compounds³ and especially cherylline (**1**)⁴ have been reported. In the course of our synthetic work on alkaloids, we have successfully developed a new approach for the synthesis of cherylline.



Cherylline **1**

The key step of our approach involved conjugate addition of the Grignard reagents to the appropriate nitrostyrene derivative. Nitroalkene chemistry⁵ has been exploited for various synthetic operations, particularly the potential usefulness of the conjugate addition of various organometallic reagents to the nitroalkenes as a means of forming carbon-carbon bonds, which may be followed by further

transformation of the derived nitroalkane products to various nitrogen heterocycles.⁶ Recent application of the nitroalkene chemistry has been extended to the asymmetric synthesis⁷ of various compounds as well as the solid phase synthesis.⁸ The strategy of our approach is illustrated for the synthesis of (±) dimethylcherylline and cherylline as shown (Scheme 1).



Scheme 1

We have found that by control of the exothermic reaction, 1,4-addition of Grignard reagent to nitrostyrene occurs smoothly at below room temperature in satisfactory yield. The Grignard reagent was prepared by addition of a solution of 4-bromoanisole in dry tetrahydrofuran to a stirred suspension of magnesium turning in dry tetrahydrofuran while maintaining a gentle reflux under nitrogen atmosphere. A solution of 3,4-dimethoxynitrostyrene in dry tetrahydrofuran was then added dropwise to the Grignard reagent which was maintained at -15 °C. The product (**3a**) was obtained in 69% yield after purification by column chromatography on silica gel. The nitro compound so obtained could be conveniently reduced by lithium aluminium hydride in ether at room temperature to give the amine (**4a**) in 75% yield. The amine was purified by preparative layer chromatography on silica gel and could be further purified by recrystallization of the derived oxalate salt. Treatment of the amine derivative with 37% formaldehyde

and formic acid at 100 °C for 3 h gave directly (±)-*O,O*-dimethylcherylline (**5a**) in 93% yield according to our previous finding that formaldehyde and formic acid promoted the cyclization and *N*-methylation of activated phenylethylamine in a "one pot" reaction.⁹

The successful preparation of (±)-*O,O*-dimethylcherylline prompted us to further investigate the generality of this method for the preparation of (±)-cherylline. Reaction of nitrostyrene (**2b**) with the Grignard reagent generated from 4-bromo-*O*-benzylphenol furnished nitro compound (**3b**) in 74% yield. Reduction of the addition product (**3b**) with LAH gave amine (**4b**) in 70% yield. The reaction of the amine with formaldehyde and formic acid gave the expected isoquinoline compound **5b** in 89% yield. The final step was accomplished by hydrogenolytic removal of the benzyl protecting groups of (±)-*O,O*-dibenzylcherylline in ethyl acetate-ethanol (1:1 by volume) containing 10 % palladium on charcoal at 1 atm. The reaction proceeded in 94 % yield to give (±)-cherylline (**1**). All the spectral data of the synthetic (±)-cherylline exhibited good correlation with those of (±)-cherylline reported in the literature.^{4d} All new compounds exhibit satisfactory analytical and spectroscopic data.¹⁰

In conclusion, we have developed an efficient synthesis of (±)-*O,O*-dimethylcherylline (**5a**) and (±)-cherylline (**1**) from readily available starting materials and the reactions involved are operationally simple which we view as a very attractive process. The key step employed in the synthesis, *i.e.*, the addition of Grignard reagents to the nitrostyrenes works well under the employed conditions. The application of this approach to the synthesis of other alkaloids is under further investigation.

ACKNOWLEDGEMENTS

We are grateful to the generous financial support of the Thailand Research Fund (TRF). We also acknowledge the facilities provided by the PERCH programme.

REFERENCES AND NOTES

1. A. Brossi, G. Grethe, S. Teitel, W. C. Wildman, and D. T. Bailey, *J. Org. Chem.*, 1970, **35**, 1100.
2. (a) J. N. Jacob, D. E. Nichols, J. D. Kohli, and D. Glock, *J. Med. Chem.*, 1981, **24**, 1013. (b) E. Zára-Kaczián, L. György, G. Deák, A. Seregi, and M. Dóda, *J. Med. Chem.*, 1986, **29**, 1189. (c) B. E. Maryanoff, J. L. Vaught, R. P. Shank, D. F. McComsey, M. J. Costanzo, and S. O. Nortey, *J. Med. Chem.*, 1990, **33**, 2793. (d) M. Kihara, M. Ikeuchi, A. Yamauchi, M. Nukatsuka, H. Matsumoto, and T. Toko, *Chem. Pharm. Bull.*, 1997, **45**, 939.
3. (a) A. P. Venkov and D. M. Vodenicharov, *Synthesis*, 1990, 253. (b) M. Kihara, M. Kashimoto, Y. Kobayashi, and S. Kobayashi, *Tetrahedron Lett.*, 1990, **31**, 5347. (c) A. P. Venkov, D. M. Vodenicharov, and I. I. Ivanov, *Synthesis*, 1991, 476. (d) M. Kihara, M. Kashimoto, Y. Kobayashi, and Y. Nagao, *Heterocycles*, 1992, **34**, 747. (e) N. Coskun and D. Sümengen, *Synth. Commun.*, 1993,

- 23, 1393. (f) R. B. Miller and J. J. Svoboda, *Synth. Commun.*, 1994, **24**, 1187. (g) H. Meda, N. Selvakumar, and G. A. Kraus, *Tetrahedron*, 1999, **55**, 943. (h) G. J. Kuster, F. Kalmoua, R. de Gelder, and H. W. Scheeren, *Chem. Commun.*, 1999, 855. (i) L. Novellino, M. D' Ischia, and G. Protà, *Synthesis*, 1999, 793 (j) M. Kihara, J.-I. Andoh, and C. Yoshida, *Heterocycles*, 2000, **53**, 359.
4. (a) A. Brossi and S. Teitel, *Tetrahedron Lett.*, 1970, 417. (b) M. A. Schwartz and S. W. Scott, *J. Org. Chem.*, 1971, **36**, 1827. (c) T. Kametani, K. Takahashi, and C. V. Loc, *Tetrahedron*, 1975, **31**, 235. (d) D. J. Hart, P. A. Cain, and D. A. Evans, *J. Am. Chem. Soc.*, 1978, **100**, 1548. (e) H. Irie, A. Shiina, T. Fushimi, J. Katakawa, N. Fujii, and H. Yajima, *Chem. Lett.*, 1980, 875. (f) S. V. Kessar, P. Singh, R. Chawla, and P. Kumar, *Chem. Commun.*, 1981, 1074. (g) T. Kametani, K. Higashiyama, T. Honda, and H. Otomasu, *J. Chem. Soc., Perkin Trans. I*, 1982, 2935. (h) H. Hara, R. Shirai, O. Hoshino, and B. Umezawa, *Heterocycles*, 1983, **20**, 1945. (i) T. Nomoto, N. Nasui, and H. Takayama, *Chem. Commun.*, 1984, 1646. (j) H. Hara, R. Shirai, O. Hoshino, and B. Umezawa, *Chem. Pharm. Bull.*, 1985, **33**, 3107. (k) A. Couture, E. Deniau, P. Woisel, P. Grandclaudeon, and J. F. Carpentier, *Tetrahedron Lett.*, 1996, **37**, 3697. (l) A. Couture, E. Deniau, S. Lebrun, and P. Grandclaudeon, *J. Chem. Soc., Perkin Trans. I*, 1999, 789.
5. For reviews see: (a) D. Seebach, E. W. Colvin, F. Lehr, and T. Weller, *Chimia*, 1979, **33**, 1. (b) A. Yoshikoshi and M. Miyashita, *Acc. Chem. Res.*, 1985, **18**, 284. (c) A. G. M. Barret, *Chem. Soc. Rev.*, 1991, 95 and references cited therein.
6. (a) R. S. C. Lopes, C. C. Lopes, and C. H. Heathcock, *Tetrahedron Lett.*, 1992, **33**, 6775. (b) C. F. Yao, K. H. Kao, J. T. Liu, C. M. Chu, Y. Wang, W. C. Chen, Y. M. Lin, W. W. Lin, M. C. Yan, J. Y. Liu, M. C. Chuang, and J. L. Shiue, *Tetrahedron*, 1998, **54**, 791. (c) H. Shiraishi, T. Nishitani, T. Nishihara, S. Sakaguchi, and Y. Ishii, *Tetrahedron*, 1999, **55**, 13957. (d) S. Lim, I. Jabin, and G. Revial, *Tetrahedron Lett.*, 1999, **40**, 4177. (e) C. S. Pak and M. Nyerges, *Synlett*, 1999, 1271. (f) J. Habermann, S. V. Ley, and J. S. Scott, *J. Chem. Soc., Perkin Trans. I*, 1999, 1253. (g) S. Mahboobi, E. Eibler, M. Koller, K. C. S. Kumar, A. Popp, and D. Schollmeyer, *J. Org. Chem.*, 1999, **64**, 4697.
7. (a) M. L. Morris and M. A. Sturgess, *Tetrahedron Lett.*, 1993, **34**, 43. (b) R. Fernandez, C. Gasch, J.-M. Lassaletta, and J.-M. Llera, *Tetrahedron Lett.*, 1994, **35**, 471. (c) A. G. M. Barrett, D. C. Braddock, P. W. N. Christian, D. Pilipauskas, A. J. P. White, and D. J. Williams, *J. Org. Chem.*, 1998, **63**, 5818. (d) S. E. Denmark and J. A. Dixon, *J. Org. Chem.*, 1998, **63**, 6178. (e) M. Avalos, R. Babiano, P. Cintas, F. J. Higes, J. L. Jiménez, J. C. Palacios, and M. A. Silva, *J. Org. Chem.*, 1999, **64**, 1494. (f) D. Enders and T. Otten, *Synlett*, 1999, 747. (g) A. Alexakis and C. Benhaim, *Org. Lett.*, 2000, **2**, 2579.
8. (a) A. W. Trautwein and G. Jung, *Tetrahedron Lett.*, 1998, **39**, 8263. (b) M. Caldarelli, G. Habermann, and S. V. Ley, *J. Chem. Soc., Perkin Trans. I*, 1999, 107. (c) G. J. Kuster and H. W. Scheeren, *Tetrahedron Lett.*, 2000, **41**, 515.

9. S. Ruchirawat, M. Chaisupakitsin, N. Patranuwatana, J. L. Cashaw, and V. E. Davis, *Synth. Commun.*, 1984, **14**, 1221.
10. All compounds have been fully characterized : Compound (**3a**), oil, IR (neat) 1605, 1590, 1545, 1510, 1460, 1375, 1250, 1140, 1025 cm^{-1} . NMR (400 MHz, CDCl_3) δ 3.78 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 4.80 (br t, 2H), 4.915 (d, 1H, $J = 7.2$ Hz), 4.918 (d, 1H, $J = 9.4$ Hz), 6.70 (d, 1H, $J = 2.0$ Hz), 6.77 (dd, 1H, $J = 8.0, 2.0$ Hz), 6.82 (d, 1H, $J = 8.0$ Hz), 6.86, 7.15 (AA'BB', 2H each, $J = 8.7$ Hz). ^{13}C (100 MHz) δ 47.83, 55.22, 55.86, 79.61, 111.14, 111.34, 114.33, 119.27, 128.58, 131.35, 131.92, 148.35, 149.23, 158.86. MS 317(M^+ , 55.43), 271(22.44), 270(100), 257(53.92), 239(15.35). Anal. Calcd for $\text{C}_{17}\text{H}_{19}\text{NO}_5$: C, 64.34; H, 6.03; N, 4.41. Found : C, 64.21; H, 6.17; N, 4.29. Compound (**3b**), oil, IR (neat) 1600, 1580, 1545, 1510, 1500, 1450, 1375, 1235, 1140, 1020 cm^{-1} . NMR (400 MHz, CDCl_3) δ 3.83 (s, 3H), 4.78 (br t, 1H), 4.888 (d, 1H, $J = 7.5$ Hz), 4.890 (d, 1H, $J = 8.7$ Hz), 5.02 (s, 2H), 5.11 (s, 2H), 6.69 (dd, 1H, $J = 8.0, 2.0$ Hz), 6.72 (d, 1H, $J = 2.0$ Hz), 6.82 (d, 1H, $J = 8.0$ Hz), 6.92, 7.13 (AA'BB', 2H each, $J = 8.7$ Hz), 7.26-7.43 (m, 10H). ^{13}C (100 MHz) δ 47.86, 56.03, 70.02, 70.99, 79.57, 111.72, 114.02, 115.24, 119.30, 127.21, 127.45, 127.86, 128.02, 128.54, 128.59, 128.65, 131.58, 132.41, 136.76, 136.98, 147.57, 149.86, 158.10. MS 469(M^+ , 5.90), 422(2.20), 331(6.13), 92(8.67), 91(100). Anal. Calcd for $\text{C}_{29}\text{H}_{27}\text{NO}_5$: C, 74.18; H, 5.80; N, 2.98. Found : C, 74.01; H, 6.10; N, 2.65. Compound (**4a**), m.p. (oxalate salt, MeOH-ether) 108-109 $^{\circ}\text{C}$. IR (KBr) 2930(br), 1610, 1590, 1515, 1250, 1150, 1025 cm^{-1} . NMR (400 MHz, CDCl_3) δ 1.56 (br s, 2H), 3.27 (br s, 2H), 3.77 (s, 3H), 3.83 (s, 3H), 3.85 (s, 3H), 3.88 (t, 2H, $J = 7.5$ Hz), 6.73 (d, 1H, $J = 1.6$ Hz), 6.79 (dd, 1H, $J = 8.0, 1.7$ Hz), 6.82 (d, 1H, $J = 8.3$ Hz), 6.85, 7.16 (AA'BB', 2H each, $J = 8.7$ Hz). ^{13}C (100 MHz) δ 7.16, 53.77, 55.19, 55.81, 55.84, 111.24, 111.53, 113.97, 119.67, 128.84, 135.01, 135.59, 147.59, 148.99, 158.14. MS 287(M^+ , 4.49), 258(26.96), 257(100). Anal. Calcd for $\text{C}_{19}\text{H}_{23}\text{NO}_7 \cdot 1/2 \text{H}_2\text{O}$: C, 59.06; H, 6.26; N, 3.62. Found : C, 59.40; H, 6.18; N, 3.61. Compound (**4b**), m.p. (oxalate salt, MeOH-ether) 114-116 $^{\circ}\text{C}$. IR (KBr) 3050-2950(br), 1605, 1580, 1505, 1250, 1225, 1140, 1020 cm^{-1} . NMR (400 MHz, CDCl_3) δ 1.4 (br s, 2H), 3.23 (d, 2H, $J = 7.7$ Hz), 3.86 (t, 1H, $J = 7.6$ Hz), 3.84 (s, 3H), 5.03 (s, 2H), 5.12 (s, 2H), 6.72 (dd, 1H, $J = 8.0, 2.0$ Hz), 6.75 (d, 1H, $J = 2.0$ Hz), 6.82 (d, 1H, $J = 8.0$ Hz), 6.92, 7.15 (AA'BB', 2H each, $J = 8.7$ Hz), 7.26-7.44 (m, 10H). ^{13}C (100 MHz) δ 7.26, 53.85, 55.99, 69.99, 71.16, 112.13, 114.03, 114.89, 119.72, 127.21, 127.44, 127.74, 127.91, 128.48, 128.54, 128.90, 135.29, 136.17, 137.28, 146.81, 149.63, 157.39. MS 439(M^+ , 1.47), 410(15.02), 409(38.12), 92(8.33), 91(100), 44(11.28). Anal. Calcd for $\text{C}_{31}\text{H}_{31}\text{NO}_7 \cdot 1/2 \text{H}_2\text{O}$: C, 69.13; H, 5.99; N, 2.60. Found : C, 68.73; H, 5.90; N, 2.82. Compound (**5a**), m.p. (ether-hexane) 97-99 $^{\circ}\text{C}$ (lit.,^{4d} 97-99 $^{\circ}\text{C}$). IR (CHCl_3) 1610, 1580, 1515, 1460, 1250, 1140, 1035 cm^{-1} . NMR (400 MHz, CDCl_3) δ 2.41 (s, 3H), 2.48 (dd, 1H, $J = 8.0, 11.4$ Hz), 2.97 (ddd, 1H, $J = 11.6, 5.5, 1.0$ Hz), 3.54 (d, 1H, $J = 14.3$ Hz), 3.66 (d, 1H, $J = 14.3$ Hz), 3.64 (s, 3H), 3.79 (s, 3H), 3.85 (s, 3H), 4.16 (br t, 1H),

6.35 (s, 1H), 6.56 (s, 1H), 6.83, 7.10 (AA'BB', 2H each, $J = 8.7$ Hz). ^{13}C (100 MHz) δ 44.64, 45.86, 55.14, 55.78, 58.07, 62.10, 108.70, 111.89, 113.65, 127.27, 129.15, 129.81, 136.82, 147.40, 147.49, 158.11. MS 313(M^+ , 35.43), 270(52.86), 269(20.66), 240(19.76); 239(100), 135(11.42). Compound (**5b**), m.p. (ethyl acetate) 141-142 °C(lit.,^{4c,4g} 144-145 °C). IR (CHCl_3) 1610, 1580, 1505, 1255, 1240, 1140 cm^{-1} . NMR (400 MHz, CDCl_3) δ 2.38 (s, 3H), 2.47 (dd, 1H, $J = 11.0, 8.0$ Hz), 2.96 (dd, 1H, $J = 11.0, 5.0$ Hz), 3.48 (d, 1H, $J = 14.5$ Hz), 3.59 (d, 1H, $J = 14.5$ Hz), 3.65 (s, 3H), 4.15 (dd, 1H, $J = 8, 6$ Hz), 5.04 (s, 2H), 5.11 (s, 2H), 6.37 (s, 1H), 6.58 (s, 1H), 6.91, 7.10 (AA'BB', $J = 8.7$ Hz), 7.27-7.46 (m, 10H). ^{13}C (100 MHz) δ 44.69, 45.84, 55.99, 57.99, 62.04, 70.01, 71.06, 111.58, 112.59, 114.64, 127.27, 127.50, 127.74, 127.91, 128.48, 128.53, 129.82, 129.90, 137.12, 137.25, 146.66, 148.23, 157.42. MS 465(M^+ , 7.28), 422(10.06), 331(15.52), 91(100).

ผลงานที่ตีพิมพ์ในวารสารวิชาการในประเทศ

Stereospecific reduction of papaveraldine and papaverinol derivatives

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Dedicated to Professor Stang Mongkolsuk on the occasion of the 30th anniversary of Prince of Songkla University

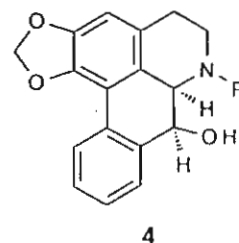
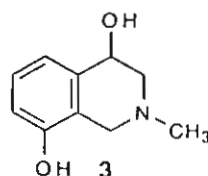
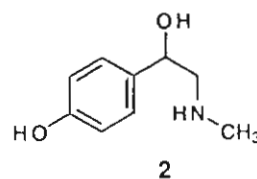
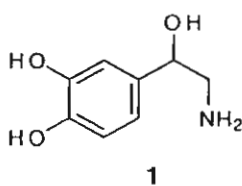
Abstract

The stereospecific reduction of various papaveraldinium salts and derivatives with sodium borohydride and potassium borohydride to give the hydroxylaudanosines was investigated. The mechanisms of the reduction were proposed to explain the stereospecificity of the reaction yielding varying ratio of the derived α and β hydroxylaudanosines.

Key words : Stereospecific reduction, β -amino alcohol, α and β laudanosines

The occurrences of diverse biologically-active amines and numerous classes of alkaloids with the hydroxyl group β to amino group are wide-spread (Hanessian *et al.*, 1996, Yuasa *et al.*, 1969). For example, norepinephrine (1) (Goodal, *et al.*, 1967) which is biosynthesized (Senoh, *et al.* 1959) from dopamine, the hydroxylation is effected by enzyme β -hydroxylase which has been isolated and used *in vitro* hydroxylation. Other simple β -hydroxy amino compounds are also known (Ranier, 1976) (2,3).

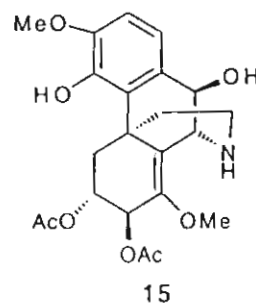
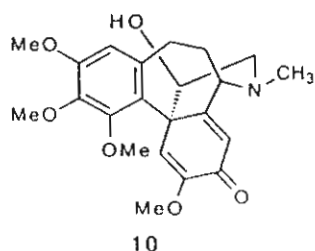
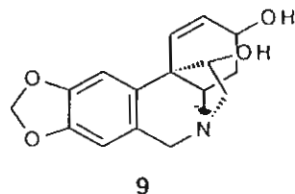
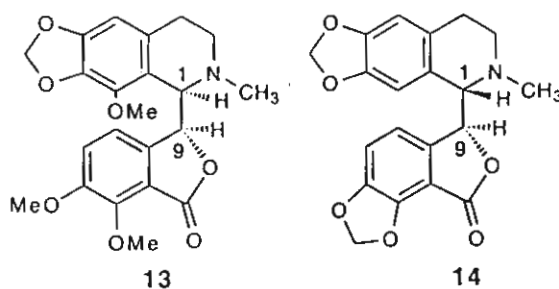
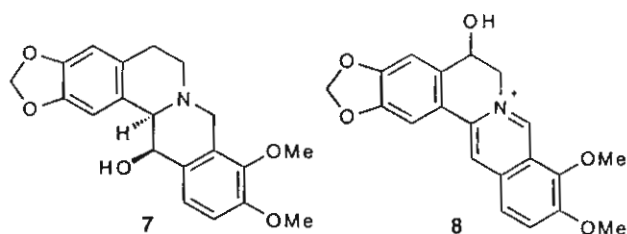
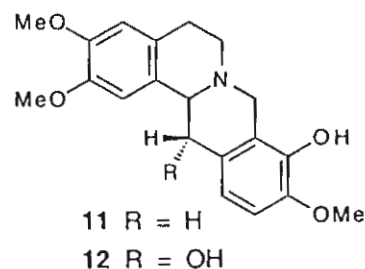
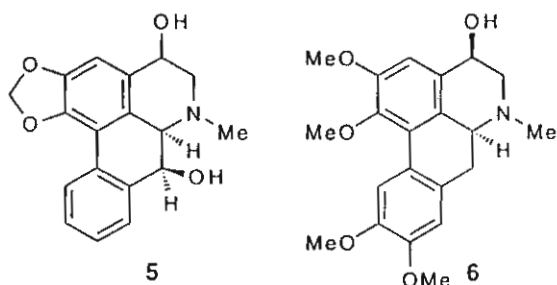
Other more complex β -hydroxy amino group in various alkaloids include ushinsunine (4), a C-7 hydroxylated aporphine, found in a variety of *Michelia* species (Yang, 1962, 1963). Two C-4 hydroxylated aporphines are known as shown in



compound (5) (Okamoto, *et al.*, 1969) and compound (6) (Ribas, *et al.*, 1972; Hoshino, *et al.* 1975). Ophiocarpine (7), Berberastine (8) are the two hydroxylated protoberberine compounds. Haemanthamine (9) (Wildman, *et al.*, 1964) also falls into the same pattern.

Apart from the isolated β -hydroxylated alkaloids, this type of compound was also postulated to be precursor in the biosynthesis of alkaloids. For example, 4-hydroxy-O-methylandrocymbine (10) was postulated from many feeding experiments to be precursor for the biosynthesis (Bat-

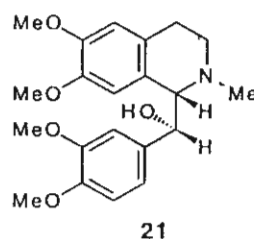
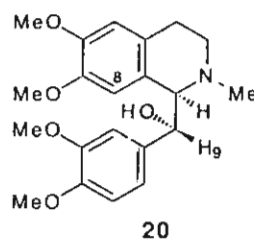
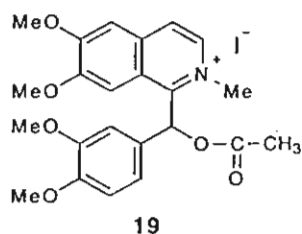
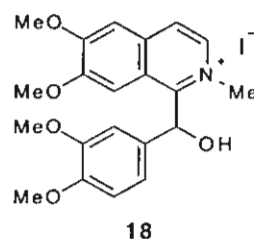
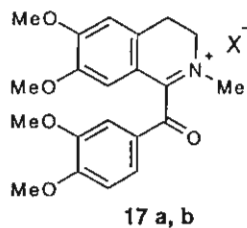
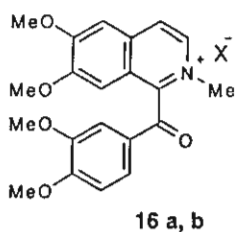
tersby, *et al.*, 1966) of colchicine. Battersby, *et al.* from extensive feeding result have proved that stereospecific hydroxylation of scoulerine (11) gives compound (12) which can then be transformed to narcotine (Battersby, *et al.*, 1975). (-)-Narcotine (13) and (+)-adalumidine (14) are two representative of the phthalideisoquinolines. Fentanyl (15), a morphinan alkaloid, has very recently been isolated (Ogino, *et al.*, 1998) from *Stephania tetrandra*, this alkaloid also exhibited the above mentioned feature.



Results and discussion

As can be seen, the stereochemistry of these β -hydroxy amino compounds could be "erythro" or "threo". In order to find a synthetic route which would produce the required isomer in the benzyloisoquinoline and the benzylisoquinoline-derived alkaloids, the reduction of C-9 oxo-isoquinolinium derivatives with sodium borohydride and potassium borohydride was investigated. Some approaches to the stereospecific synthesis of β hydroxy amino compounds have recently been reported (Morita, *et al.*, 1998).

N-methylpapaveraldinium salts (**16a** X^- = sulfate, **16b** X^- = iodide), N-methyl-3,4-dihydro-papaveraldinium salts (**17a** X^- = sulfate, **17b** X^- = iodide), papaverinol methiodide (**18**) and papaverinol acetate methiodide (**19**) were used as substrates in the reduction. The reduction of these salts with borohydride salts gave α - and β -hydroxylaudanosines (**20**, **21**).



In this reduction step, two asymmetric centers were created thus two pairs of diastereoisomers were expected. The ratio of α - and β -hydroxylaudanosines could be obtained from the integration ratio of the NMR spectrum of the reduction product. Four significant peaks i.e. C-8 aromatic proton, methoxyl group attached to C-7,

N-methyl group and proton attached to C-9 including coupling constants, were considered to assign each diastereoisomer. These chemical shifts are shown in table I.

Table I.

compounds	C8-H	C7-OMe	N-Me	H-C9-OH
α -hydroxy-laudanosine	5.49	3.35	2.57	4.29-4.46 doublet J : 10Hz
β -hydroxy-laudanosine	5.89	3.48	2.65	5.10 broad doublet J : 3Hz

The coupling constant of H-C1 and H-C9 in α -hydroxylaudanosine is 10 Hz. indicating a trans relationship between these two protons. α -Hydroxylaudanosine is shown in conformation (22) to illustrate the trans relationship of these two protons.

Conformation (22) suggests a high chemical shifts of C-8 proton and C-7 methoxyl group with normal N-methyl group, all these facts are observed as shown in the table 1, this is due to anisotropy effect induced by ring C. Similarly, conformation (23) may be written for β -hydroxylaudanosine. The coupling constant of H-C9 and H-C1 in β -hydroxylaudanosine is 3 Hz. suggesting a dihedral angle of ca. 50°- 60°. Our finding is in full agreement with the reported coupling constant of phthalideisoquinoline (J H-C9 and H-C1 = 3.5 Hz) (Safe, *et al.*, 1964; Shamma, *et al.*, 1976).

The ratio of α - and β -hydroxylaudanosines obtained from the reduction of papaveraldine methosulfate, papaveraldine methiodide, 3,4-dihydropapaveraldine methosulfate and 3,4-dihydropapaveraldine methiodide were shown in table 2.

In the table 2, we can see that the metal attached to the borohydride and the anion (i.e. sulfate or iodide) have no effect on the ratio of the product. Significant increase in the ratio of β : α hydroxylaudanosine has been obtained when the substrate was changed to 3,4-dihydro derivatives. In the

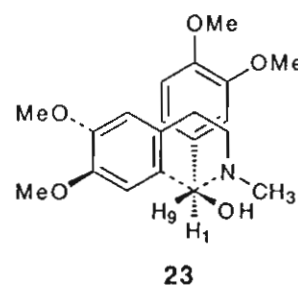
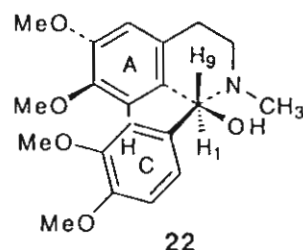


table 2 ethyl alcohol was used as the solvent in the reduction step.

We have also studied the effects of solvents on the ratio of β : α hydroxylaudanosines produced in the reduction step. The results are shown in table 3.

Higher ratios were obtained when methanol and 2-propanol (IPA) were used as solvents compared with when ethyl alcohol was used as solvent and lower ratio was obtained when t-butanol was used as solvent.

Table 2.

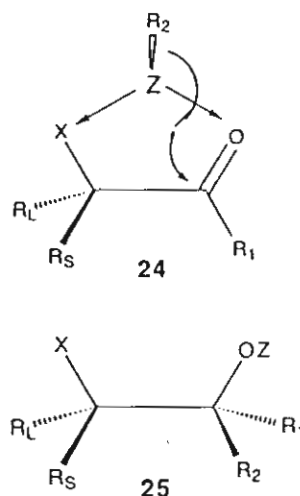
run	starting material	reducing agent	ratio of β : α hydroxylaudanosine
1	Papaveraldine methosulfate	NaBH ₄	2.30 : 1
2	Papaveraldine methosulfate	KBH ₄	2.28 : 1
3	Papaveraldine methiodide	NaBH ₄	2.26 : 1
4	Papaveraldine methiodide	KBH ₄	2.38 : 1
5	3,4-Dihydropapaveraldine methosulfate	NaBH ₄	3.27 : 1
6	3,4-Dihydropapaveraldine methosulfate	KBH ₄	3.10 : 1
7	3,4-dihydropapaveraldine methiodide	NaBH ₄	3.23 : 1
8	3,4-dihydropapaveraldine methiodide	KBH ₄	3.29 : 1

Table 3.

run	starting material	solvent	ratio of $\beta : \alpha$ hydroxylaundanosine
1	Papaveraldine methiodide	EtOH	2.26 : 1
2	Papaveraldine methiodide	MeOH	2.70 : 1
3	Papaveraldine methiodide	MeOH	2.85 : 1
4	Papaveraldine methiodide	MeOH	2.68 : 1
5	Papaveraldine methiodide	IPA	2.42 : 1
6	Papaveraldine methiodide	IPA	2.48 : 1
7	Papaveraldine methiodide	IPA	2.36 : 1
8	Papaveraldine methiodide	t-BuOH	1.80 : 1

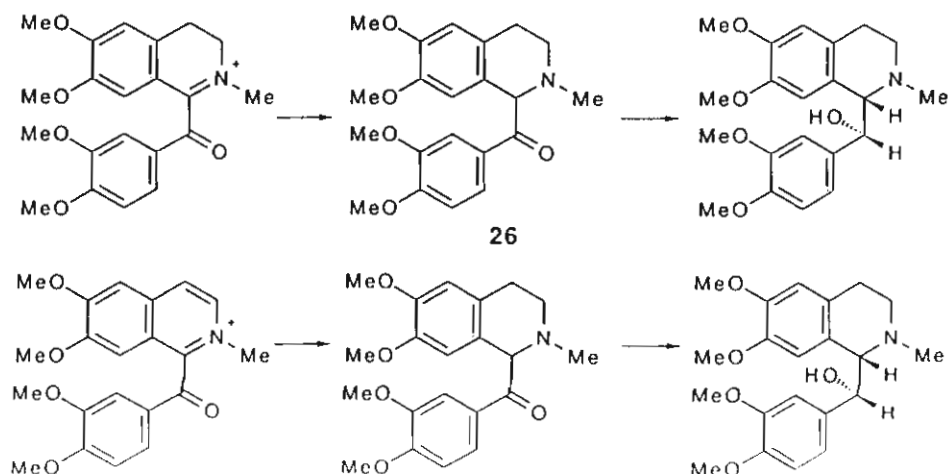
Reactions of achiral reagents with chiral aldehydes and ketones have been well studied. Many models (Morrison, *et al.*, 1971; Boyd, *et al.*, 1968) have been proposed to account for the stereoselectivity of the reaction. It was recognised that in circumstances in which one of the groups on the adjacent chiral center, for instance hydroxy or amino, could complex with the reagent. For these special cases a "cyclic model" was proposed to account for this stereoselectivity (Cram, *et al.*, 1959)

Application of the "cyclic model" to our case (24), $X=N$, $R_2 = H$. Since R_2 (in our case = H) will be added *cis* to the smallest group ($R_S = H$ in our case), so the product (25) by this mechanism will be β -hydroxylaundanosine. So the interpretation of the results could be as follows.



Reduction of N-methyl-3,4-dihydropapa-

veraldinium salt and N-methylpapaveraldinium salt could give the ketolaundanosine (26) which

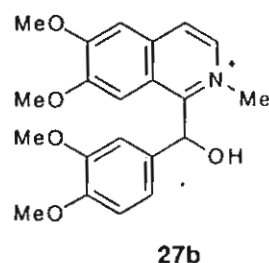
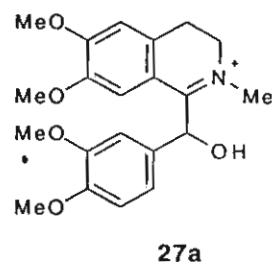


could be reduced by the "cyclic model" mechanism to β -hydroxylaundanosine (21).

If the above pathway is the only pathway in the reduction, one would expect 100% of the β -hydroxylaundanosine, however, as previously shown, reduction of 3,4-dihydropapaveraldine methiodide and papaveraldine methiodide gave β - and α -hydroxylaundanosine in the ratio of 3.23 : 1 respectively.

This suggested that there would be competing reaction (s) in the reduction. We thought that the main competing reaction would be the reduction of the carbonyl group to the corresponding alcohol before the reduction of the iminium group i.e. the formation of compounds (27a and 27b).

Complex formation of the hydroxyl group with borohydride necessitated the transfer of hydride to the iminium group to be on the same side



of the hydroxyl group thus the two hydrogens at C-1 and C-9 protons had to be threo. By the above proposal, one would expect the α -hydroxylaundanosine would be the product of the reaction.

In order to test the validity of the above proposal, a reduction of papaverinol methiodide (18) and papaverinol acetate methiodide (19) was studied. The results are shown in table 4.

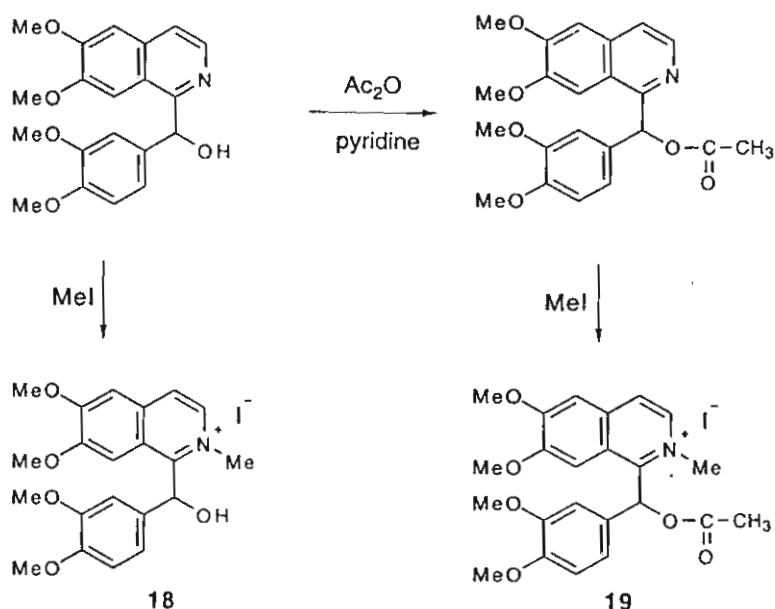


Table 4.

run	starting material	reducing agent	ratio of β : α hydroxylaundanosine
1	papaverinol methiodide	NaBH ₄	1 : 2.0
2	papaverinol methiodide	KBH ₄	1 : 2.0
3	papaverinol-acetate methiodide	NaBH ₄	1 : 1.5
4	papaverinol-acetate methiodide	KBH ₄	1 : 1.5

The results were in full accord with our proposed mechanism, α -hydroxyaudanosine was the main product of the reaction, Runs 3 and 4 demonstrated that acetyl group can complex with borohydride, even though it is less effective.

Synthesis of the compounds used in the stereospecific reductions

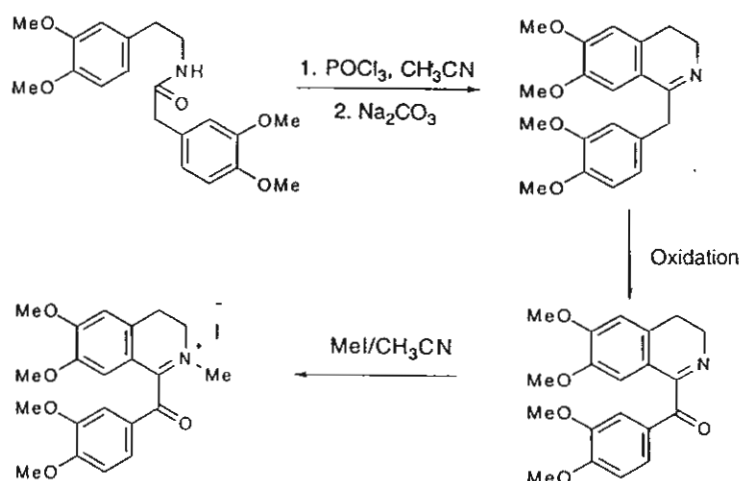
N-Methylpapaveraldinium iodide was prepared by quaternization of papaveraldine with methyl iodide in acetonitrile. Papaveraldine was synthesized by the oxidation of papaverine with molecular oxygen in base. Recently, we have found (Ruchirawat, *et al.*, 1976) in our laboratory that papaveraldine could be most conveniently prepared by oxidation of papaverine with oxygen and triton B in pyridine. However, in order to seek for other phase-transfer reagent to effect the same transformation, we have found that cetyltrimethylammonium bromide could be used in place of benzyltrimethylammonium chloride. This is signi-

ficant, in view that cetyltrimethylammonium bromide is much cheaper than benzyltrimethylammonium chloride. We found that papaveraldine could be obtained in 90% yield by oxidation of papaverine with oxygen, cetyltrimethylammonium bromide and 50% sodium hydroxide solution. The papaveraldine so obtained could be methylated with methyl iodide to give the required N-methylpapaveraldinium iodide in good yield.

Reduction of papaveraldine with sodium borohydride gave papaverinol in quantitative yield, papaverinol so obtained can be acetylated with acetic anhydride in pyridine to give the acetylated product in 98% yield.

Papaverinol and papaverinol acetate was methylated with methyl iodide in acetonitrile to give the corresponding methiodide salt in good yield. The results are summarized in the scheme.

The synthesis of N-methyl dihydropapaveraldinium iodide was accomplished as shown in the scheme.



Bischler-Napieralski reaction of homoveratroyl-homoveratryl amine with phosphorus oxychloride in acetonitrile gave dihydropapaverine hydrochloride in good yield. Oxidation of dihydropapaverine with lead tetracetate or oxygen gave dihydropapaveraldine which could be methylated to the corresponding methiodide in good yield.

Our study of the borohydride reduction of some papaverinol and papaveraldine derivatives has given some insight into the mechanism of the

reduction. The findings also give us a clue as to the choice of the starting materials depending on which isomer we are interested in.

Acknowledgements

We are grateful to Thailand Research Fund (TRF) for the generous support of our research programme.

References

- Battersby, A.R., Herbert, R.H., McDonald, E. and Ramage, R. 1966. Chem. Comm., 603.
- Battersby, A.R., Staunton, J., Willyshire, H.R., Bircher, B.J. and Claudio Fuganti. 1975. J. Chem. Soc. Perkin Trans I., 1162.
- Boyd, D.R. and McKevey, M.A. 1968. Quart. Rev., 95.
- Cram, D.J. and Kopecky, K.R. 1959. J. Amer. Chem. Soc., 81 : 2748.
- Goodal, M. and Krishnev, N. 1967. J. Biol. Chem., 226.
- Hanessian, S. and Ninkovic S. 1996. J. Org. Chem., 61 : 5418.
- Hoshino, O., Hara, H., Ogawa, M. and Umezawa, B. 1975. J. Chem. Soc. (Chem. Comm.), 306.
- Morita, T., Matsunaga, H., Sugiyama, E., Ishizuka, T. and Kunieda, T. 1998. Tetrahedron Lett., 7131.
- Morrison, J.D. and Mosher, H.S. 1971. in "Asymmetric Organic Reactions" Chapter 3, Prentice Hall Inc.
- Ogino, T., Katsuhara, T., Sato, T., Sasaki, H., Okada, M. and Maruno, M. 1998. Heterocycles, 48 : 311.
- Okamoto, J., Yuge, E. and Nagai, Y. 1969. Tetrahedron Lett., 3287.
- Ranier, R.L. and McLaughlin, J. L. 1976. J. Org. Chem., 41 : 319.
- Ribas, I., Sueiras, J. and Castedo, L. 1972. Tetrahedron Lett., 2033.
- Ruchirawat, S., Somchitman, V., Tongpenyai, N., Lertwanawattana, W., Issarayangyuen, S., Prasitpan, N. and Prempre, P. 1976. Heterocycles, 4 : 1917.
- Safe, S. and Moir, R.Y. 1964. Can. J. Chem., 42 : 160.
- Senoh, S., Crevelling, R., Udenfriend, S. and Witkop, B. 1959. J. Am. Chem. Soc., 81 : 6236.
- Shamma, M. and Georgier, V. St. 1974. Tetrahedron Lett., 2339. and 1976. Tetrahedron, 32 : 211.
- Wildman, W.E. and Fales, H.M. 1964. J. Am. Chem. Soc., 86 : 294.
- Yang, T.H. 1962. J. Pharm. Soc. (Japan), 82 : 798.
- Yang, T.H. 1963. J. Pharm. Soc. (Japan), 83 : 216.
- Yuasa, Y., Ando, J. and Shibuya, S. 1966. J. Chem. Soc. Perkin Trans I., 793 and references cited therein.

The compounds exhibited the following physical data.

Compound 16a: m.p. 137° C decomposed ; IR (nujol) 1651, 1149, 1114, 864 cm^{-1} ; NMR (CDCl_3) 3.74 (s, N- CH_3), 3.92, 4.42, 4.12, 4.3, 4.48 (4Xs, 4x OCH_3), 7.06 (s, H_5), 7.21, 7.35 (d, ABq, H_5 , J : 9 Hz), 7.48, 7.62 (d, ABq, H_6 , J : 9 Hz), 7.48, 7.51 (d, ABq, H_6 , J : 2 Hz), 7.92, 7.95 (d, ABq, H_2 , J : 2 Hz), 8.84, 8.95 (d, ABq, H_4 , J : 5 Hz), 9.13, 9.24 (d, ABq, H_3 , J : 5 Hz)

Compound 16b: m.p. 140° C decomposed; IR (nujol) 1661, 1580, 1229, 1141, 1019, 992 cm^{-1} ; NMR (CDCl_3) 3.94 (s, N- CH_3), 4.12, 4.36, 4.61 (3Xs, 4x OCH_3), 7.09 (s, H_5), 7.18, 7.33 (d, ABq, H_5 , J : 9 Hz), 7.6, 7.76 (d, ABq, H_6 , J : 9 Hz), 7.76, 7.79 (d, ABq, H_6 , J : 2 Hz), 7.94, 7.79 (d, ABq, H_2 , J : 2 Hz), 8.33 (s, H_4), 9.02, 9.12 (d, ABq, H_4 , J : 6 Hz), 9.38, 9.48 (d, ABq, H_3 , J : 6 Hz)

3,4-Dihydropapaveraldine: m.p. 189° C - 190° C; IR (nujol) 1627, 1511, 1266, 1024, 866 cm^{-1} ; NMR (CDCl_3) 2.68, 2.8, 2.93 (t, 2x H_2 , J : 7 Hz), 3.79 (s, 1x OCH_3), 3.96 (1xs, 3x OCH_3), aromatic protons appeared as multiplets from 6.79 - 7.73

Compound 17a: m.p. 95° C decomposed; IR (nujol) 1659, 1274, 1013, 874 cm^{-1} ; NMR (CDCl_3) 3.68 (s, N- CH_3), 3.75 (s, 3x OCH_3), 4.23 (s, 1x OCH_3), aromatic proton appeared as complex multiplets from 6.63 - 7.83

Papaverinol: m.p. 136° - 137.5° C; IR (nujol) 3360, 1604, 1522, 896, 874 cm^{-1} ; NMR (CDCl_3) 3.78 (s, 1x OCH_3), 3.84 (s, 2x OCH_3), 4.01 (s, 1x OCH_3), 6.2 (s, $\text{H}_{\text{benzylic}}$), 6.88, 6.9 (d, H_5 , H_6 , J : 1 Hz), 7.12 (s, H_4), 7.18 (s, H_2), 7.35 (s, H_3), 7.51, 7.6 (d, ABq, H_4 , J : 4 Hz), 8.42, 8.51 (d, ABq, H_3 , J : 4 Hz)

Compound 18: m.p. 198° - 199° C; IR (nujol) 3245, 1293, 1131 cm^{-1} ; NMR (CDCl_3) 3.88, 3.97, 4.08, 4.22, 4.6 (5Xs, 4x OCH_3 , N- CH_3), 6.16 - 7.38 (m, H_2 , H_5 , H_6 , $\text{H}_{\text{benzylic}}$), 7.59, 7.79 (2Xs, H_4 , H_3), 8.36, 8.47 (d, ABq, H_4 , J : 6 Hz), 8.6, 8.72 (d, ABq, H_3 , J : 6 Hz)

Papaverinol acetate: m.p. 151° - 153° C; IR (nujol) 1709, 1604, 1244, 849, 804 cm^{-1} ; NMR (CDCl_3) 2.24 (s, O-CO- CH_3), 3.79, 3.82 (2xs, 2x OCH_3), 3.93, 3.82 (2x OCH_3), 6.93 (s, $\text{H}_{\text{benzylic}}$), 7.09 (broad s, H_5 , H_6 , H_4), 7.53 (m, H_4 , H_6 , H_5), 8.43, 8.53 (d, H_4 , J : 5 Hz)

Compound 19: m.p. 196° - 197° C; IR (nujol) 1731, 1230, 1152, 1034 cm^{-1} ; NMR (CDCl_3) 2.24 (s, O-CO- CH_3), 3.86 (s, 3x OCH_3), 4.2 (s, 1x OCH_3), 4.73 (s, N- CH_3), 6.58, 6.69 (d, ABq, H_4 , J : 4 Hz), 7.05, 7.18 (d, ABq, H_3 , J : 4 Hz), other aromatic protons and C-9 $\text{CH}_2\text{-OAc}$ appeared as a set of peaks from 6.69 - 7.95

Biodiversity conservation and drug discovery

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Dedicated to Professor Stang Mongkolsuk on the occasion of the 30th anniversary of Prince of Songkla University

Abstract

The threat to biodiversity through the destruction of terrestrial and marine ecosystems coupled with the urgent need to find novel, new chemotherapeutic agents as well as active chemotypes as leads for effective drug development, make natural products research in drug discovery and development a top priority. The paper will highlight the research of selected natural products aiming at the discovery of therapeutic agents from Thai plants. Attention will be focussed on selected plants that possess cytotoxic properties.

Key words : biodiversity, bioactive natural products, cytotoxicity

Biodiversity - the diversity of living forms - has lately attracted a great deal of interest and concern since biological resources constitute an asset with a great deal of immediate as well as potential benefit for the quality of life. Ironically, just as we begin to recognize some of the potential benefits that might accrue from our having a large number of species, we are also coming to a realization that there is a current decline in the number of species and that this may have catastrophic consequences. This decline in biodiversity is largely the result of human activities such as drastic transformation of natural landscapes or deforestation. These phenomena pose a serious threat to sustainable development since species diversity may well be our planet's most important and irreplaceable resource. Once depleted, species regeneration, if at all possible, might take 5 to 10 million years. Its

loss would thus have profound negative effects on the overall quality of life on our planet and on our potential development (Chivian, 1993).

Estimated present status

It was believed until the early 1980s that there were about 3 million species (approximately 1.5 million known species and about the same number of unknown species). In the early 80s however, when the tropical rain forests began to be studied systematically, these estimates were altered immensely. Conservative estimates suggest that there are more than 10 million species, while other less conservative estimates suggest there are between 20 and 80 million species. To illustrate this: of the species of flowering plants, more than half are found in the tropics where tropical rain forests contain the richest concentration of biota. In

the tropics, many species may be found in very limited areas. For example, a one hectare plot in Kalimantan, Indonesia, yielded approximately 700 species of plants. In the Amazon region up to 500 different species of trees grow on a single hectare of forest. And a single tree in Peru yields 43 ant species in 26 genera. The enormous consequential losses are obvious when one realizes that approximately 42 million acres of rain forest are permanently lost every year.

Since the turn of the century, more than one-half of the world's tropical forests have been lost. Not surprisingly, most of the disappearing tropical forests belong to the developing world. At the current rate of deforestation, it is predicted that between five and ten percent of plant and animal species will become extinct. It is believed that approximately 27,000 species were lost in tropical rain forests last year alone as a result of deforestation. Edward O. Wilson, the most prominent figure in the biodiversity campaign, estimates that deforestation results in the loss of half of one percent of the existing species each year. If this current trend continues, millions of species will vanish without ever even having been discovered by man.

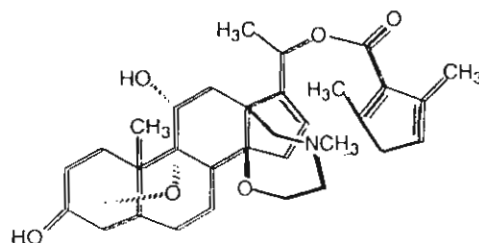
Consequences of species loss

The consequences of a reduction in biodiversity through loss of species constitute a serious threat to human survival. The loss of species could reduce the availability of natural products used as raw materials for manufacturing and industry. It could also diminish the future availability of new genetic resources and wild germ plasm essential for breeding crop varieties with higher productivity and with greater resistance to insects, diseases, and adverse climatic conditions. In medical science, the loss of species could reduce the opportunity for treatment of diseases through the loss of medical models and new medicines as a result of reduction of the availability of natural products which have potential medicinal properties.

Animals and human beings share certain similarities as well as differences in a number of biochemical processes and mechanisms. It is through the knowledge and research findings

obtained from studies in animals that many physiological and biochemical mechanisms are unravelled, particularly those underlying the etiology of diseases that lead to development of therapeutic methods and discovery of agents currently employed. Because of these similarities, valuable medical models can be found among animals as different from our own as the amphibian, fish and other mammals. Such models can assist in our understanding of human physiology and illness. Some animal species that are currently at risk have been shown to be valuable medical models offering windows for greater understanding of human physiology and biochemistry which may lead to successful treatments of diseases that are at present incurable. This subject has been superbly reviewed by Dr. Eric Chivian in "Critical Condition : Human and the Environment." (1993)

An example of medical model developed from animals species that would appear to be far apart in the biosystem is highlighted by research on the active constituents of frog toxins. The research undertaken by John Daly of the National Institute of Health, USA, and colleagues since the early 1970's deserves special mention (Daly, 1998). Certain types of frogs found in tropical rain forests in Central and South America produce alkaloids, which are nitrogen - containing ring compounds which make the dart-poison frogs (*Dendrobati-* *dae*) an extremely important medical model in our understanding of cross-species biochemistry. These brightly coloured, toxin producing frogs are, however, in danger of extinction because of the destruction of their habitat. Alkaloids, which are the active ingredients of the frog toxins, work by binding to specific ion channels and pumps at the

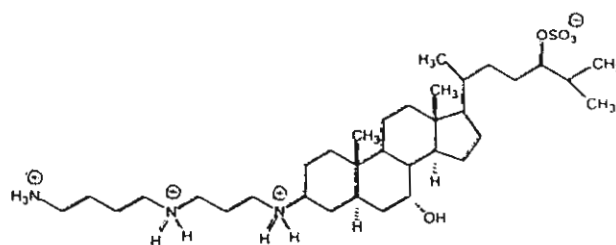


Batrachotoxin

synaptic junctions in neurons. These alkaloids are important scientific tools in the study of the basic unit of membrane function and neurophysiological research.

Specifically, batrachotoxin, which is one of the toxins produced by dart-poison frogs, binds to a specific site in sodium channels and has been of critical importance in defining the action of this fundamental molecular structure. The study of the mechanism of action of frog toxins may provide clues for the production of new therapeutic agents for treatment of diseases such as Alzheimer's disease and other neurological disorders. Unfortunately, continued deforestation will likely result in the extinction of the dart-poison frog and the resulting loss of a possible tool for studying neurological disorders.

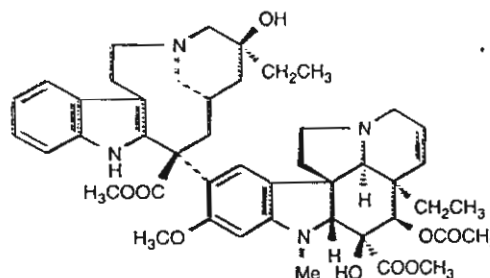
Another example of the loss of a medical model caused by species extinction is the reduction in number of sharks which are being slaughtered by man to the point of extinction; very unfortunately, perhaps because "shark-fin soup" is considered an exotic dish and a delicacy on the Chinese menu! The immune systems of certain kinds of sharks are very good at warding off cancerous tumors and infections. It has been shown that tumors resulting from known potent carcinogens cannot be induced in some types of sharks. This observation is very significant in cancer research since cancer is a very complex disease characterized by uncontrolled growth and the ability to invade and metastasize to other tissues of the body and therefore highly malignant. The sharks have a type of substance found in their cartilage which prevents the growth of tumors by slowing the growth of new blood vessels toward tumors. How these sharks' immune systems work to prevent the growth of tumors may never be discovered however, since sharks are in jeopardy of becoming extinct. In addition, a compound, squalamine, has been isolated from the tissues of the dogfish shark. Squalamine has demonstrated potent activity in laboratory tests against a variety of bacteria, fungi, and parasites, leading to the exciting possibility that new antibiotics might be developed for infectious organisms that have become resistant to drugs in current use.



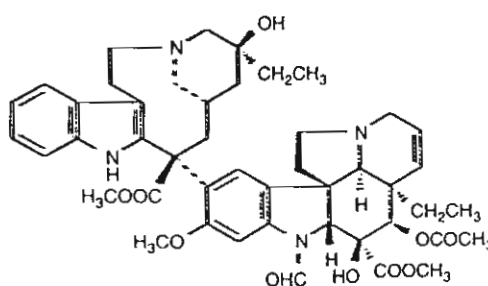
Squalamine

Squalamine is a water-soluble aminosterol and exhibits the same potency as ampicillin. The compound is stable to heat and protease enzymes and can be easily absorbed by the digestive tract, indicating that the compound should be capable of being taken orally.

The loss of other species could have immediate negative effects on the use of medicines derived from these endangered species. The rapid dying out of the periwinkle plant illustrates the negative result of deforestation.



Vinblastine
(from *Catharanthus roseus*)



Vincristine
(from *Catharanthus roseus*)

Both Vinblastine and Vincristine (Neuss,

et al., 1964), derivatives of the periwinkle plant, have proved to be effective against tumor growth. Vinblastine has been shown to be a very effective treatment for Hodgkin's disease, and has also been used to treat breast cancer, Kaposi's sarcoma, and other diseases. Vincristine is well known for its 90% success rate in treating different types of childhood leukemia. Extinction of the periwinkle plants as a result of deforestation would thus result in a loss of treatments which have proven effective in treating certain illnesses.

Throughout the ages, humans have exploited the cornucopia of nature as a source of medicines for the treatment of a variety of diseases. Plants have formed the basis for traditional medicine for thousand of years. It is clear that demand for drugs, disposable consumer products, biological agents and insecticides will continue to increase for the foreseeable future. But unless we take specific action to protect and develop our environment under sustainable conditions, the window of opportunity for the discovery of new medicinal and biological agents will be shut forever.

Importance of natural products in drug development

In industrialized nations at the present time, some fifty percent of all prescribed drugs are derived or synthesized from natural products, the only available sources for which are animals, marine, plants, and micro-organisms. It is considered that because of the structural and biological diversity of their constituents, terrestrial plants offer a unique and renewable resource for the discovering of potential new drugs and biological entities. However, only 5,000 of the world's 250,000 flowering plants have as yet been analysed for their possible medicinal uses (Balandrin, *et al.*, 1993). The most alarming cause for concern is that, by the turn of this century, it is expected that some 25,000 species of plants will have ceased to exist. This represents about five plant species a day between now and the year 2000. Moreover, in developing countries, medicinal plants continue to be the main source of medication. In China alone, 7,295 plant species are utilized as medicinal agents. The World Health Organization has esti-

mates that for some 3.4 billion people in the developing world, plants represent the primary source of medicine. This represents about 88% of the world's inhabitants who rely mainly on traditional medicine for their primary health care (Farnsworth, *et al.*, 1977, 1985). It is thus a matter of utmost concern to public health and indeed to human life that urgent action is taken to prevent further diminution of actual and potential availability of medicinal and biological agents (O'Niell, *et al.*, 1993).

Natural remedies that, although undocumented, may have been used for many thousands of years by the human race must be appropriately catalogued to ensure that vital ethnomedical information is not lost forever. Our country, as a tropical country, has long enjoyed the luxury of an innumerable variety of tropical plants. In this regard, the Thais have a long tradition of folklore medicine, utilizing alleged medicinal herbs and plants. However, most of these "claimed" curative properties have neither been scientifically proved nor properly investigated and it is not uncommon to find a herb cited as a cure for "all" ailments. It is apparent that expeditious research is urgently needed to explore the potential of these natural products before opportunities become forever lost through advancing deforestation.

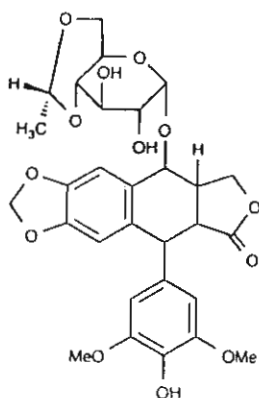
Farnsworth reported that at least 119 compounds derived from 90 plants species can be considered as important drugs currently in use in one or more countries, with 77% of these being derived from plants used in traditional medicine. The importance of natural products is also evidenced by the fact that in 1991 nearly half of the best selling drugs were either natural products or their derivatives. Newman and Cragg (1997) of NCI reported some interesting statistics that the new approved drugs during the period of 1983 to 1994 consisted of 30 drugs derived from unmodified natural products and 127 from modified natural products (i.e. semisynthetics) and 289 compounds derived from synthesis. In the new approved drugs for all disease types those derived from synthesis are approximately twice the number of those derived from natural products both modified and unmodified. However, of the 93 new approved anti-infective drugs, 7 are the unmodified natural pro-

ducts and 45 are the modified natural products giving 63% of drugs derived from natural sources. The same trend is observed in the cancer area where the number of anticancer drugs derived from natural products both modified and unmodified is higher than the synthetic drug i.e. 62% of the 87 approved anticancer drugs. It is very interesting to note that for the pre-new drug application anticancer drugs show a high number of 50 compounds from natural sources indicating the increasing importance of natural products as anticancer agents.

It is my conviction that research on natural products is still most worthwhile despite some sign that the interest in natural product research is waning with the explosive growth of combinatorial chemistry. It is obvious from the above statistics that natural products play a major role as drugs for the treatment of cancer and infectious diseases.

Natural products as anticancer agents

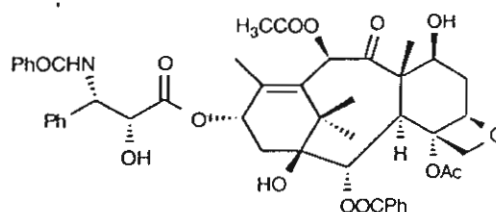
Apart from being an excellent source of anti-infectious drugs (Mahidol, *et al.*, 1997; Phillipson, *et al.*, 1991), plants are also good source of anticancer agents (Cordell, *et al.*, 1993; Hostettmann, *et al.*, 1991, 1998; Kinghorn, *et al.*, 1995; Pezzuto, 1996; Suffness, *et al.*, 1995; Valeriote, *et al.*, 1995.) National Cancer Institute (NCI), USA has launched an extensive program for the development of natural products for the treatment of various forms of cancer. Many clinically useful drugs have been discovered from various plants.



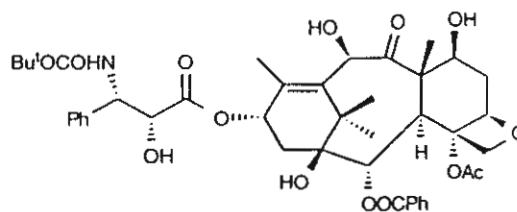
Etoposide

These include the earlier mentioned vin-

blastine and vincristine from *Catharanthus roseus*, and the semisynthetic, etoposide, as well as the recently discovered taxol and the semisynthetic taxotere.



Taxol
(from *Taxus brevifolia*)

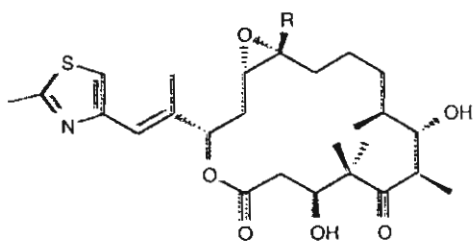


Taxotere

Taxol (paclitaxel) has been isolated from the bark of the Pacific or American yew tree, *Taxus brevifolia*. The discovery of taxol has indeed heightened the interest in plant-derived anticancer drug. Discovered in 1962, Taxol appears to be an exceptionally promising drug. It exhibited a very broad spectrum of activity against leukaemias and solid tumors and taxol has been found to be beneficial in treatment of refractory ovarian, breast, and other cancers and the drug was recently approved for marketing. It is estimated that four mature yew trees which take about 100 years to reach full maturity are needed to produce enough taxol to treat a single case of ovarian cancer. At present, although the synthesis of this extremely complicated taxol molecule has been accomplished, it has not yet become commercially feasible. It is likely that taxol will be produced semisynthetically for commercial production using intermediates related to baccatin III thus avoiding direct extraction of the plants.

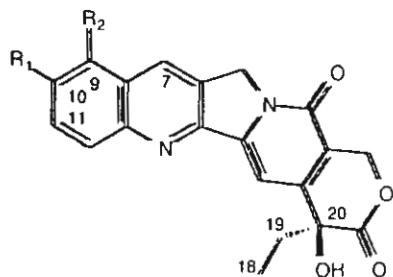
The addition of taxol to the list of anticancer

drug is testimony to the synergism of broadly based contributions from multidisciplinary scientific endeavour. The isolation and structure elucidation led the way to the pharmacological and toxicological testings. The finding of baccatin III from other phytochemical sources coupled with the synthetic organic chemistry make the drug available for human trials.



Epothilone

The finding that taxoids act through the stabilization of microtubules has led to the search of new agents that function by a comparable mechanism. Towards this end, new compounds have been discovered. Epothilones are a new class of macrocyclic natural products which were first isolated from myxobacteria (Gerth, *et al.*, 1996). Epothilones (Finlay, 1997) are more potent than taxol in some cell lines and they hold great promise for further investigation. Like taxol, epothilones have captured the interest of many synthetic organic chemists and the syntheses of these compounds have recently been accomplished.

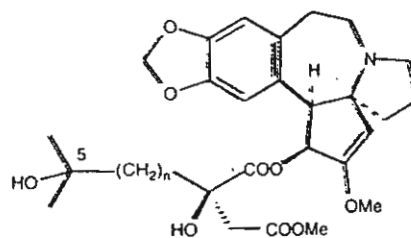


Camptothecin $R_1 = R_2 = H$

Topotecan $R_1 = OH, R_2 = -CH_2 - NMe_2$

In addition to the above mentioned clinically approved drugs and promising drug candidates,

some other plant-derived compounds show a great deal of promise for future use as anticancer agents. Camptothecin (Wall *et al.*, 1966) was originally isolated from the Chinese tree *Camptotheca acuminata* and a number of camptothecin analogues (Wall, *et al.*, 1993) are currently being developed as anticancer agents. Camptothecin was established as having *in vivo* activity against the murine leukemia and rat Walker carcinosarcoma 256 models. While early clinical trials on the parent alkaloid and camptothecin sodium were not particularly successful due to toxicity problems, interest in camptothecin intensified once it was discovered that it exhibits a novel mechanism of action by inhibiting the enzyme DNA topoisomerase I. Accordingly, a number of camptothecin analogues have been developed in an attempt to reduce toxicity, optimize efficacy, and improve water solubility without opening the lactone ring present in the parent molecule. Topotecan is one of the camptothecin analogue which is under clinical trial.



$n = 2$

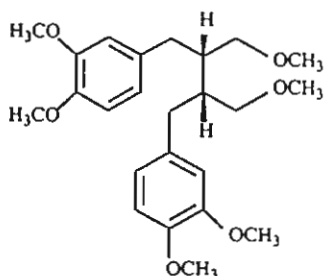
Harringtonine

Another plant derived alkaloid which is also under clinical trial is homoharringtonine, a cephalotaxine alkaloid (Feldman, *et al.*, 1992; Powell, *et al.*, 1972). The compound was originally isolated from *Cephalotaxus harringtonia*, it shows anti-neoplastic activity, especially against murine lymphocytic leukaemias. It was found to be more active than Vincristine against mouse leukaemias and melanomas.

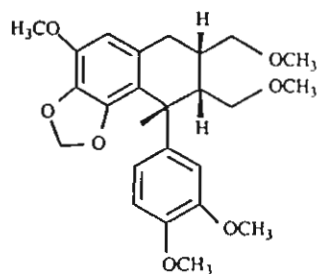
Chulabhorn Research Institute has initiated "The Cancer Research Programme" as one of the comprehensive research programmes. This particular programme encompasses the study of the mechanisms of carcinogenesis, modifiers and risk

factors in carcinogenesis, identification of specific tumor markers, development of monoclonal antibody for early detection of certain tumors, and screening of Thai medicinal plants for anticancer properties. At Natural Product Laboratory of CRI, the plant *Phyllanthus amarus* Schum. & Thonn. of the *Euphorbiaceae* family, locally known as Look Tai Bai, has been investigated for cytotoxicity activity, in collaboration with Professor Geoffrey Cordell of the University of Illinois at Chicago.

Phyllanthus amarus (Somanabandhu, *et al.*, 1993) has been traditionally used for the treatment of jaundice and other hepatic diseases. Although the antihepatotoxic potential of the plant has been controversial, the major chemical components were known to be *Phyllanthin* and *Hypophyllanthin*, with the structures as shown.



Phyllanthin



Hypophyllanthin

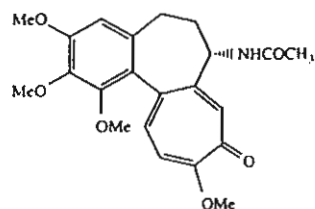
Apart from the structural studies, the biological activities of these compounds have also been investigated.

Apart from *Phyllanthus amarus*, we have also investigated *Gloriosa superba* Linn. for anticancer activity. *Gloriosa superba* Linn. is known in Thai as "Dong Dueng" or "Dao Dueng", a climber plant

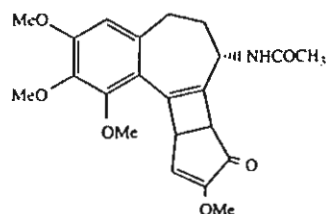
in the family "Colchicaceae", which is widely distributed in the tropical plants of Asia and Africa, with many varieties presented in Thailand.

The active principle of *Gloriosa superba* is the alkaloid "Colchicine", which is isolated from dried tuber of the plant. The alkaloid colchicine, itself has long been used for the treatment of arthritis.

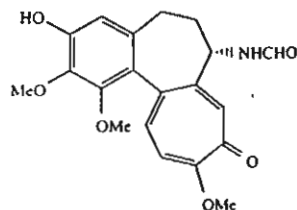
From the dried tuber of *Gloriosa superba*, four tropolone alkaloids (Bentley, 1998; Capraro, *et al.*, 1984; Engprasert, 1995) were isolated, and the structures are as shown.



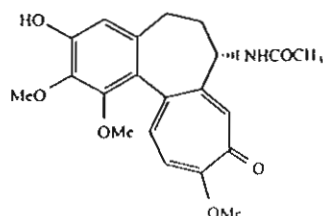
Colchicine



Lumicolchicine



3-Demethyl-N-Formyl-N-Deacetylcolchicine



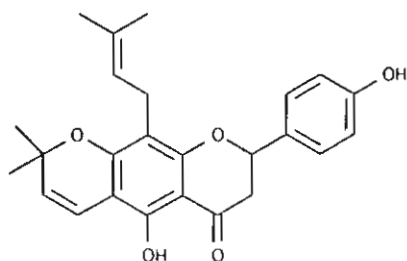
3-Demethylcolchicine

The structures were elucidated using various spectroscopic techniques, such as UV, IR, MS, one- and two dimensional NMR. The cytotoxicity testings of various colchicines with various cell lines have also been established.

Another cancer cell line of our interest is the Cholangiocarcinoma cell lines. Cholangiocarcinoma, a form of bile duct cancer, is a rare type of cancer in the Western world but it is highly prevalent in Thailand and in many other Asian countries in our geographical region. The cause of the disease is believed to be associated with infestation of *Opisthorchis viverrini* (O.V.) or liver fluke and exposure to a chemical carcinogen in food or in the environment, presumably, dimethylnitrosamine (DMN).

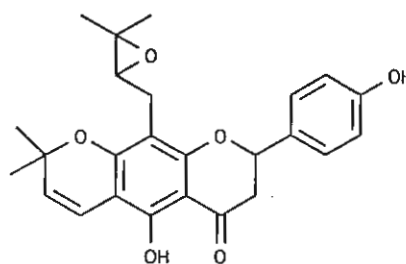
At CRI, evaluation of the effectiveness of some new anticancer agents against Cholangiocarcinoma was conducted. This process was carried out by the *in vitro* testing of the cholangiocarcinoma (HuCCA-1) cell line (Sirisinha, *et al.*, 1991, 1993) that was previously established in the Laboratory of Immunology. Some Colchicine derivatives have been subjected to cytotoxicity testing, using micro-culture protein assay.

We have recently investigated another plant called *Derris reticulata* locally known as Cha-aim thai (Mahidol, *et al.*, 1997). Cha-aim thai is a medicinal plant of Thailand used for the relief of thirst and as an expectorant. Our studies led to the isolation of two new pyranoflavanone compounds. Lupinifolin, a known flavanone was isolated as the major constituent of this plant. Detailed analysis of NMR spectra through COSY, NOSEY, APT, HETCOR and selective INEPT confirmed the structure of Lupinifolin as the flavanone derivative as shown.



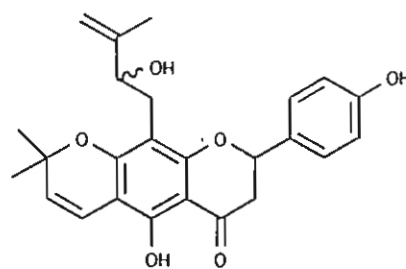
Lupinifolin

The first unknown isolated, named epoxyepinifolin, was proved to be the 2,3 epoxide of lupinifolin by spectroscopic methods. The structure of the epoxide was further confirmed by successful epoxidation of lupinifolin with magnesium monoperoxyphthalate hexahydrate (MMPP)



epoxyepinifolin

The other new compound isolated was named dereticulin and the structure was found to be hydroxy derivatives through the analysis of the NMR spectra of the corresponding triacetate



Dereticulin

We have also carried out the *in vitro* bio-assay evaluation of lupinifolin, epoxyepinifolin and dereticulin triacetate. The results are shown that each of them inhibited the P-388 cell line at 0.4-0.5 microgramme per ml, but were inactive against the KB cell line.

Conclusion

Presently we are seeing a selfish drive to achieve material wealth at the expense of the earth's biological resources. There is no doubt that preserving these resources is of paramount import-

ance. The question thus becomes how could we achieve the goal. It seems ironic that while patent laws protect corporations as well as individuals from having their ideas and inventions stolen, nature's biodiversity and ethnomedical knowledge are largely unprotected. Ethnomedical knowledge has been passed down from generation to generation and now is considered an important factor in the discovery of new drugs and biological agents. This knowledge will be irretrievably lost without the sustainable development of the tropical rain forests. To rectify this problem, the pharmaceutical community needs to accept the responsibility of paying equitable compensation to the source country for discoveries of biological medicines which lead to profit. On a small scale, this has already begun. For example, Merck Research Laboratories is paying compensation in order to receive greater access to medicinal plants in Costa Rica. The United States National Cancer Institute has also agreed to pay compensation if they make discoveries which result in profit. Another point of equal importance is better enforcement of uncontrolled deforestation practices. This may involve helping to provide alternative work for individuals involved in the timber trade as well as enactment of more careful control over endangered areas. Along this line, the Thai Government and the Thai Royal Family are striving to reach the goal. Also international agreements for open access to native resources along with reasonable compensation for this access must be made.

Now is the time to accept responsibility of taking care of the earth's natural resources; otherwise, it will be too late -- many valuable resources have already been lost or are being lost even as we discuss this matter. Every minute we wait to act we lose species diversity and knowledge of the uses of plants and animals -- two of our planet's most valuable resources.

Acknowledgements

One of us (S.R.) is grateful to Thailand Research Fund (TRF) for the award of "TRF Senior Research Scholar".

References

- Balandrin, M.F., Kinghorn, A.D. and Farnsworth, N.R. 1993. In Human Medicinal Agents from Plants (Kinghorn, A.D. and Balandrin, M.F. eds.), Symposium Series No. 534, American Chemical Society, Washington, D.C. 2-12.
- Bentley, K.W. 1998. In The Isoquinoline Alkaloids, chapter 20, 389-445. Harwood Academic Publishers
- Capraro, H.G. and Brossi, A. 1984. In The Alkaloids (Brossi, A. ed.) 23 : 1-70. Academic Press
- Chivian, E. 1993. In Critical Condition : Human Health and the Environment (Chivian, E., McCally, M., Hu, H. and Haines, A. eds.) M.I.T. Press.
- Cordell, G.A., Farnsworth, N.R., Beecher, C.W.W., Soejarto, D.D., Kinghorn, A.D., Pezzuto, J.M., Wall, M.E., Wani, M.C., Brown, D.M., O'Neill, M.J., Lewis, J.A., Tait, R.M., and Harris, T.J.R. 1993. In Human Medicinal Agents from Plants (Kinghorn, A.D. and Balandrin, M.F. eds.), Symposium Series No. 534, American Chemical Society, Washington, D.C. 193-204.
- Cragg, G.M., Newman, D.J. and Snader, K.M. 1997. J. Nat. Prod., 60 : 52-60.
- Daly, J.W. 1998. J. Nat. Prod., 61 : 162-172.
- Engprasert, S., thesis, M.Sc. 1995. Mahidol University.
- Farnsworth, N.R., Akerele, O., Bingel, A.S., Soejarto, D.D. and Guo, Z. 1985. Bull. WHO 63 : 965-981.
- Farnsworth, N.R. and Bingel, A.S. 1977. In New Natural Products and Plant Drugs with Pharmacological, Biological or Therapeutic Activity (Wagner, H. and Wolff, P. eds), 61-73. Springer, New York.
- Feldman, E., Arlin, Z., Ahmed, T., Mittelman, A., Pucchio, C., Chun, H., Cook, P. and Baskind, P. 1992. Leukemia, 6 : 1189-1191.
- Finlay, R. 1997. Chem. Ind. (London), 24 : 991-996.
- Gerth, K., Bedorf, N., Hofle, G., Irschik, H. and Reichenbach, H. 1996. J. Antibiot., 49 : 560-563.
- Hamburger, M. and Hostettmann, K. 1991. Phytochemistry, 30 : 3864-3874.
- Hostettmann, K., Poterat, O. and Wolfender, J.L. 1998. Chimia, 52 : 10-17.
- Kinghorn, A.D., Farnsworth, N.R., Beecher, C.W.W., Soejarto, D.D., Cordell, G.A., Pezzuto, J.M., Wall, M.E., Wani, M.C., Brown, D.M., O'Neill, M.J., Lewis, J.A. and Besterman, J.M. 1995. Int. J. Pharmacog., 33 : 48-58.
- Mahidol, C., Prawat, H., Ruchirawat, S., Likhitwitayawuid, K., Long-ZeLin and Cordell, G.A. 1997. Phytochemistry, 45 : 825-829.

- Mahidol, C., Prawat, H. and Ruchirawat, S. 1997. In *Biocative Natural Products from Thai Medicinal Plants*, chapter "Phytochemical Diversity : A Source of New Industrial Products", published by The Royal Society of Chemistry, RSC, England 96-105.
- Neuss, M., Gorman, M., Hargrove, W., Cove, N.J., Biemann, K., Buchi, G., and Manning, R.E. 1964. *J. Am. Chem. Soc.*, 86 :1441.
- O'Niell, M.J., and Lewis, J.A. 1993. In *Human Medicinal Agents from Plants* (Kinghorn, A.D. and Balandrin, M.F. eds.), Symposium Series No. 534, American Chemical Society, Washington, D.C. 48-55.
- O'Niell, M.J., Lewis, J.A. and Besterman, J.M. 1995. *Int. J. Pharmacog.*, 33 : 48-58.
- Pezzuto, J.M. 1996. *Biochemical pharm.*, 53 : 121-133.
- Phillipson, J.D., and Wright, C.W. 1991. *Planta Med.*, 57 : S53-59.
- Powell, R.G., Weisleder, D. and Smith, Jr. C.R. 1972. *J. Pharm. Sci.*, 61 : 1227-1230.
- Sirisinha, S., Petmir, S., Utaisincharoen, P. and Tengchaisri, T. 1993. *Asian J. Surg.* 19 :198-206.
- Sirisinha, S., Tengchaisri, T., Boonpucknavig, S., Prempracha, N., Ratanarapee, S. and Pausawasdi, A. 1991. *Asian Pacific J Allergy Immunol.*, 9 153-157.
- Somanabandhu, A., Nitayangkura, S., Mahidol, C., Ruchirawat, S., Likhitwitayawuid, K., Shieh, H.L., Chai, H., Pezzuto, J.M. and Cordell, G.A. 1993. *J. Nat. Prod.*, 56 : 233-239.
- Suffness, M., Cragg, G.M., Grever, M.R., Grifo, F.J., Johnson, G. Mead, J.A.R., Schepartz, S.A., Venditti, J.M. and Wolpert, M. 1995. *Int. J. Pharmacog.*, 33 : 5-16.
- Valeriote, F., Corbett, T., LoRusso, P., Moore, R.E., Scheuer, P., Patterson, G., Paul, V., Grindey, G., Bonjouklian, R., Pearce, H. and Suffness, M. 1995. *Int. J. Pharmacog.*, 33 :59-66.
- Wall, M.E., and Wani, M.C. 1993. In *Human Medicinal Agents from Plants* (Kinghorn, A.D. and Balandrin, M.F. eds.) Symposium Series No. 534, American Chemical Society, Washington, D.C. 149-169.
- Wall, M.E., Wani, M.C., Cook, C.E., Palmer, K.H., McPhail, A.T. and Sim, G.A. 1966. *J. Am. Chem. Soc.*, 88 : 3888-3890.

การเสนอผลงานในที่ประชุมวิชาการนานาชาติ

EFFICIENT SYNTHESSES OF THE POLYCYCLIC ISOINDOLONE ALKALOIDS

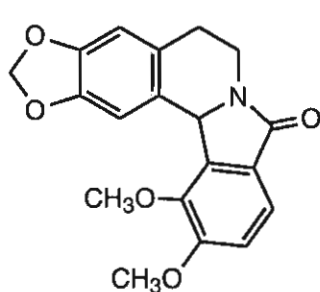
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¹Chulabhorn Research Institute, Vibhavadee-Rangsit Highway,
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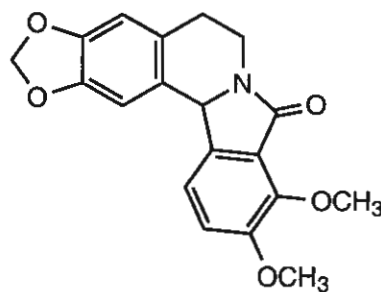
²Department of Chemistry, Faculty of Science and Chulabhorn Research Centre,
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(±)-Nuevamine was recently isolated from *Berberis darwinii* Hook, procured in southern Chile. It was the first isoindoloisoquinoline alkaloid isolated from natural source. Castedo *et al.*¹ reported the assignment of a revised structure (1) for the alkaloid (±)-nuevamine from the originally proposed isomeric structure (2).

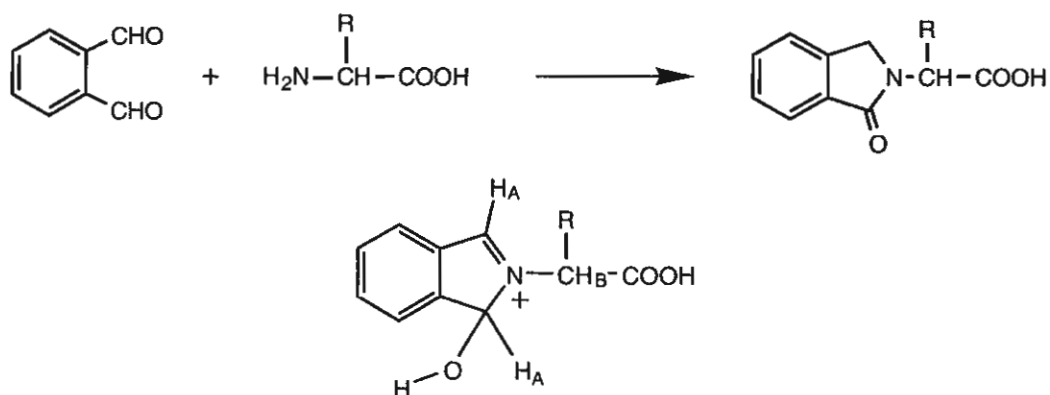


(1)

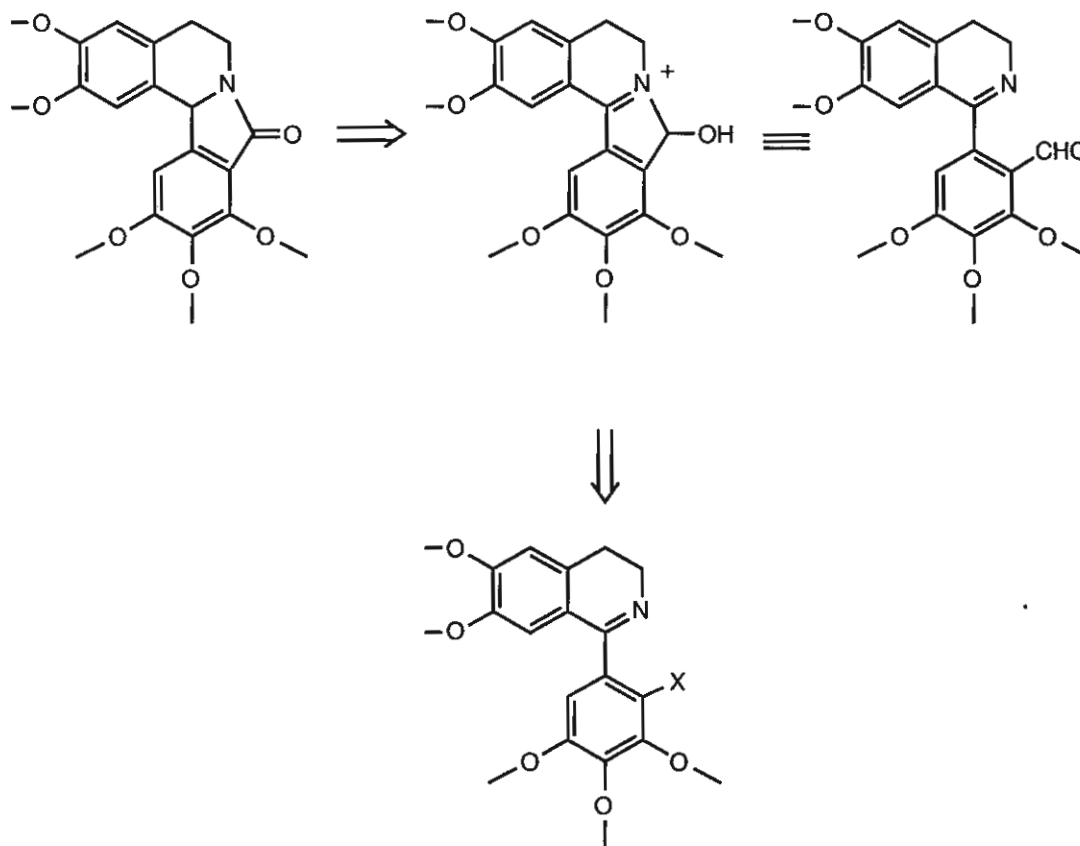


(2)

The novelty of nuevamine skeleton prompted us to seek a synthetic route to this type of the isoquinoline alkaloids. To this end, we exploited Grigg's finding to synthesize the compound. In 1985, Grigg *et al.*² reported a reaction of o-phthalaldehyde with α-amino acids in boiling acetic acid to give N-substituted isoindolin-1-ones in good yields. The reaction was proposed to pass through isoindolinol intermediate

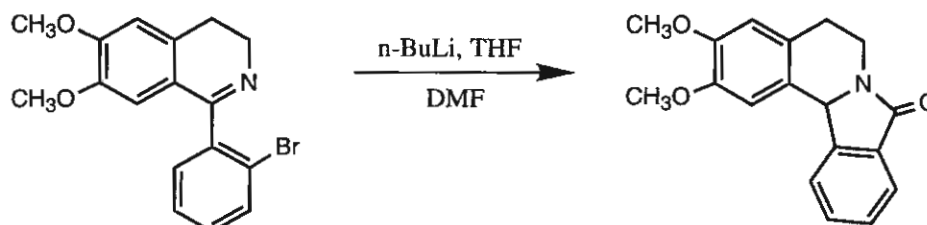


It occurred to us that we could exploit this finding for the synthesis of nuevamine and other isoindoloisoquinoline alkaloids. The retrosynthetic analysis of the synthetic approach is outlined.

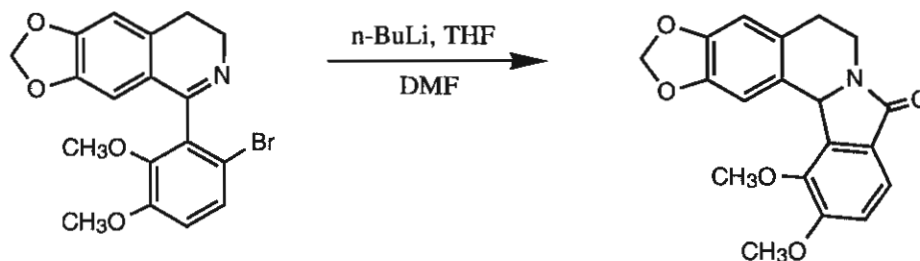


The isoindolone could be envisaged to arise from the isoindolinol. The synthetic equivalent of the isoindolinol is the amino-aldehyde. With the above idea in mind, we then tested the feasibility of such a proposal.

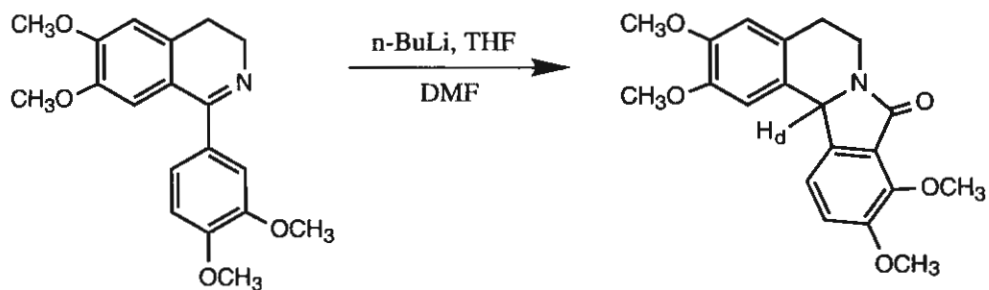
Reaction of 1-(2-bromophenyl)-3,4-dihydro-6,7-dimethoxyisoquinoline with *n*-butyllithium in tetrahydrofuran at -78°C under nitrogen atmosphere gave the carbanion which was then reacted with *N,N*-dimethylformamide to give a product in 41% yield. It was gratifying to find that the product was indeed the expected 2,3-dimethoxyisoindoloisoquinoline.



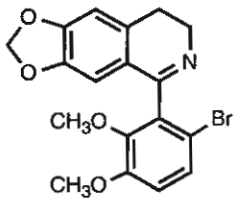
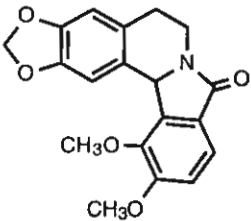
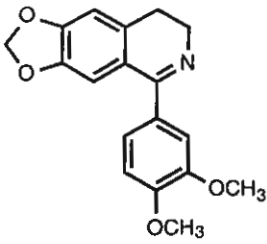
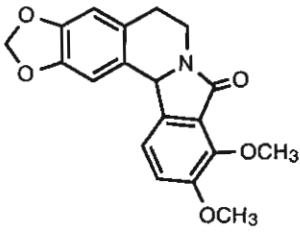
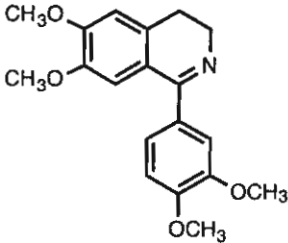
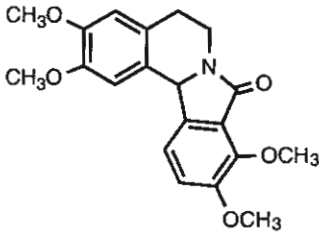
Similar reaction was also applied to other imine to give nuevamine directly in 53% yield.



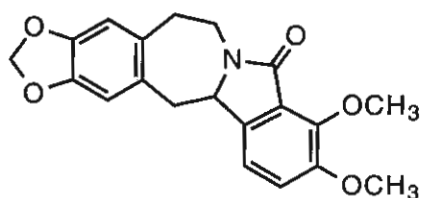
In the above approach the bromine atom was used as a means to direct the formyl group into a specific point i.e. specific formation of the aryl carbanion. However in some special cases specific lithiation can also be accomplished without the bromine atom to direct the exchange as shown.



The isoindoloisoquinoline derivatives that have been successfully synthesized by this route are shown in the table.

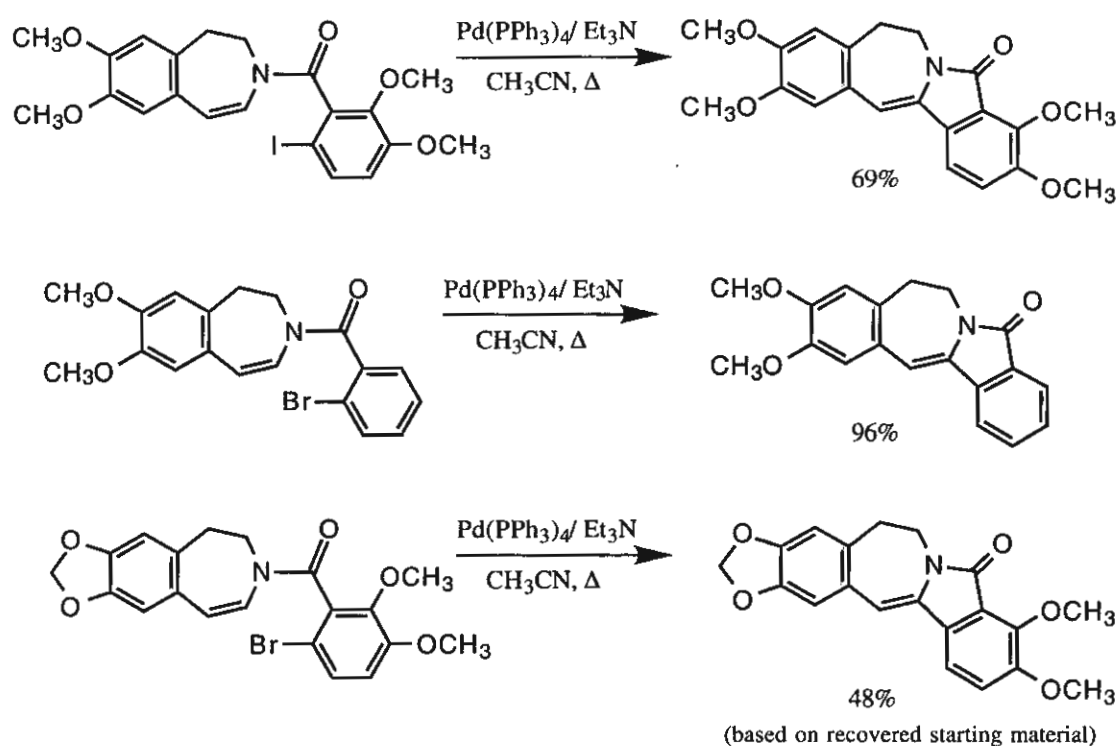
imine	isoindoloisoquinoline	yield (%)
		53
		53
		61

Lennoxamine is also the first representative of the isoindolobenzazepine alkaloids³. Both lennoxamine and nuevamine contain the unique isoindolone ring system in the molecules.

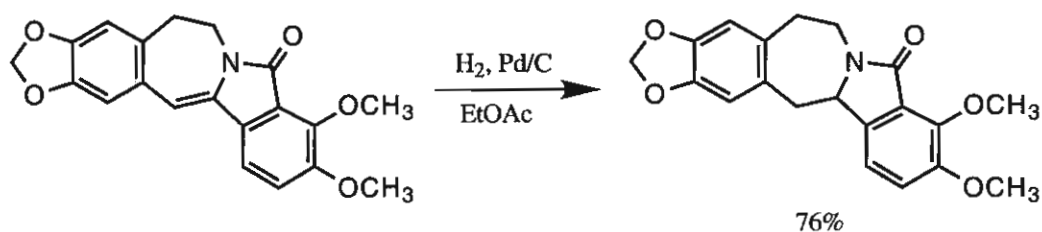


Lennoxamine

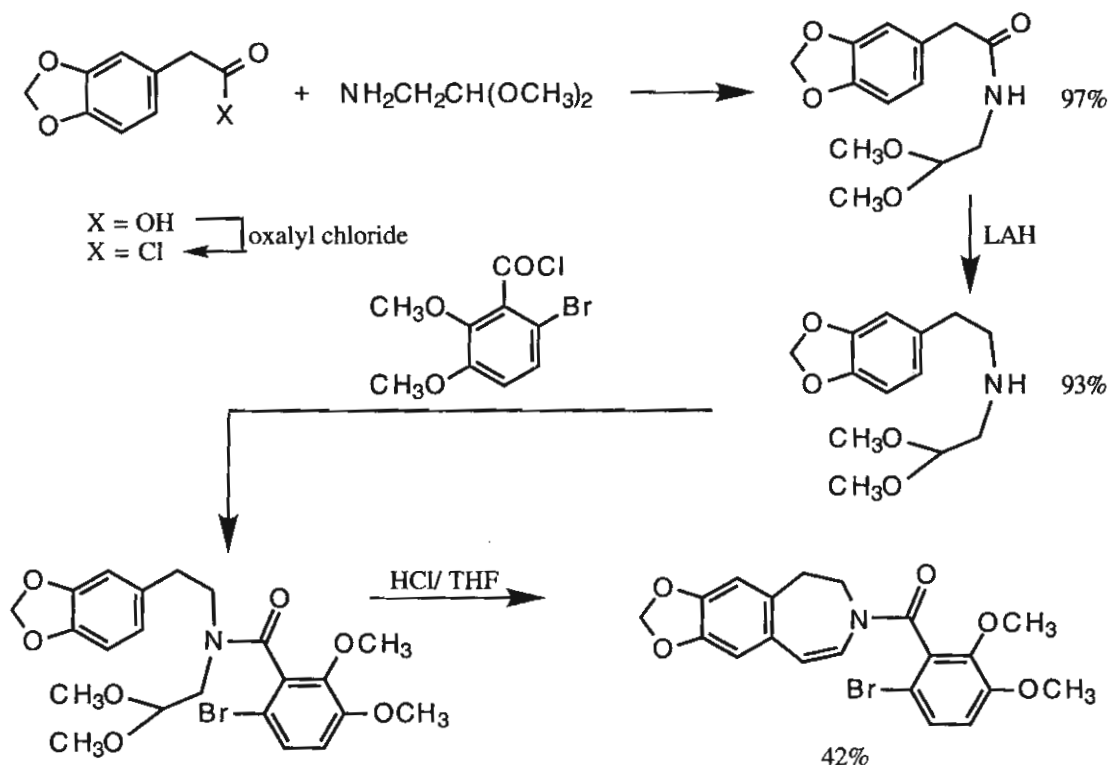
Our approach was based on the organopalladium^{4,5} mediated reaction and the key step in the approach is the Heck reaction and this is illustrated in the synthesis of lennoxamine analogue. Reaction of the halobenzazepine derivative with tetrakis(triphenylphosphine) palladium(0), triethylamine in acetonitrile, gave the isoindolobenzazepine intermediates after purification with PLC. The reaction can be extended to the synthesis of key intermediate for the synthesis of lennoxamine.



The product so obtained could be converted to lennoxamine by catalytic hydrogenation.



The sequence of the reaction in the synthesis of lennoxamine is shown below.



Thus we have devised a very efficient synthesis of lennoxamine and its analogues.

Acknowledgements: We are grateful to Thailand Research Fund (TRF) for the generous support for our research programme.

References

1. Castedo L., Alonso R., Dominguez D. **Tetrahedron Lett.** 1985; **26**(24): 2925.
2. Grigg R., Gunaratne H.Q.N., Sridharan V. **J. Chem. Soc., Chem. Commun.** 1985; 1183.
3. Fajardo V., Elango V, Cassels B.K and Shamma M, **Tetrahedron Letters**, 1982, 23(1), 39-42.
4. Cacchi S, Torre F.L and Palmieri G. **J. Organomet. Chem.**, 1984, 268: C48.
5. Suau R, Ruiz I, Posadas N and Valpuesta M, **Heterocycles**, 1996, 43(3), 545-550.

SYNTHESIS OF NAPHTHOL AND 1,3-DIMETHYLISOQUINOLINE DERIVATIVES

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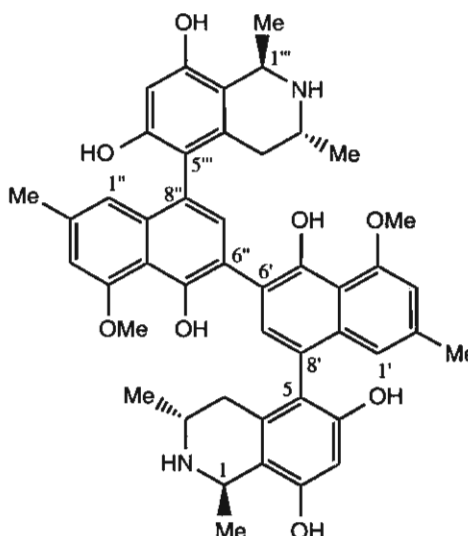
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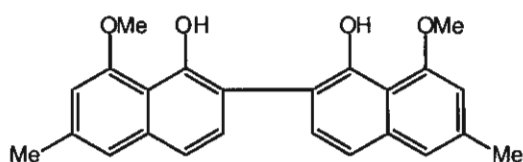
Introduction

Michellamines A (1)^{1,2}, B (2)^{1,2}, and C (3)² isolated from *Ancistrocladus korupensis*, constitute a family of anti-HIV, atropisomeric, naphthylisoquinoline alkaloids³. Michellamine B, the most studied and most prevalent of the group, completely protects MT-2 cells from both AZT-resistant and pyridone-resistant strains of HIV-1.

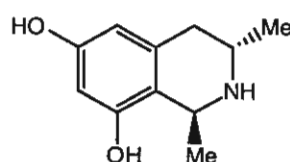
	Stereogenic Axis Configuration	
	5/8'	8''/5'''
1 Michellamine A	S	S
2 Michellamine B	S	R
3 Michellamine C	R	R



The structure of michellamine B is composed of 2 units, the binaphthol unit and the 1,3-dimethylisoquinoline unit as shown.



Binaphthol unit



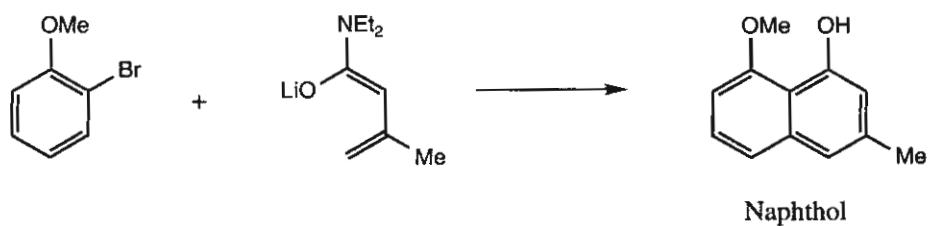
1,3-Dimethylisoquinoline unit

We have studied the synthetic routes leading to the binaphthol units as well as the 1,3-dimethylisoquinoline derivatives.

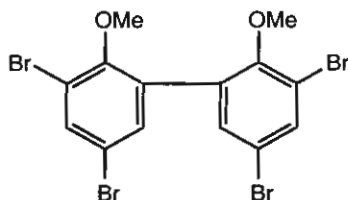
Results and Discussion

The binaphthol unit.

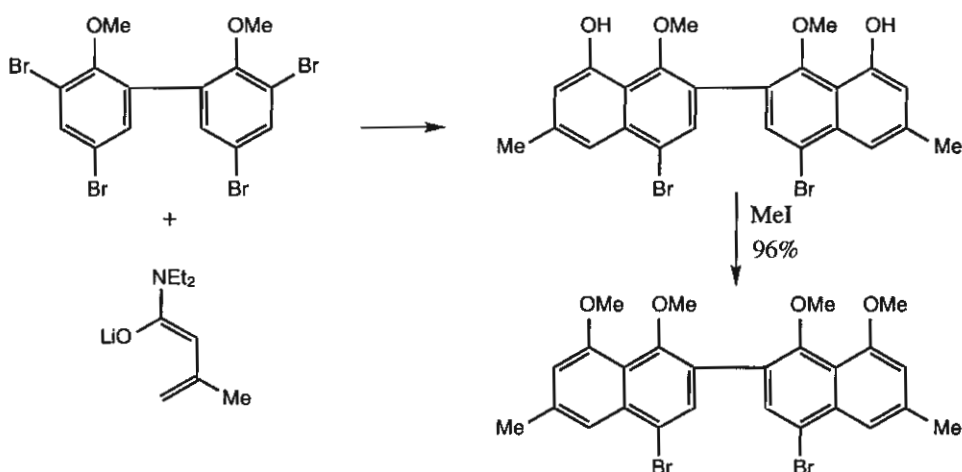
One synthetic route used for constructing a naphthol unit is the regiospecific benzyne annulation reaction. Treatment of o-bromoanisole with an excess of lithium cyclohexylisopropylamide and N,N-diethyl seneciamide gave naphthol⁴.



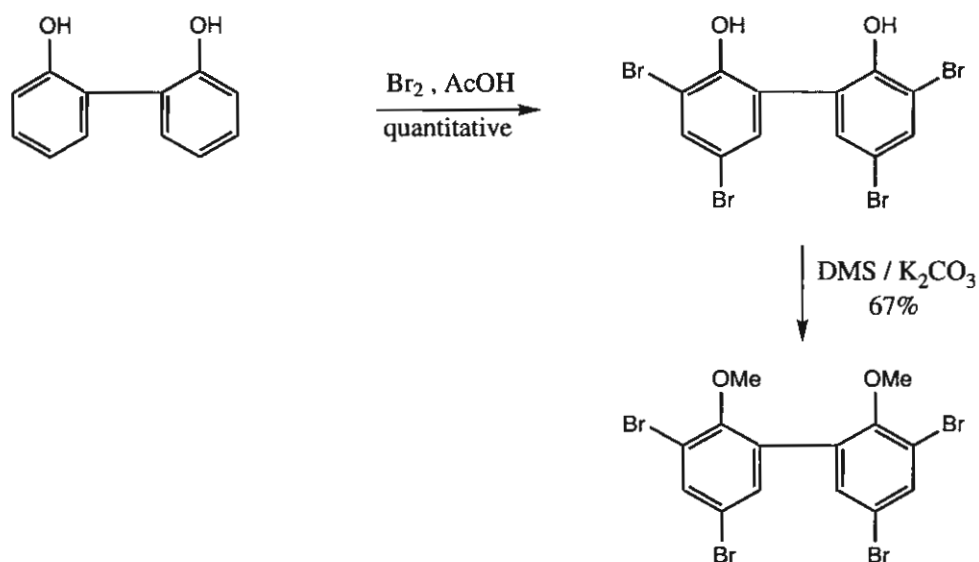
We have extended the above reaction to build up a binaphthol unit by starting from biphenyl derivative.



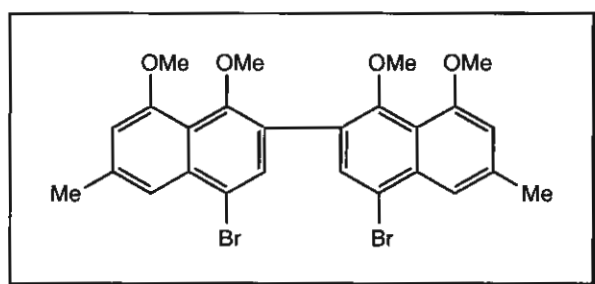
Reaction of biphenyl derivative with lithium enolate of N,N-diethyl seneciamide gave the required binaphthol in 20% yield.



Methylation of the resulting binaphthol gave dibromotetramethoxydiospyrol derivative. Biphenyl derivative can be easily prepared in 2 steps from biphenol.

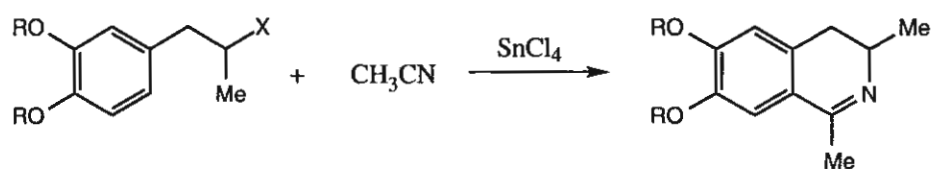


The dibromotetramethoxydiphenyl ether derivative so obtained contains a dibromo molecule in the right position which can be used as a handle for coupling with the 1,3-dimethylisoquinoline unit under metal-mediated coupling reaction.



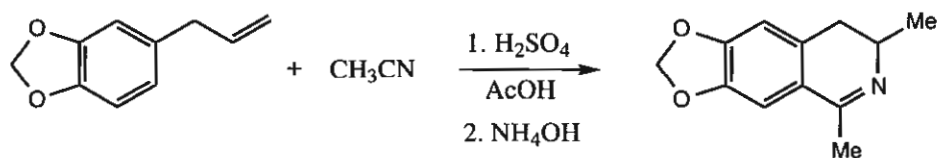
The 1,3-dimethylisoquinoline unit

Lora-Tamayo⁵ *et al* in 1961 reported a construction of 1,3-dimethyldihydroisoquinoline skeleton *via* Ritter reaction by treatment of β -haloethylbenzene and acetonitrile in the presence of SnCl_4 .



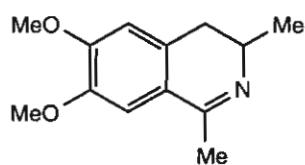
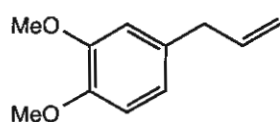
With the above idea, we planned to synthesize the 1,3-dimethyldihydroisoquinoline by treatment of the allylbenzene derivatives with acetonitrile in the presence of acid. Safrole was our first allylbenzene selected in the reaction.

Reaction of safrole with a mixture of conc. sulfuric acid and glacial acetic acid and acetonitrile (1:1:1) at 43°C overnight gave the expected 1,3-dimethyldihydroisoquinoline in 43% yield.

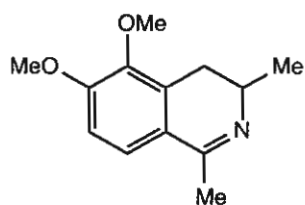
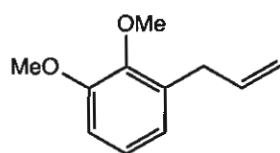


4 more non-phenolic allylbenzene were investigated and the result were summarized as shown.

Allylbenzene	Product	Yields(%)
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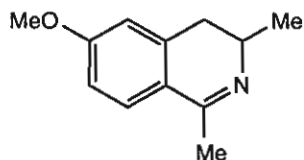
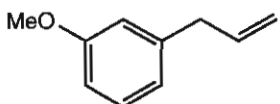


46

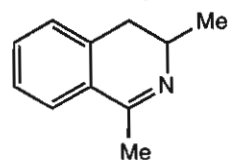
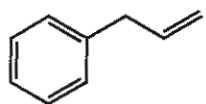


40

Allylbenzene	Product	Yields(%)
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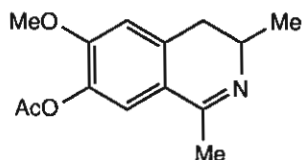
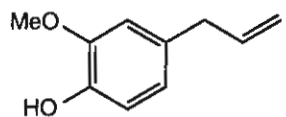


11



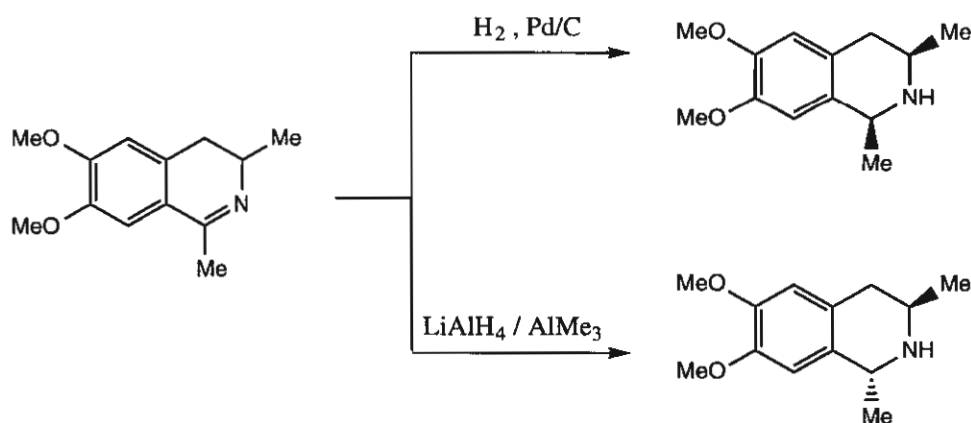
0

Phenolic allylbenzene was also studied.



36

The resulting 1,3-dimethyldihydroisoquinoline derivatives could be reduced to both cis- and trans- diastereomer depending on reagent used.^{6,7}



Conclusion

2-Units of michellamine derivatives, binaphthol unit and 1,3-dimethylisoquinoline unit, were successfully synthesized by simple and very short routes. Coupling of the two units under metal-assisted condition to give the michellamine derivatives will be further investigated.

Acknowledgement : We are grateful to Thailand Research Fund for generous support to our research programme.

References

1. Manfredi, K.P.; Blunt, J.W.; Cardellina, II, J.H.; McMahon, J.B.; Pannell, L.K.; Cragg, G.M.; Boyd, M.R. *J. Med. Chem.* **1991**, 34, 3402.
2. Boyd, M.R.; Hallock, Y.F.; Cardellina, II, J.H.; Manfredi, K.P.; Blunt, J.W.; McMahon, J.B.; Buckheit, R.W., Jr; Bringmann, G.; Schaffer, M.; Cragg, G.M.; Thomas, D.W.; Jato, J.G. *J. Med. Chem.* **1994**, 37, 1740.
3. For a review see: Bringmann, G.; Francois, G.; Assi, L.A.; Schlauer, J. *Chimia* 1998, 52, 18-28.
4. Watanabe, M.; Hisamatsu, S.; Hotokezaka, H.; Furukawa, S. *Chem. Pharm. Bull.* **1986**, 34, 2810-2820.
5. Lora-Tamayo, M.; Madronero, R.; Garcia Munoz, G.; Martinez Marzal, J.; Stud, M. *Chem. Ber.* **1961**, 94, 199-207.
6. Bringmann, G.; Jansen, J.R.; Rink, H.-P. *Angew. Chem. Int. Ed. Engl.* **1986**, 25, 913-915.
7. Hoye, T.R.; Chen, M. *Tetrahedron Lett.* **1996**, 37, 3099-3100.

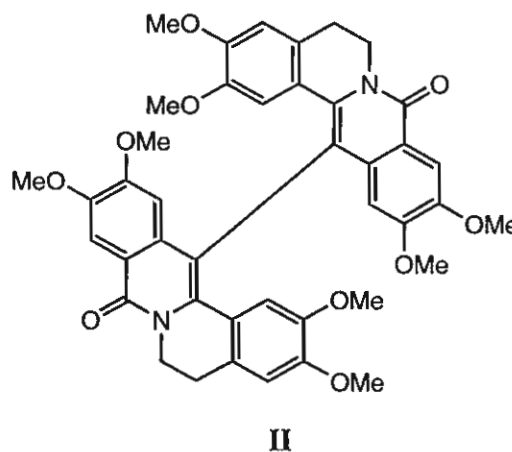
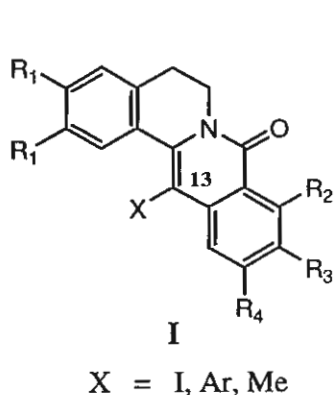
THE SYNTHESIS OF C-13 SUBSTITUTED PROTOBERBERINE ALKALOIDS

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Mahidol University, Bangkok THAILAND

The protoberberine alkaloids exhibit a broad array of biological activities.[1] The core unit of the protoberberine alkaloids has been synthesized by a wide variety of synthetic methods.[2] In order to increase the diversity of the structures of these alkaloid, we have developed different approaches to the syntheses of C-13 substituted protoberberine alkaloids (I) as well as the synthesis of Ilcifoline (II), a dimeric protoberberine alkaloids isolated from *Berberis ilicifolid*. [3]



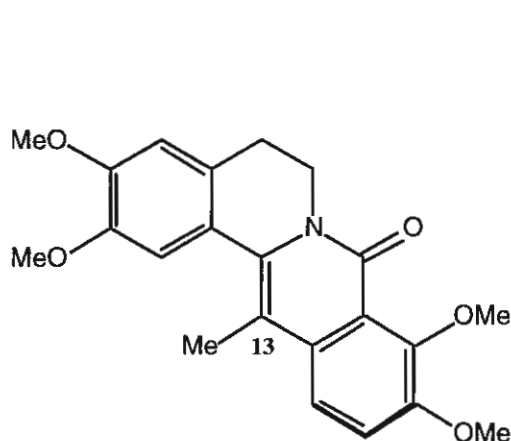
Acknowledgement : We are grateful to Thailand Research Fund for generous support to our research programme.

References :

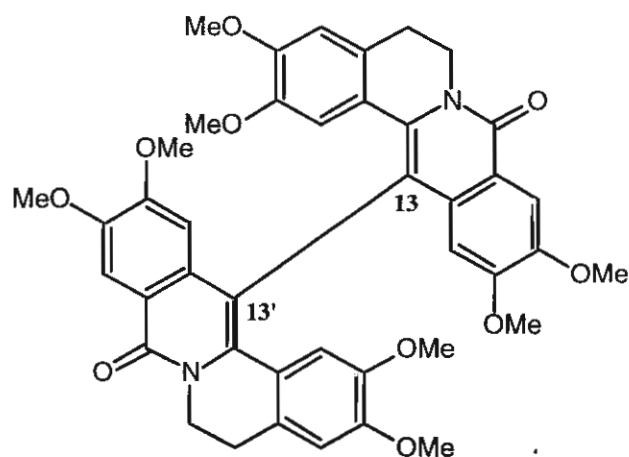
- [1] S.Simeon, J.L.Rios and A.Villar, *Plant Med. Phytother.*, 1989, **23**, 202.
- [2] D.A. Bhakuni and S.Jain, *The Alkaloids*, Academic Press, 1986, vol 28, p.95; M.Shamma and J.L.Moniot, *Isoquinoline Alkaloids Research 1972-1977*, Plenum Press, New York and London, 1978, p.209 ; M.Shamma, *The Isoquinoline Alkaloids, Chemistry and Pharmacology*, Academic Press, New York and London, 1972, p.268 ; Y.Kondo, *Heterocycles*, 1976, **4**, 197 ; K.W.Bentley, *The Isoquinoline Alkaloids*, Harwood Academic Publishers, 1998, p.219.
- [3] V.Fajardo, C.Carcamo and B.Moreno, *Heterocycles*, 1996, **43**(5), 949.

Introduction

Protoberberines are the isoquinoline derived alkaloids whose natural occurrences and pharmacological properties^{1,2} have been reviewed and the alkaloids continued to attract the interests of several groups. Yuanamide **1**, an oxodihydroprotoberberine alkaloid, was isolated from *Calydalis bulbosa* DC³. Recently, the new dimeric protoberberine, ilicifoline **2**, was isolated from *Berberis ilicifolia* (Berberidaceae)⁴.



Yuanamide
1



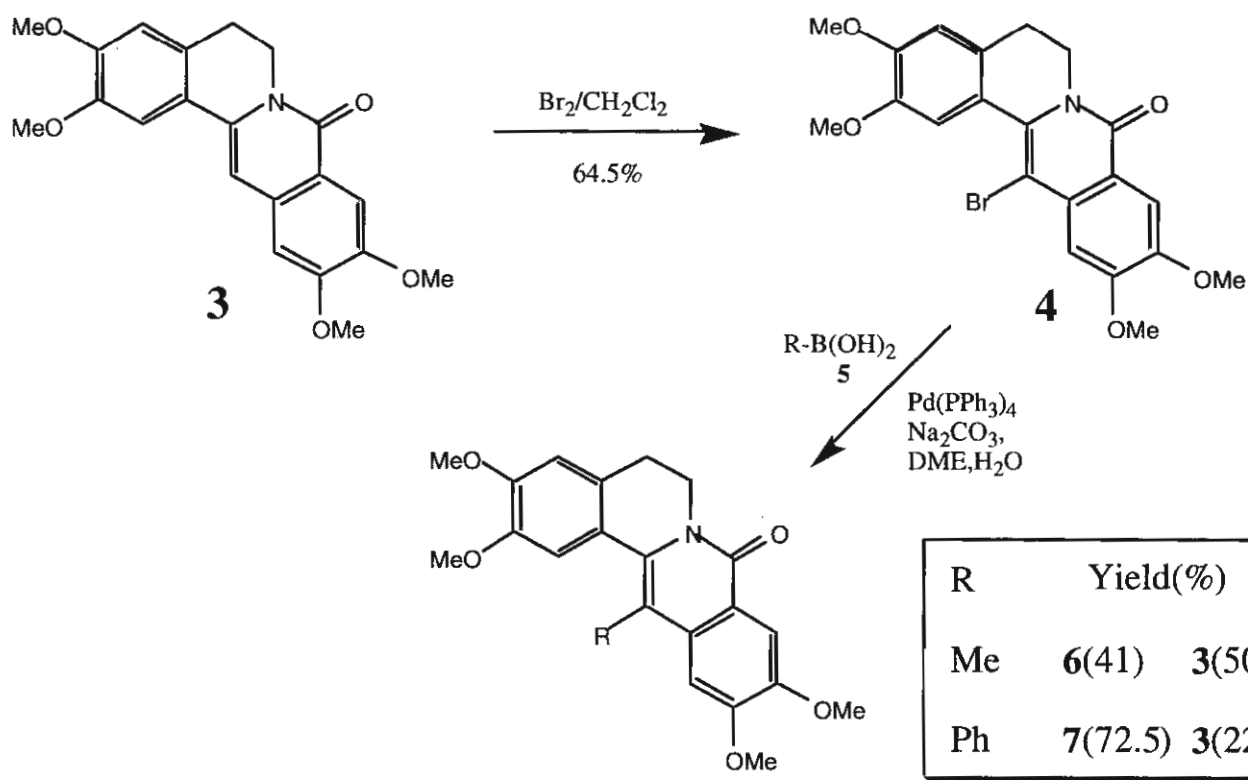
Ilcifoline
2

Both of these compounds are substituted at position 13. In order to increase the diversity of the structure of these alkaloids, we have developed different methodology to the syntheses of C-13 substituted protoberberine alkaloids.

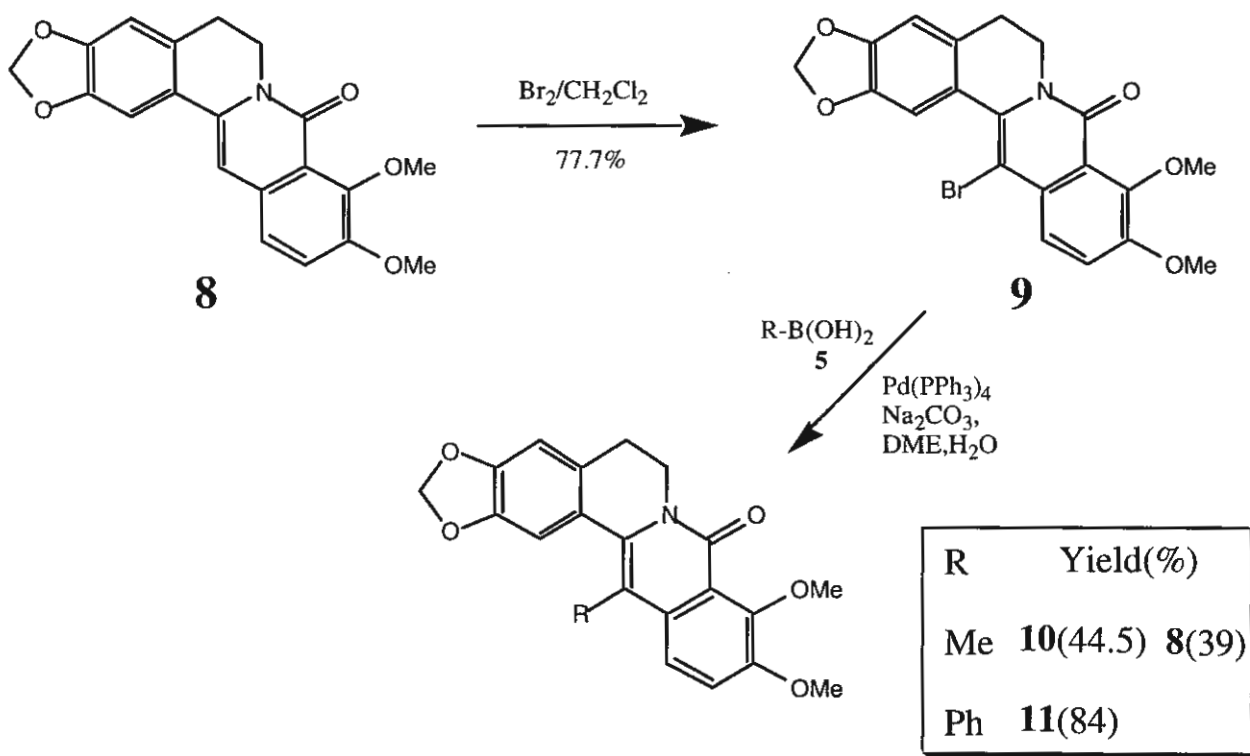
Results and Discussion

The Suzuki reaction has proved to be extremely versatile and has found extensive use in natural product synthesis. Boronic acids are the usual substrates in this reaction together with alkyl or aryl halides. To that end, the first stage of our approach was the synthesis of appropriate bromoprotoberberine. The brominated 8-oxopseudopalmatine **4** was obtained in 64.5 % yield by direct bromination ($\text{Br}_2/\text{CH}_2\text{Cl}_2$) of 8-oxopseudopalmatine **3**.

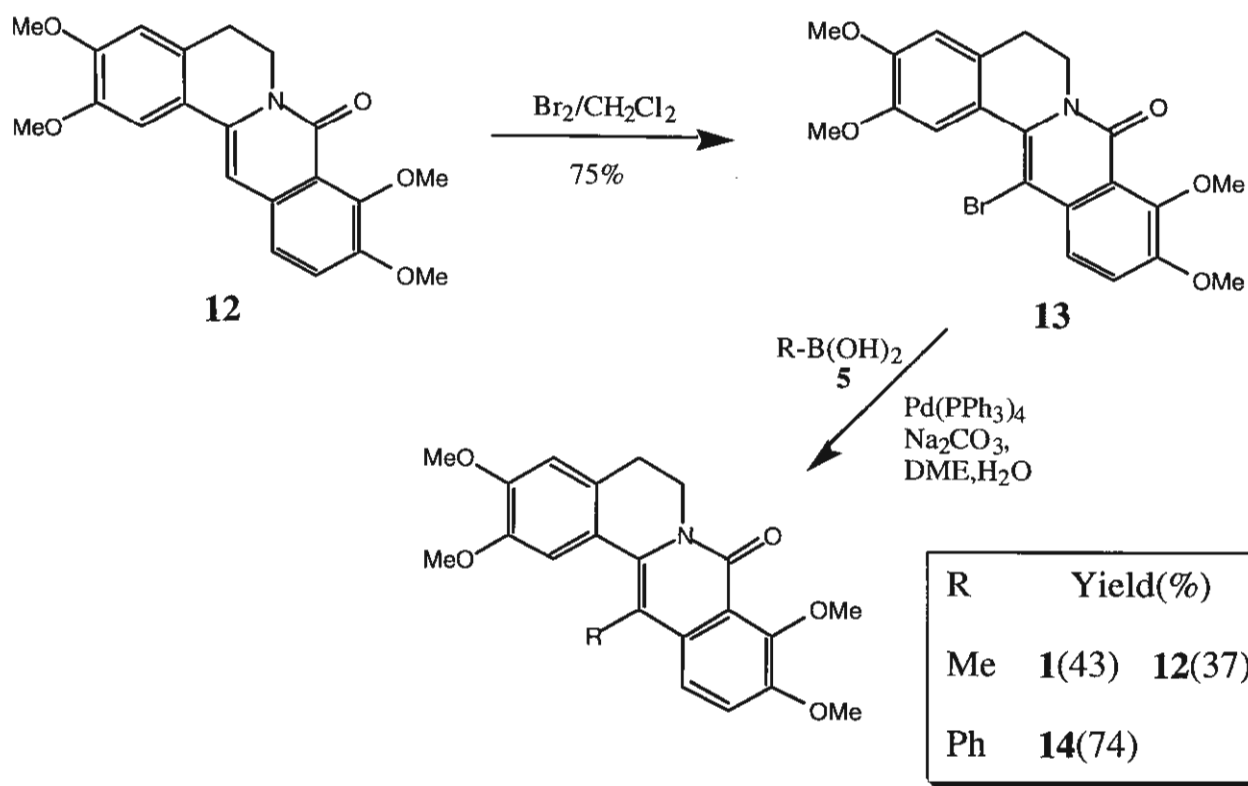
Having the required 13-bromo-8-oxopseudopalmatine **4** in hands, we were in the position to try Suzuki-type reaction. Coupling reaction between 13-bromo **4** and boronic acids **5** was carried out in the presence of tetrakis(triphenylphosphine)palladium (0) and sodium carbonate to afford 13-substituted-8-oxopseudopalmatine **6** and **7** in 41 and 72.5 % respectively.



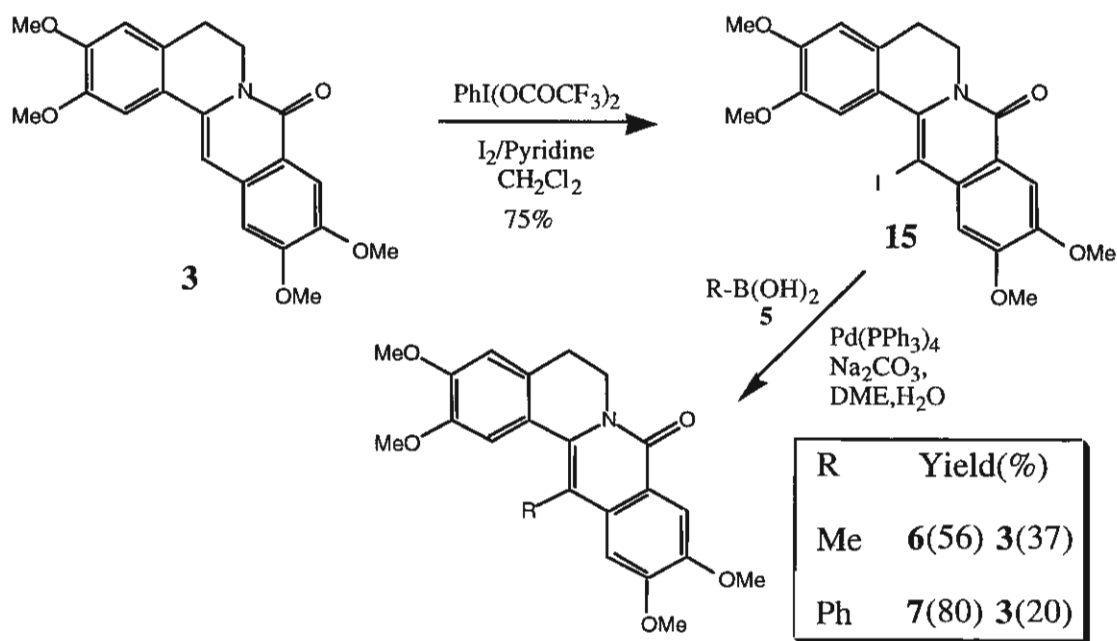
Applying the above procedure, we successfully obtained 13-substituted-8-oxyberberine **9** and **10** in 44.5 and 84 % yield respectively.



In the same manner, when using 8-oxypalmatine **11** as the starting material, we obtained 13-substituted-8-oxypalmatine **12** and **13** in 43 and 74 % yield respectively.

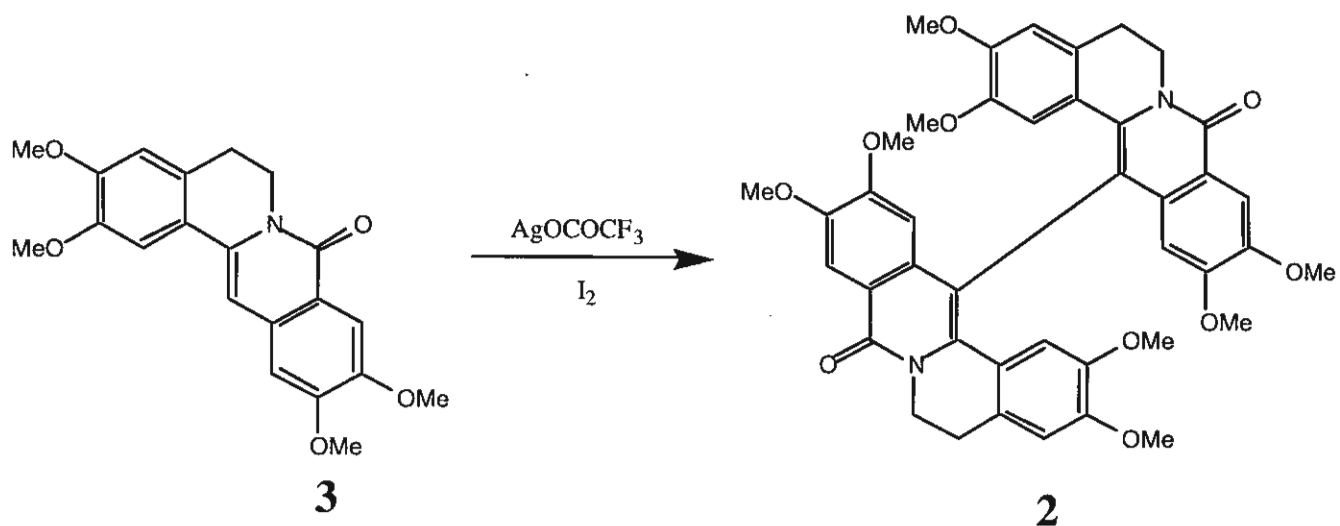


Since iodo derivatives are generally more reactive than bromo derivatives in metal-catalyzed coupling reaction, the iodo analogue **4** was prepared from **3**. Using the bis-(trifluoroacetoxy)iodobenzene/iodine/pyridine reagent system⁵, 13-iodo **15** was synthesized in 75% yield. Treatment of this iodo compound as in the above condition gave the 13-substituted-8-oxopseudopalmitine **6** and **7** in 56 and 80 % yield respectively.



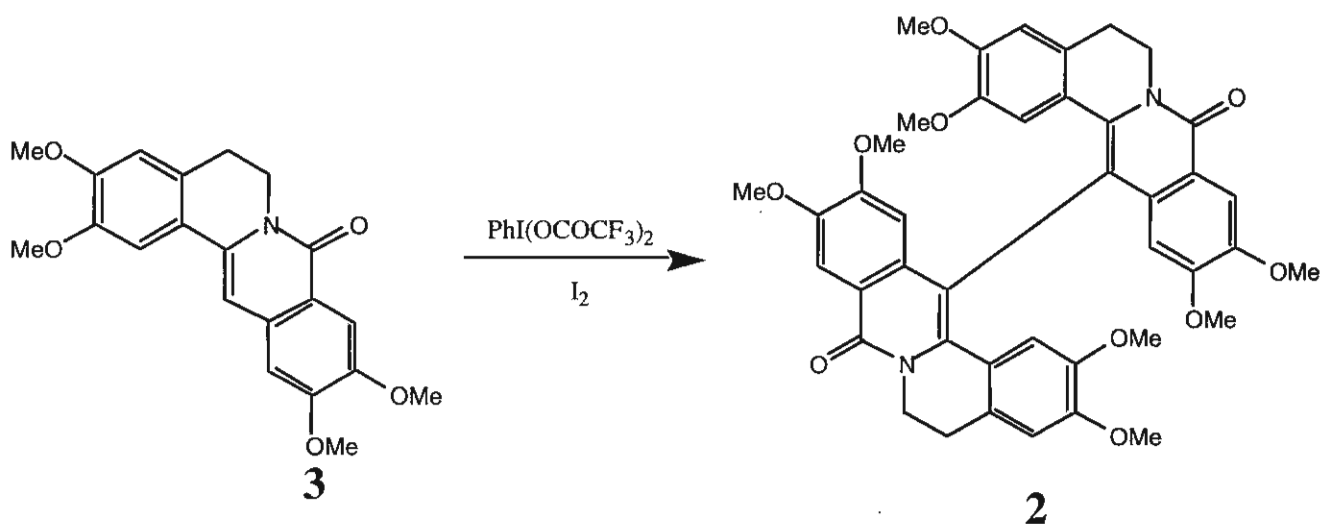
Our another aim was to dimerize the protoberberine monomer to the bisprotoberberine alkaloid. The synthesis began with our finding that when 8-oxopseudopalmitine **3** was treated with AgOCOCF_3 and I_2 in dichloromethane, the

yellow solid of ilicifoline **2** was obtained in 71% yield, accompanying with 17% of 13-iodo-8-oxopsedopalmatine **15**.

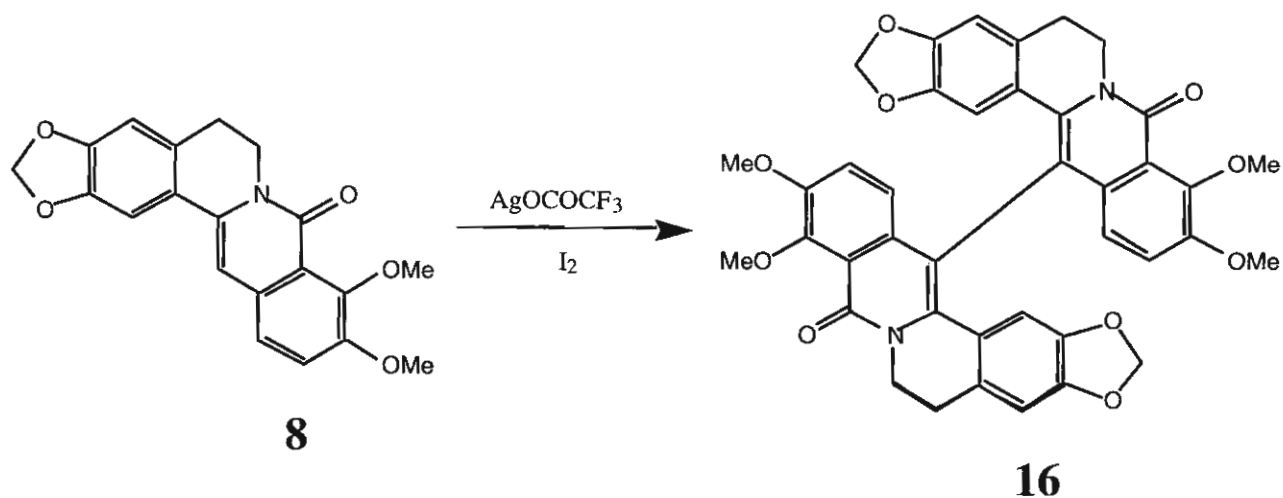


We also found that 8-oxopsedopalmatine **3** could react with AgOAc and I_2 , ilicifoline **1** was obtained in 70% yield. When AgNO_3 was used instead of AgOAc , the product was obtained in poor yield (37%).

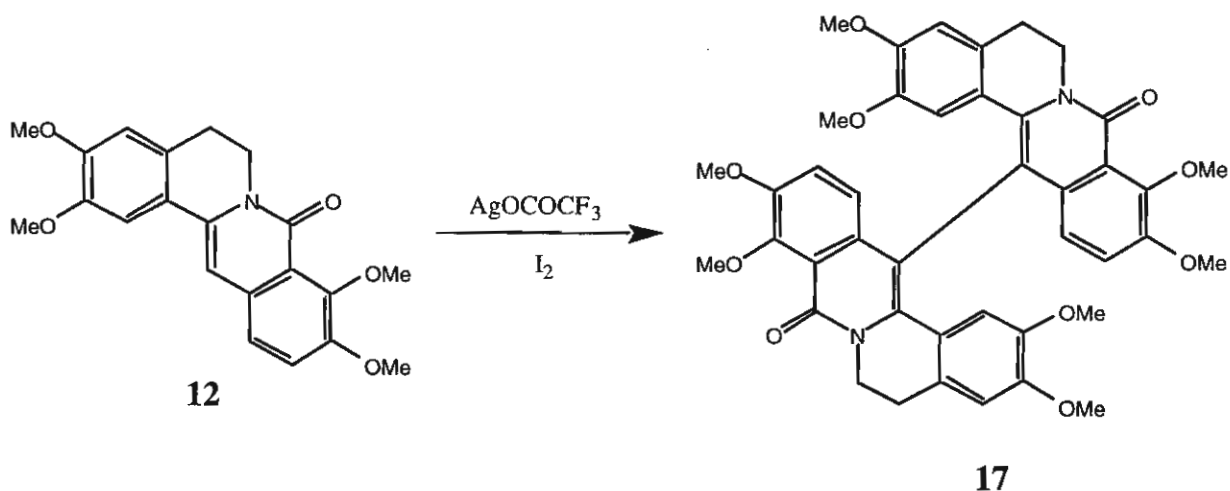
Lately the hypervalent iodo compounds have been used in various oxidation reactions. We have found that 8-oxopsedopalmatine could be oxidized with [bis(trifluoroacetoxy)iodo]benzene to give the product in 66.6 % yield.



In addition, other related dimeric protoberberines have been synthesized by this method. When 8-oxyberberine **8** was treated with AgOCOCF_3 and I_2 , bisoxyberberine **16** was obtained in 88% yield.

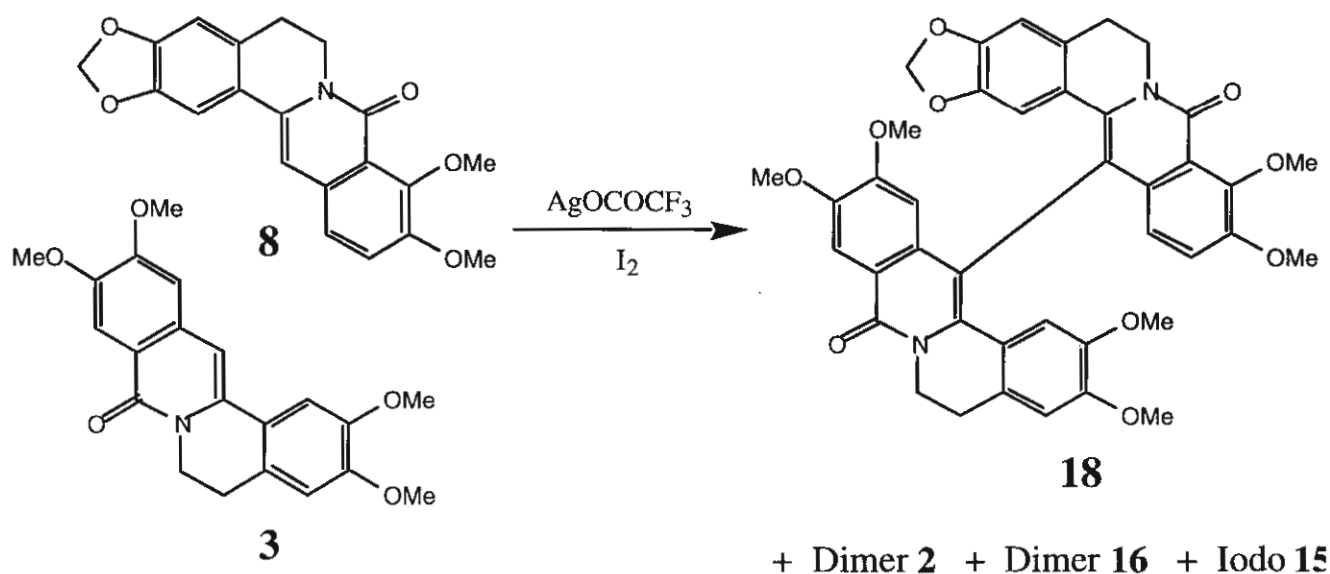


Similarly, the dimer **17** was obtained in 66.4 % yield when treated 8-oxypalmatine with AgOCOCF₃ and I₂.



In the course of our work, we have become interested in the cross coupling reaction. We have investigated this reaction by using a mixture of 8-oxopseudopalmatine **3** and 8-oxyberberine **8** as starting materials.

The mixture of 8-oxopseudopalmatine and 8-oxyberberine was treated with AgOCOCF₃ and I₂, the cross coupling product **18** was obtained in 63% yield, accompanying with ilicifoline **2** (17%), bisoxyberberine **16** (9%) and iodo compound **15** (9%).



In conclusion, we have developed a convenient method for the synthesis of the 13-substituted protoberberine alkaloids by using the Suzuki coupling reaction. The various methods for the oxidative coupling of protoberberines were also investigated. The desired yuanamide **1**, ilicifoline **2** and others related protoberberines could be obtained. We have also introduced for the first time the use of hypervalent iodo compound $[\text{PhI}(\text{OCOCF}_3)_2]$ in the oxidative dimerization of the protoberberine alkaloids to the corresponding bisprotoberberine alkaloids.

Acknowledgement : We are grateful to Thailand Research Fund for generous support to our research programme.

References

1. Simeon, S., Rios, J.L. and Villar, A. *Plant Med. Phytother.*, 1989, **23**, 202.
2. Bhakuni, D.A. and Jain, S. *The Alkaloids*, Academic Press, 1986, vol 28, p 95; Shamma, M. and Moniot, J.L. *Isoquinoline Alkaloids Research 1972-1977*, Plenum Press, New York and London, 1978, p.209; Shamma, M. *The Isoquinoline Alkaloids, Chemistry and Pharmacology*, Academic Press, New York and London, 1972, p.268; Kondo, Y. *Heterocycles*, 1976, 4, 197; Bentley, K.W. *The Isoquinoline Alkaloids*, Harwood Academic Publishers, 1998, p.219.
3. Ito, C., Mizuno, T., Wu, T.S. and Furukawa, H. *Phytochemistry* 1990; **29**(6): 2044.
4. Fajardo, V., Carcamo, C. and Moreno, B. *Heterocycles* 1996; **43**(5): 949-951.
5. Benhida, R., Blanchard, P. and Fourrey, J.L. *Tetrahedron Lett.* 1998; **39**: 6849.

THE SYNTHETIC OF FREE RADICAL REACTION IN THE SYNTHESES OF ALKALOIDS

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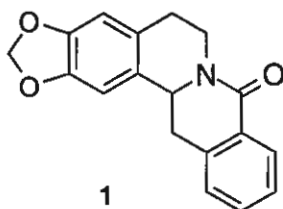
Department of Chemistry, Faculty of Science, Mahidol University³

Introduction

Organotin hydrides have proved to be capable and versatile agent for radical generation and they are widely used for the formation of carbon-carbon bond in many organic transformations. Using the applications of tributyltin hydride-induced intramolecular radical cyclization¹, we have studied the construction of ring systems in three classes of isoquinoline derived alkaloids: protoberberine, erythrina and pavine.

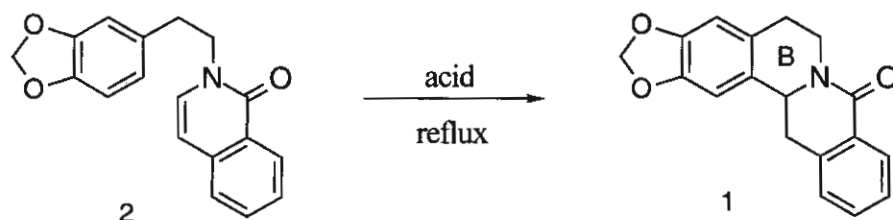
Results and Discussion

Numerous protoberberine alkaloids and their derivatives have been isolated and characterized. Gusanlung D², **1**, an 8-oxotetrahydroprotoberberine alkaloid, was isolated from *Acangelisia gusanlung* H.S.Lo, a small shrub distributed in Hainan Island of Sounthern China and the whole stem was used in folk medicine.



Gusanlung D

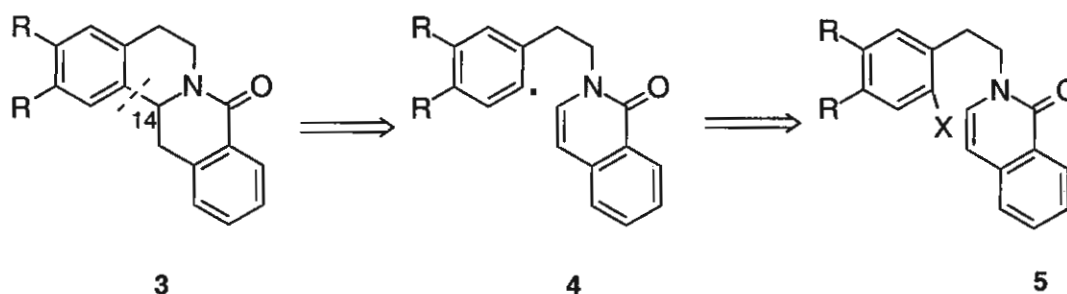
In our laboratory, the cyclization of isoquinolone derivative, **2**, to the gusanlung D has been achieved. However, the acid-catalyzed cyclization to construct ring B as shown in Scheme 1, gave unsatisfactory yield.



- condition :
1. trifluoroacetic acid /reflux 24 h/20%yield
 2. 1:1 acetic acid:o-phosphoric acid/reflux 5 h/20% yield
 3. 1:1 formic acid:trifluoroacetic acid/reflux 12 h/20% yield

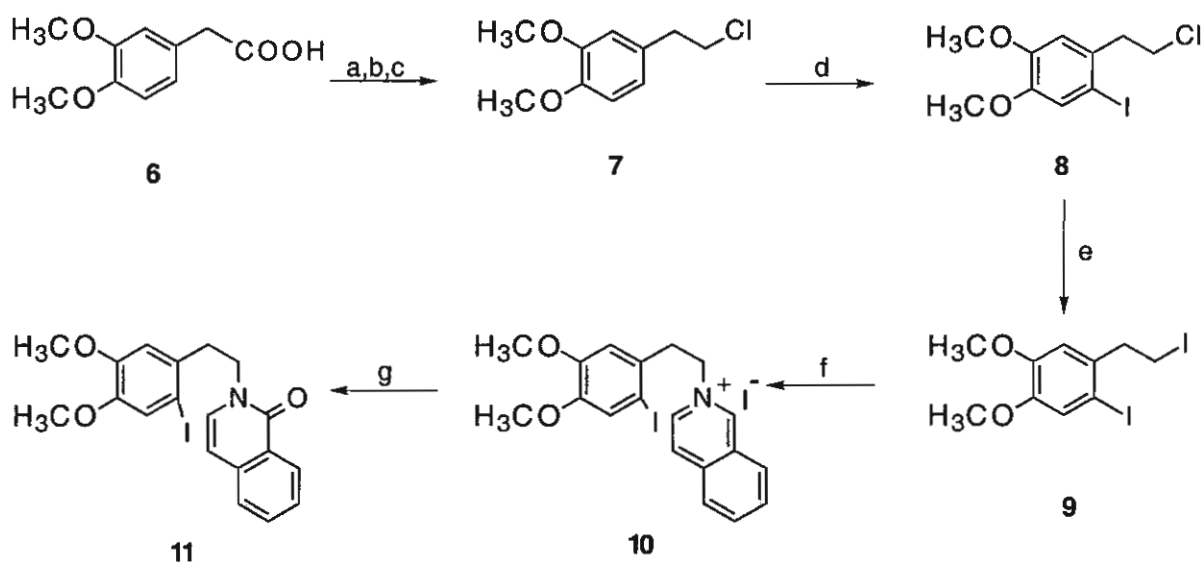
Scheme 1

Therefore, the alternative route of synthetic approach, the application of radical cyclization, was investigated. We planned to construct ring B by carbon-carbon bond formation of C14 and C14a using tributyltin hydride-induced intramolecular radical cyclization reaction as shown in the retrosynthetic analysis Scheme 2.



Scheme 2

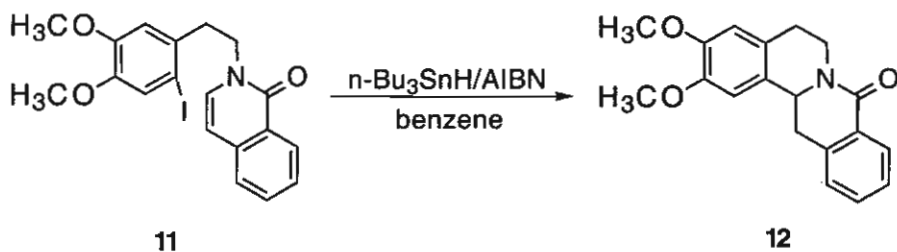
To test the proposed synthetic validity, starting *N*-(4',5'-dimethoxy-2'-iodophenyl-ethyl)-1*H*-isoquinolin-1-one, **11**, was first synthesized by modification of the widely used methods as shown in Scheme 3.



condition: a. $\text{SOCl}_2, \text{CH}_3\text{COOH}$ (76%)/ b. LAH, THF (75%)/ c. $\text{SOCl}_2, \text{CH}_2\text{Cl}_2$ (61%)/
 d. $\text{I}_2, \text{CF}_3\text{COOAg}$ (82%)/ e. NaI (72%)/ f. isoquinoline, CH_3CN (79%)/
 g. $\text{O}_2, t\text{-BuOH}, \text{KOH}$ (75%)

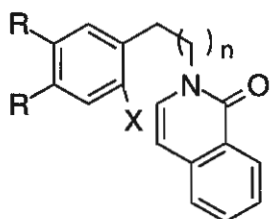
Scheme 3

With the isoquinolone **11** in hand, we then first studied the intramolecular cyclization reaction using tributyltin hydride as a cyclizing reagent. We were very pleased to find that when the isoquinolone **11** was heated under reflux with tributyltin hydride and azoisobutyronitrile in dry benzene for 6 hours, the cyclization product, tetrahydropprotoberberine **12**, was obtained in 70% yield (scheme 4).



Scheme 4

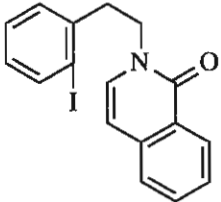
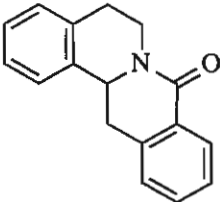
It is also our plan to investigate some factors, which might affect the cyclization reactions, such as substituents, type of halide and the magnitude of the ring size. To test our approach, we designed four suitable 2-substituted isoquinolones **11**, **13**, **14** and **15** as starting materials for the investigation of the key step in the tin-catalyzed cyclization.

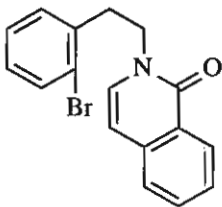
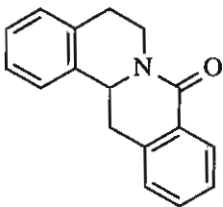
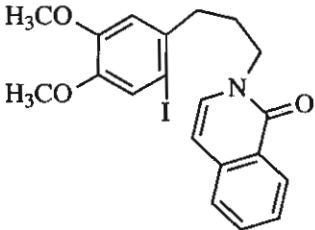
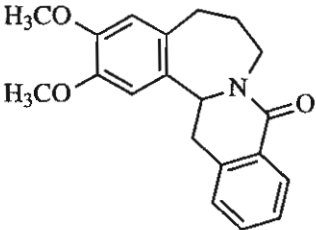


	X	R	n
11	I	OCH ₃	1
13	I	H	1
14	Br	H	1
15	I	OCH ₃	2

The cyclization products of halo isoquinolone compounds **11**, **13**, **14** and **15** with tributyltin hydride under the standard condition are shown in the table.

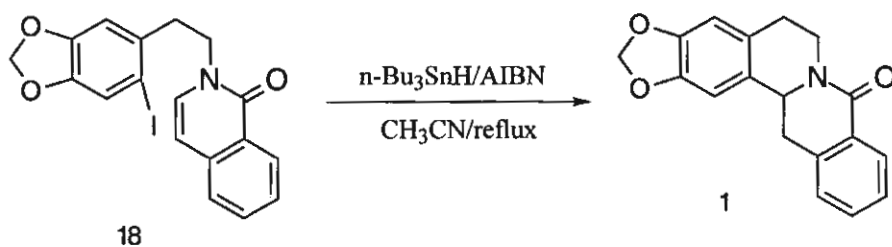
Entry	Starting	Product	Yield (%)
1	<p style="text-align: center;">11</p>	<p style="text-align: center;">12</p>	70

2	 <p style="text-align: center;">13</p>	 <p style="text-align: center;">16</p>	67
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Entry	Starting	Product	Yield (%)
3	 14	 16	46
4	 15	 17	68

It was found that *para*-methoxy substituent and the magnitude of the ring size were not the crucial factors in this radical cyclization (Entry 1, 2 and 4). Furthermore, the reactivity of halogen atom also played an important role in the reaction. As seen in entry 2 and 3, the bromo compound gave lower yield of the tetrahydroprotoberberines.

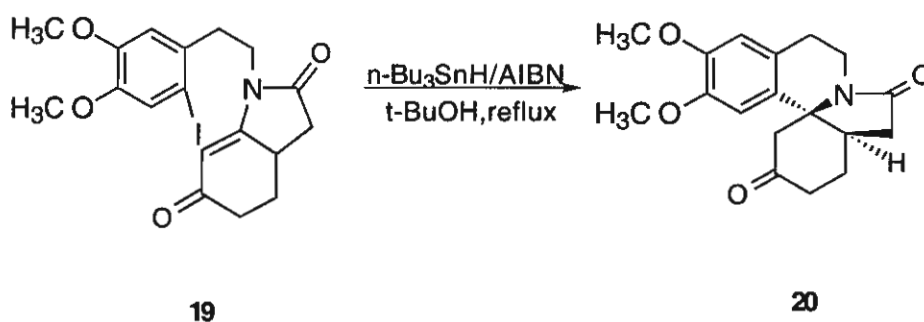
By applying the above finding, gusanlung D,1, could be synthesized from iodo-isoquinolone derivative **18**.



Scheme 5

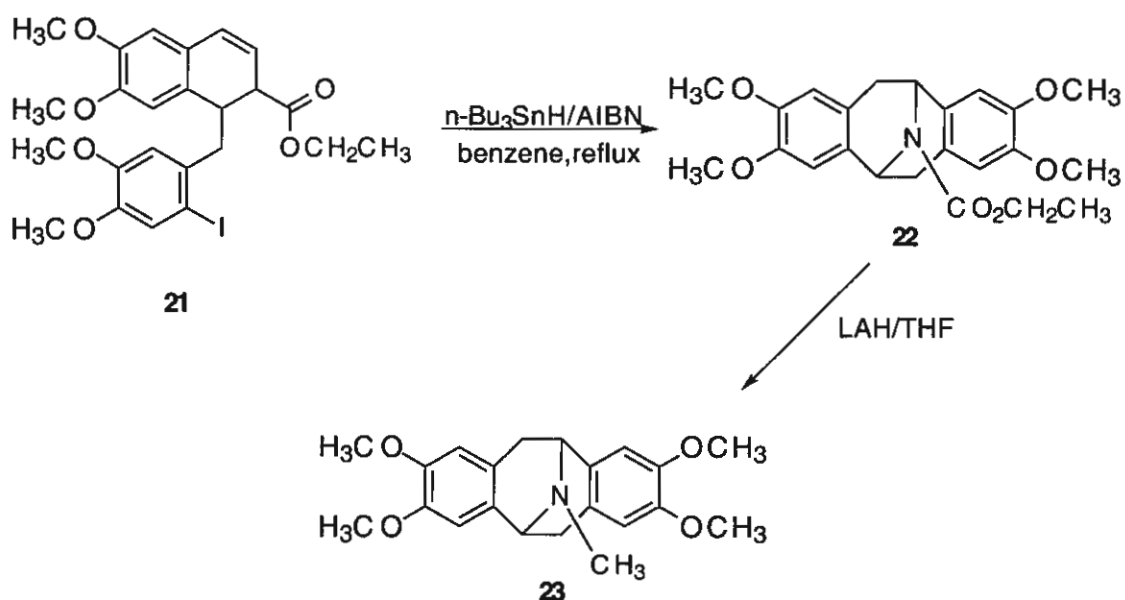
Indeed, it was found that gusanlung D,1, could be synthesized from the radical cyclization reaction of the refluxing acetonitrile solution of iodo-isoquinolone derivative **18**, in the presence of tributyltin hydride and azoisobutyronitrile in 78.6% yield.

According to the successful results obtained from tetrahydroprotoberberine, we extended our work to the synthesis of erythrina alkaloids³. The key intermediates were depicted as **19**, that cyclized smoothly under radical condition to give the erythrina alkaloid **20** in 33.5% yield.



Scheme 6

Similarly, tributyltin hydride-catalyzed radical cyclization was applied to the transformation of iodobenzylisoquinoline **21** to pavine alkaloid derivative **22** in 42% yield, which is applicable to the synthesis of (+)argemonine⁴ **23**, the natural product.



Scheme 7

Conclusion

We have successfully devised a radical method for the synthesis of protoberberine, erythrina and pavine alkaloids. The synthetic strategy involves the intramolecular radical coupling of the halo compounds, catalyzed by tributyltin hydride. The yields of the products depended on the reactivity of halogen atom.

Acknowledgment

Financial support from Thailand Research Fund (TRF) is gratefully acknowledged.

Reference

1. Bimal, K.B., Gottumukkala, V.S., Maghar, S.M., and Ajay, K.B.; **Tetrahedron Lett.** 1996; 37: 1363-1366.
2. Zhang, J.S., Men-Olivier, L.L., and Massiot, G.; **Phytochemistry** 1995 ; 39(2) : 439-442.
3. Chawla, A.S., and Jackson, A.H., **Nat. Prod. Rep.** 1986; 3: 555.
4. Gozler, B., Lantzand, M.S., and Shamma, M., **J. Nat. Prod.** 1983; 46: 239-309.

DIOSPYROL REVISITED : NEW SYNTHETIC STUDIES

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and Somsak Ruchirawat^{1,2,3}

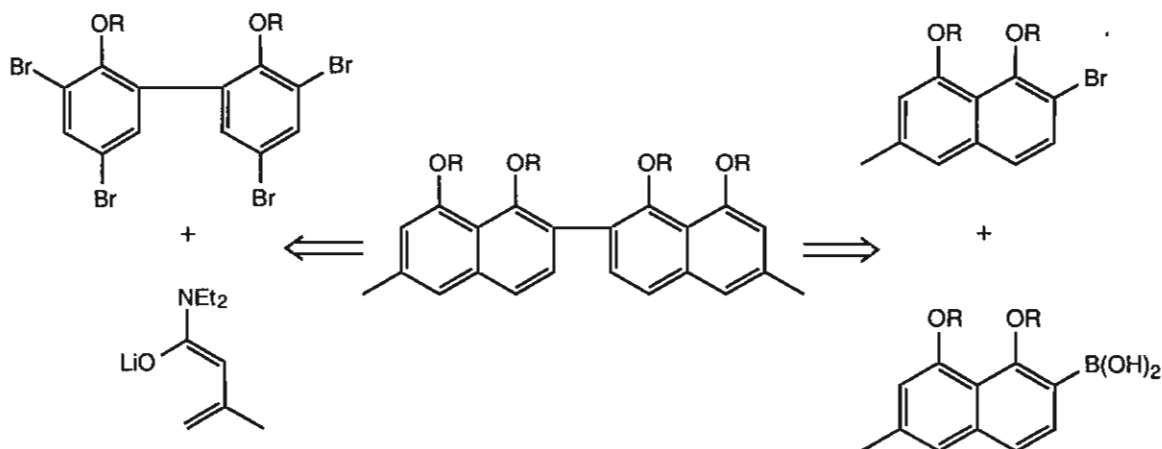
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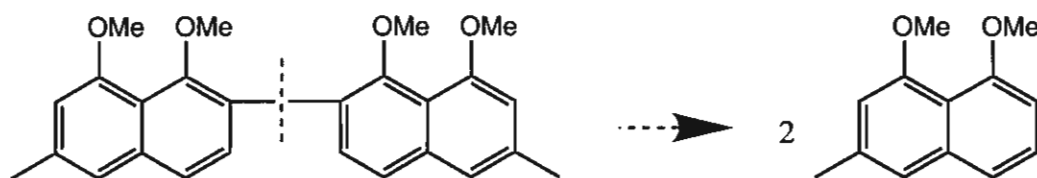
Introduction

Diospyrol, a dimeric naphthol derivative, has been isolated from the berry of *Diospyros mollis* which has been used in Thailand as anthelmintics. Two syntheses of diospyrol derivatives were accomplished by benzyne-annulation reaction and palladium-mediated coupling reaction (Suzuki reaction) as the key steps. Details of the approaches are presented below.

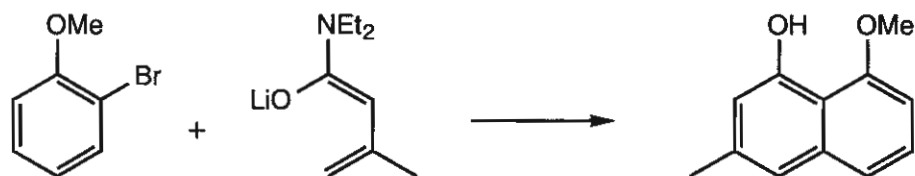


Results and discussion

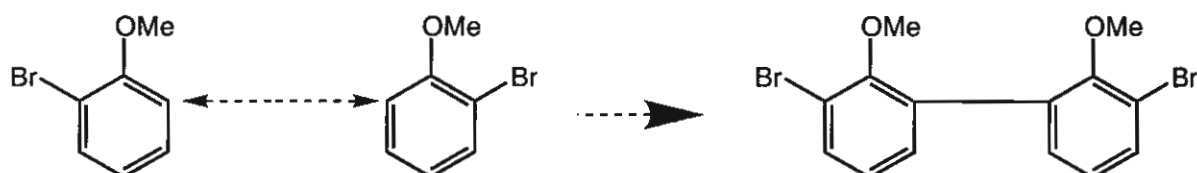
1. Diospyrol derivative *via* benzyne annulation reaction.



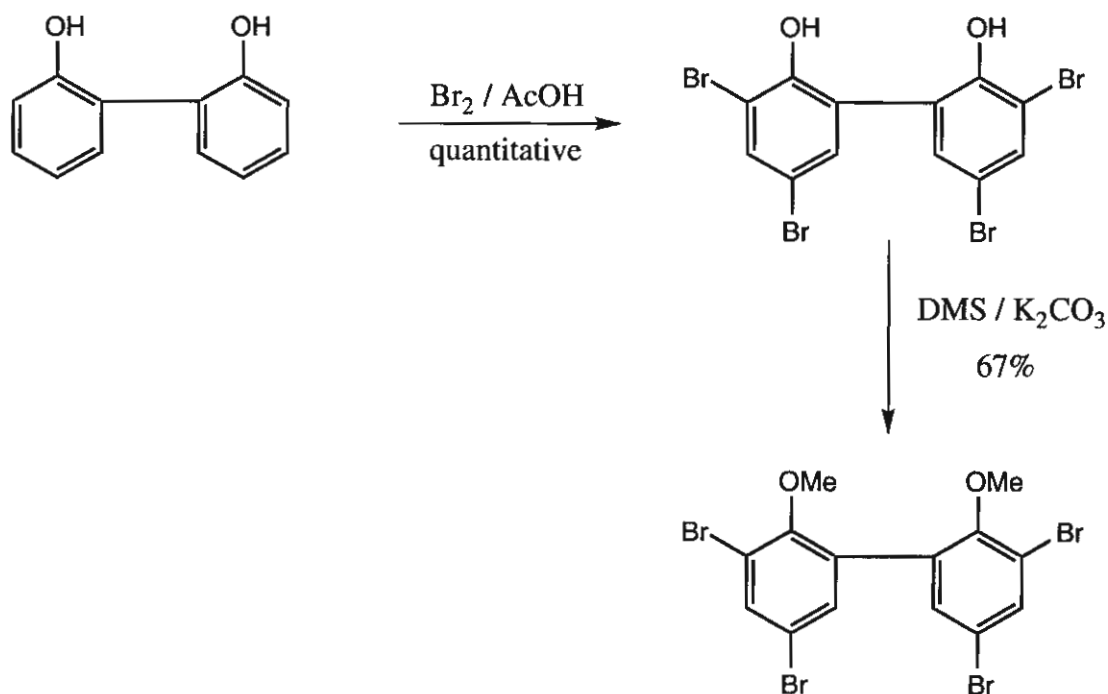
Diospyrol is a symmetrical dimer of naphthol derivative. This unique naphthol derivative could be conveniently prepared in one step utilizing regiospecific benzyne annulation reaction. Thus, reaction of o-bromoanisole with an excess of lithium cyclohexylisopropylamide and N,N diethyl seneciamide gave the expected naphthol in 20%.



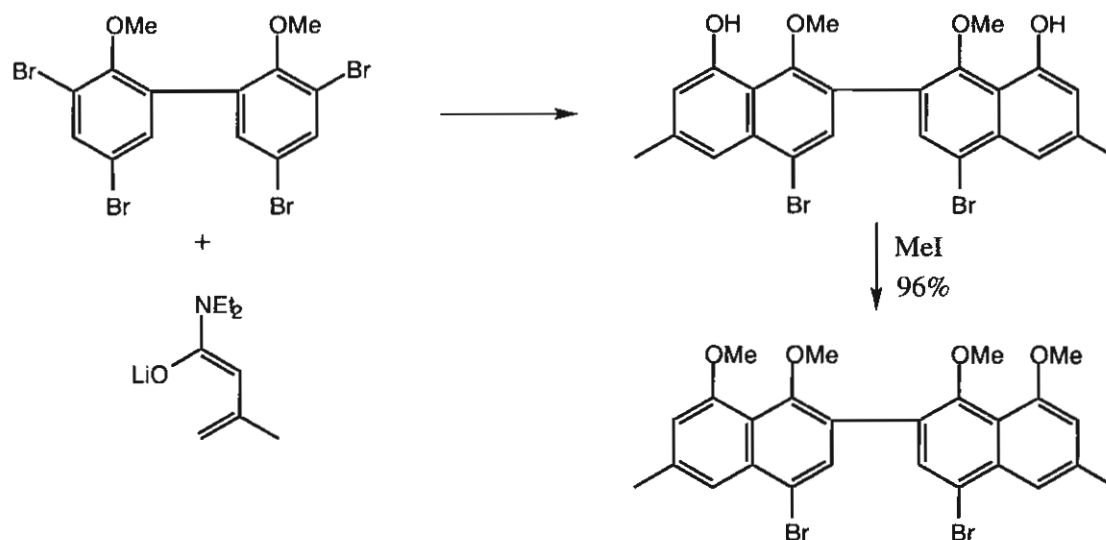
If two units of the starting material, o-bromoanisole, are linked at C-6 position, our modified starting material will be the biphenyl derivative. It is expected that this biphenyl will undergo regiospecific benzyne annulation in the same manner as in the monomer to give the required diospyrol derivative.



The dibromo biphenyl derivative which we planned to use as starting material was not convenient to synthesize due to the over bromination. The most suitable starting material should be a fully brominated compound that could be easily prepared in 2 steps from biphenol derivative.

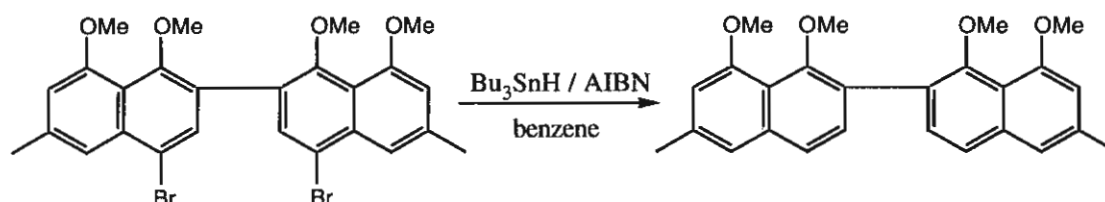


Treatment of the so obtained tetrabromobiphenyl derivative with lithium enolate of N,N-diethyl seneciamide gave the required diospyrol derivative in 20% yield.



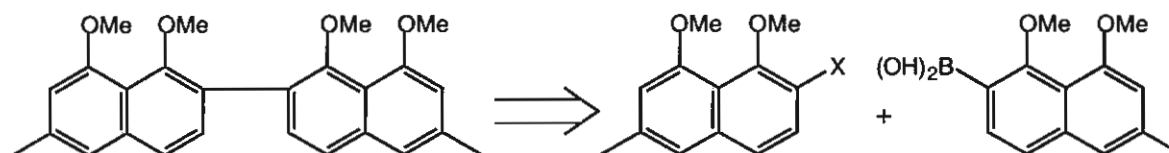
Methylation of the resulting binaphthol gave dibromotetramethoxydiospyrol derivative.

Removal of the dibromo molecule proceeded smoothly under radical condition to obtain the target tetramethoxydiospyrol derivative in 86% yield.

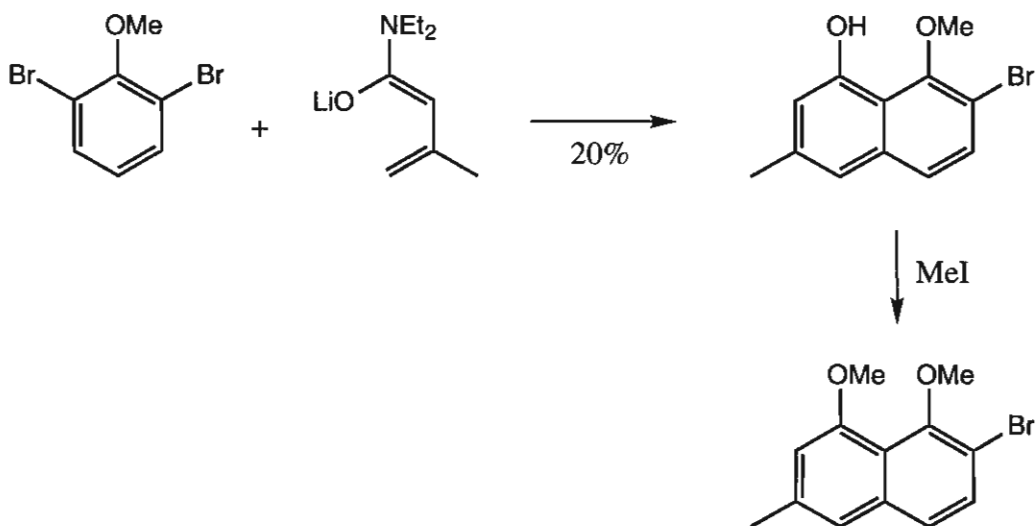


2. Diospyrol via Suzuki coupling reaction.

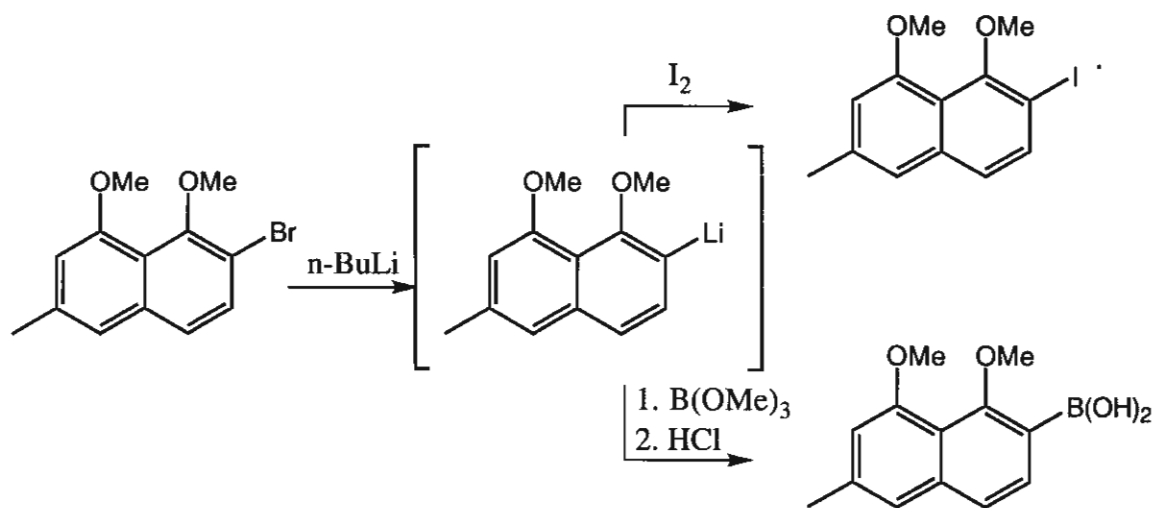
The most suitable starting material for the synthesis of diospyrol derivative under Suzuki coupling condition were halonaphthol and naphthol boronic acid.



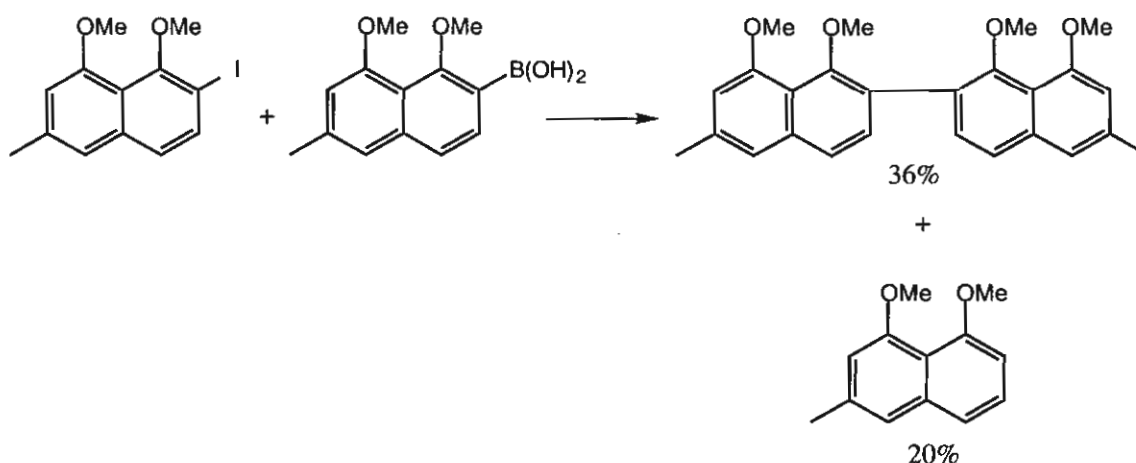
The bromonaphthol could be synthesized under regiospecific benzyne annulation reaction between 2,6-dibromoanisole and lithium cyclohexylisopropylamide and N,N-diethyl seneciamide to give naphthol derivative in 20% yield followed by methylation under standard condition.



The resulting bromonaphthol could be transformed to both iodonaphthol and naphtholboronic acid by metal-bromine exchange in the first step followed by quenching with iodine and trimethylborate respectively.



With both starting materials are in hand, the Suzuki Coupling reaction was carried out (condition : $\text{Pd(PPh}_3)_4$ / K_2CO_3 / toluene : EtOH : water / $115-120^\circ\text{C}$ / 19h) to obtain the required tetramethoxydiospyrol in 36% yield together with debrominated naphthol in 20% yield.



We have also performed an in situ Suzuki coupling reaction by pre-generating the boronic acid and then let it react with the remaining bromonaphthol under the identical condition as above to obtain the tetramethoxydiospyrol (21%) and debrominated naphthol (26%) as well as recovered starting material (37%).

Conclusion

Two new routes for the synthesis of diospyrol derivative were accomplished. The key step of the first method involved regiospecific benzyne annulation between biphenyl derivatives and seneciamide and the another method utilized Suzuki coupling reaction between halonaphthol and naphtholboronic acid.

Acknowledgement : Financial support from Thailand Research Fund (TRF) is gratefully acknowledged.

Synthesis and Pharmacology of a Potent new Antimalarial

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Introduction

Malaria and other protozoal diseases of humans and their livestock continue to have a devastating impact worldwide¹. However, non of these protozoal diseases has had the enormous effect upon civilization, either historically or in modern times, as has malaria.

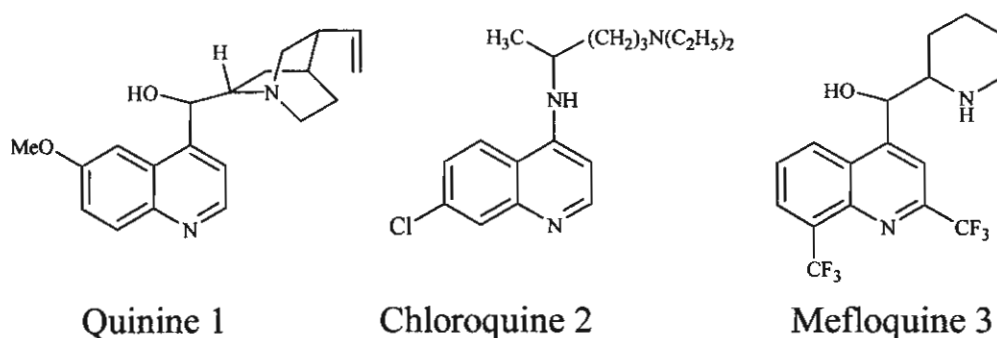
Malaria in humans is caused by four species of *Plasmodium* protozoa, which spend half their life cycle in female Anopheles mosquitoes.

The four *Plasmodium* species are as follows: *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*.

Hence, new antimalarial drugs with novel actions are being sought. For many years Cinchona species have been used for the production of quinine (1) used as the major drug for the treatment of malaria and in more recent times clinicians have turned again to quinine therapy because resistance of *Plasmodium* strains is low in comparison to other drugs. Most drugs used in modern malaria chemotherapy-chloroquine (2) act primarily at the erythrocytic stage in the malaria life cycle.

Mefloquine² (3) is the most promising of a group of a new antimalarial drugs. Since its FDA approval in 1989, mefloquine has become a drug of choice for malaria

suppression, particularly in areas of known chloroquine resistant strains. An improved method of synthesis.

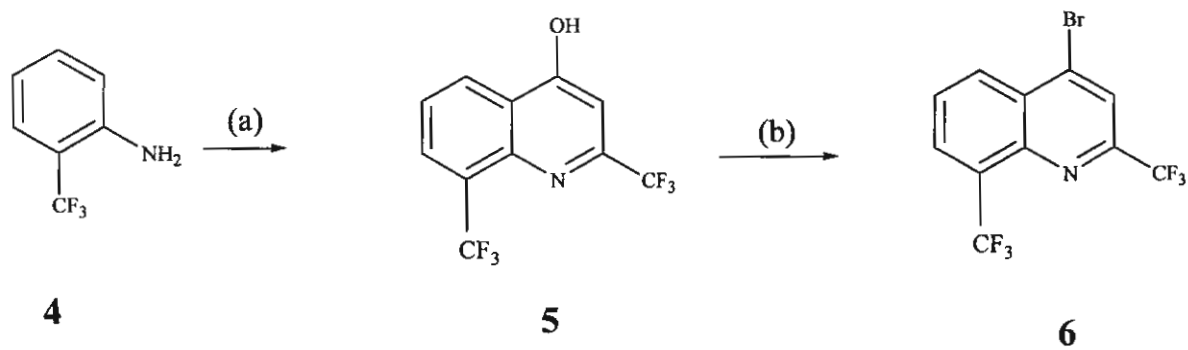


Scheme 1

Results and Discussion

Chemical Investigation

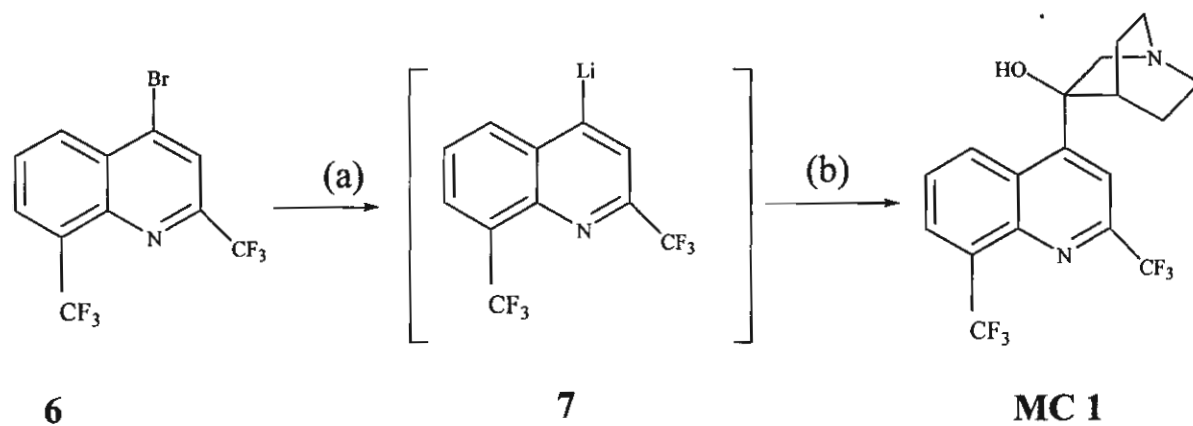
It is also noteworthy that almost all of the commercially available antimalarial agents have one common structural feature a quinoline ring. Many of them have been developed synthetically by using the quinine molecule as a template, at present there do not seem to be any similar natural alkaloids which have been used for malaria treatment. An improved method of synthesis, condensation of 2-aminobenzotrifluoride 4 with ethyl-4,4,4-trifluoroacetoacetate in the presence of polyphosphoric acid gave only the 4-quinolinol 5. This reaction with ethyl acetoacetate itself gives a mixture of the 2- and 4-quinolinols 5, depending upon whether the amine reacts with the ester or the β -keto group. The electron withdrawing power of the trifluoromethyl group apparently leads to exclusive reaction of the electronegative β -keto group with the amine³. involved treating the lithio derivative of 4-bromo-2,8-bis(trifluoromethyl) quinoline 6 with 3-quinuclidone 8. It is illustrated in scheme 3.



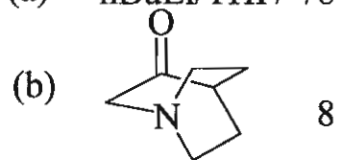
(a) $\text{CF}_3\text{COCH}_2\text{COOC}_2\text{H}_5, [\text{H}_3\text{PO}_4]_n$

(b) POBr_3

Scheme 2



(a) $n\text{BuLi/THF/-78}^\circ\text{C}$



Scheme 3

These analysis of structure (IR, NMR, MS and Analysis)-activity relationship should serve as a guide for synthesis and investigations of additional analogs. Newer and more effective drugs are required because of the relentless emergence of strains of human plasmodia (especially *P. falciparum*) resistance to currently available antimalarial drugs.

Pharmacological Investigation

2. Pharmacological Investigation

2.1 The in vitro study of antimalarial activity of MC1 on *P. falciparum*

Human erythrocytes (type O) infected with *P. falciparum* K1 strain (Chloroquine resistant) was maintained in continuous culture, according to the method described by Trager (Trager, W and Jensen, J.B. science 193, 673-675, 1976). RPMI 1640 culture medium (Gibco, USA) supplemented with 25 mM of HEPES (sigma, USA), 40 mg/L gentamicin sulfate (Government Pharmaceutical Organization, Thailand) and 10 ml of human serum was used in continuous culture. Before starting experiment, *P. falciparum* culture was synchronized by sorbital hemolysis according to the method of Lambros (Lambros C. and Vanderberg J.P., J. Parasitol 65, 418-420, 1979) to get only ring-infected cells and then incubated for 48 hours.

The experiment were started with synchronized suspension ring infected (0.5% parasitemia). Parasites were suspended with culture medium supplemented with 15% human serum to get 10% cell suspension. The parasite suspension was put into 96-wells microtiter plate ; 50 ul in each well and then add 50 ul of various tested drug concentration. Incubate for further 48 hours in the atmosphere of 5 % CO₂ at 37 °C. Examine percent parasitemia between control and every drug concentration by methanol-fixed Giemsa stained thin smear.

The test result was showed in table 1.

Table 1: Effect of MC1 on *P. falciparum* (K_1) growth.

Chemicals	Experriment number	IC ₅₀ ^a (M)
MC1 chloroquine ^b	1	4.0 x 10 ⁻⁸
	2	1.5 x 10 ⁻⁷
	3	2.0 x 10 ⁻⁸
		6.1 x 10 ⁻⁷

a = concentration that inhibit 50 % growth of *P. falciparum* (K_1)

when compare with control.

b = standard drug for treatment of malaria.

\bar{x} = mean value

From the experiment show that MC1 inhibit *P. falciparum* growth better than chloroquine which is used as standard drug for treatment of malaria (IC₅₀ = 6.1 x 10⁻⁷ M). From three experiments show that IC₅₀ of MC1 lower than chloroquine 15 folds (IC₅₀ = 4.0 x 10⁻⁸ M), 4 folds (IC₅₀ = 1.5 x 10⁻⁷ M) and 30 folds (IC₅₀ = 2.0 x 10⁻⁸ M). Mean value of IC₅₀ MC1 is 7.0 x 10⁻⁸ M which is lower than IC₅₀ of chloroquine 16.3 folds.

2.2 Antimalarial activity study of MC1 on the inhibition of *P. berghei* in animal model.

Materials and methods : Male Swiss Albino mice weighed 25-30 gm were used in this study.

3. **% parasitemia** % parasitemia was determined microscopically on thin-film Giemsa-stained preparations. Blood was drawn from tail-vein to determine % parasitemia. This information was necessary to determine the efficiency of the test compounds as antimalarial drugs.

This values were recorded continuously for 30 days.

Results

1. The effects of MC1 on % cumulative death and mean survival time.

The results were showed in Figure 1. It was found that distilled water and 20% DMSO in control groups were not different results. The 100% deaths of infected-mice were observed on day 7 of *P. berghei* infection and the mean survival time were 6.0 ± 0.58 and 6.6 ± 0.24 day in distilled water group and 20% DMSO group, respectively.

Chloroquine at the dose of 7.5 mg/kg.BW. could increase the mean survival time to 14.0 ± 0.00 day but 100% cumulative death was observed.

The results demonstrated that MC1 at the dose of 7.5 mg/kg.BW.,the same dosage as chloroquine treatment and 15 mg/kg.BW. could decrease % cumulative death to 33.33% and 40.00% and prolong the mean survival time to 28.0 ± 2.00 day and 26.2 ± 2.33 day, respectively.

Note: Assign 100% cumulative death in 30 days of the experiment for determination of mean survival time.

2. The effects of MC1 on % parasitemia.

The results were showed in Figure 2. The continuously increase % parasitemia in control groups (both distilled water and 20% DMSO) was observed after 2 days of infection and increase to 70-80 % after day 6 which % cumulative

death of the test mice reach to 100%. The administration of chloroquine could decrease % parasitemia during day 5 to day 10 but after that % parasitemia increase up to 70% on day 13 of the experiment and 100% deaths of the test mice was observed on day 14. MC1 at the dose of 7.5 and 15 mg/kg.BW. could decrease % parasitemia longer than chloroquine, 0 % parasitemia were still observed until day 18 and day 13, respectively. Furthermore, % parasitemia of the survival mice in MC1 treated group was 0 % upto the end of the experiment (day 30).

The results from this study revealed that MC1 has more potential on antimalarial activity than chloroquine in *P. berghei* infected mice. It could decrease % parasitemia and % cumulative death and increase survival time significantly as compared to the control group and chloroquine administration group.

References

1. Ross WJ *Chemotherapy of Trypanosomiasis and other Protozoan Disease*. In **Burger's Medicinal Chemistry, Part II**, 4th ed. Edited by Wolff M.E. New York: A Wiley Interscience Publication, 1979:439-479.
2. Rozman RS, Canfield CJ. New Experimental Antimalarial 7-Bis(fluoromethyl) α -(2-piperidyl)-4-quinolinemethanols. **J med Chem** 1971; 14(10):926-928.:
3. Day AS, Joulie MM. *Synthesis and Properties of Fluorine-containing Heterocyclic compounds. I. Trifluoromethyl Quinolines(1)*. **J Heterocycle Chem** 1965; 2(2):115-119.

Financial support from Thailand Research Fund (TRF)

is gratefully acknowledged.

THE SYNTHETIC UTILITIES OF ORGANO PALLADIUM IN THE SYNTHESES OF ALKALOIDS

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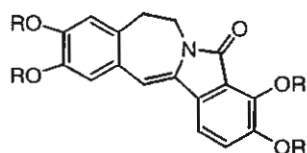
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Organometallic in general and organopalladium in particular are widely used in organic synthesis for constructing a carbon-carbon bond. Various organic compounds as well as diverse classes of natural products including alkaloids were synthesized utilizing palladium mediated conditions. The application of the organopalladium compounds in the synthesis of isoindolobenzazepine and other alkaloids will be presented.

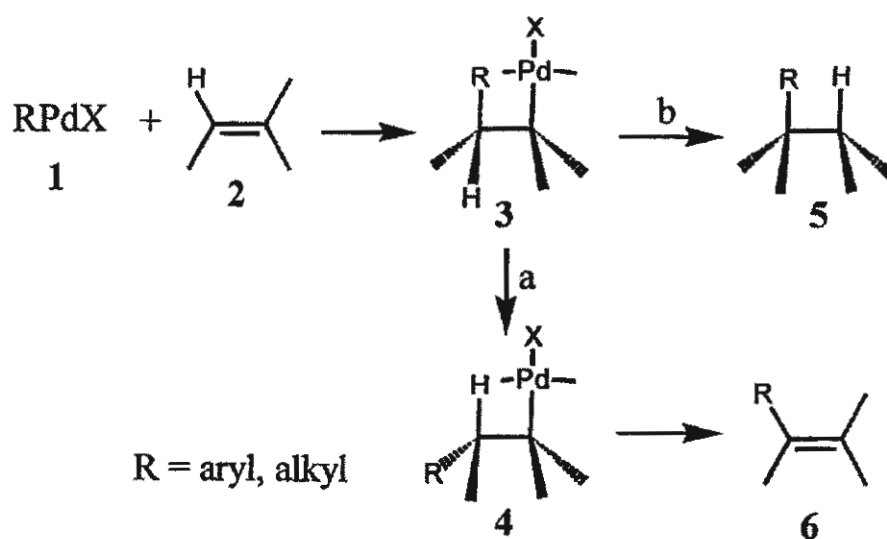


Financial support from Thailand Research Fund (TRF) is gratefully acknowledged.

Introduction

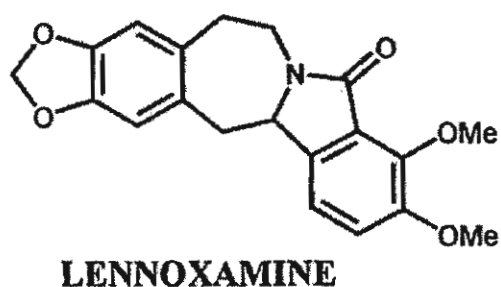
The Heck reaction plays an important role in both inter and intramolecular carbon-carbon bond formation method. The well-known example is illustrated in scheme 1, the alkene **2** reacted with palladium compound **1** to give both vinylic substitution product **6** and addition product **5**. The reaction involved the *syn*-addition of palladium reagent to alkene to provide σ -aryl and σ -vinylpalladium complex **3** Which further converted to complex **4** and then underwent *syn*- β -elimination of HPdX to form substitution product **6** (path a).

In addition, σ -alkylpalladium **3**, under suitable reaction conditions, could undergo carbon-palladium bond cleavage to give the new carbon-hydrogen bond (path b) resulting in the formation of the addition product **5**¹.

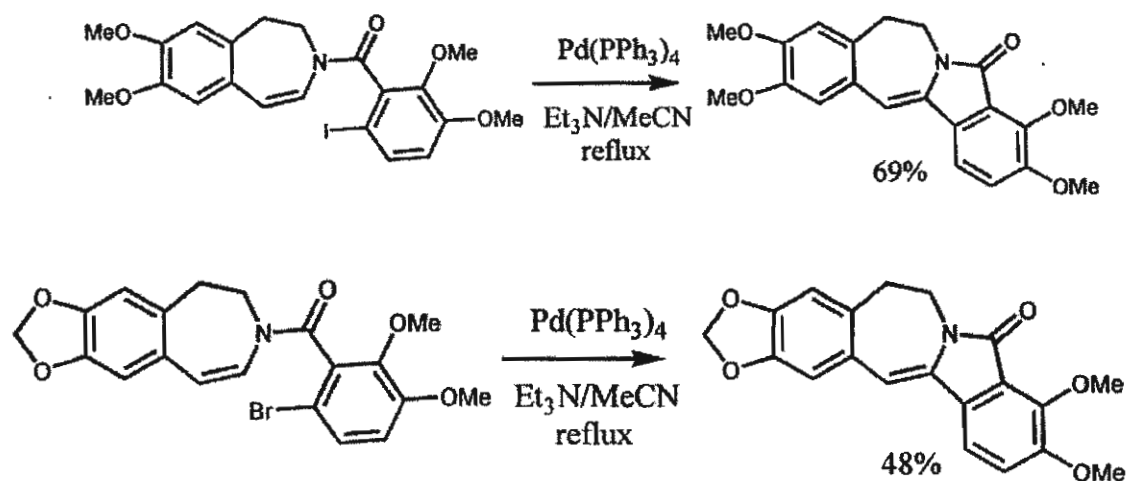


Results and Discussion

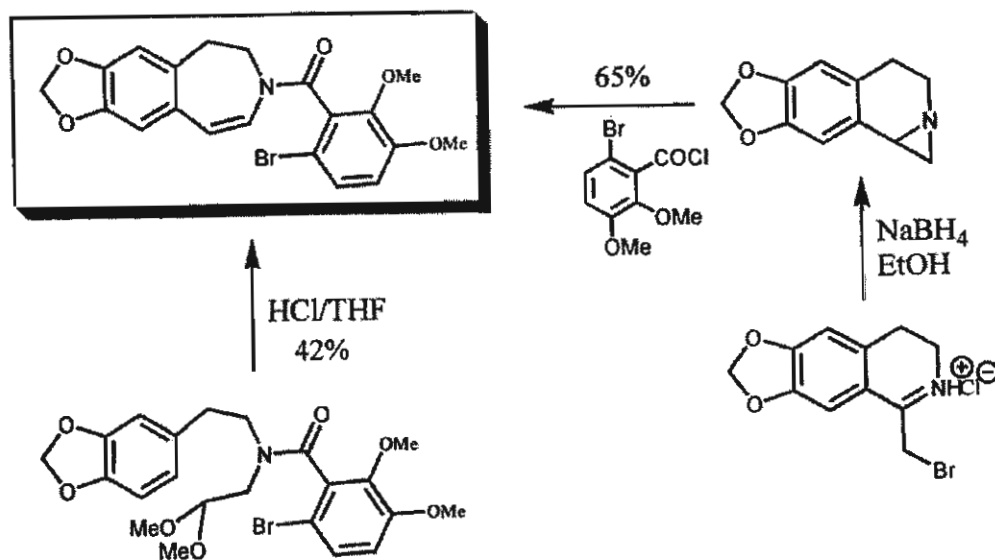
We have developed an efficient pathway for the synthesis of natural lennoxamine²: the first representative of the isoindolobenzazepine alkaloids and its analogues. The approach was based on the organopalladium mediated reaction and the key step is the Heck reaction.



Reaction of the halobenzazepine derivative with tetrakis (triphenylphosphine)palladium (O), triethylamine in acetonitrile, gave the isoindolobenzazepine intermediates after purification with preparative layer chromatography. These halobenzazepine derivatives have been investigated. The yields and the condition of the reaction are listed.



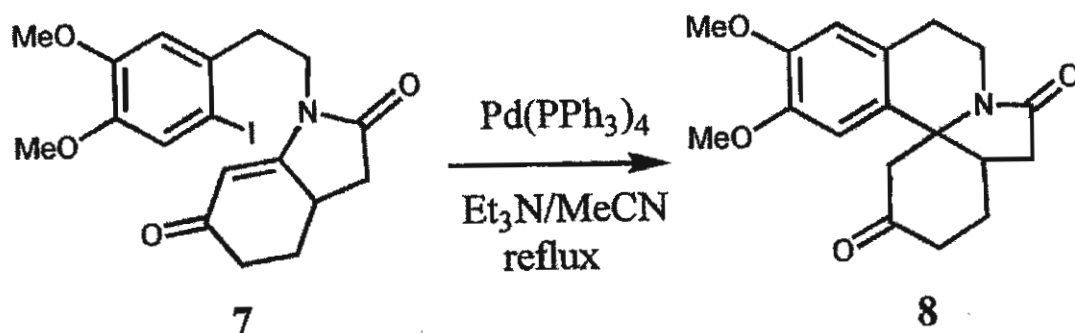
The key intermediate of the reaction can be synthesized by two synthetic routes as shown.



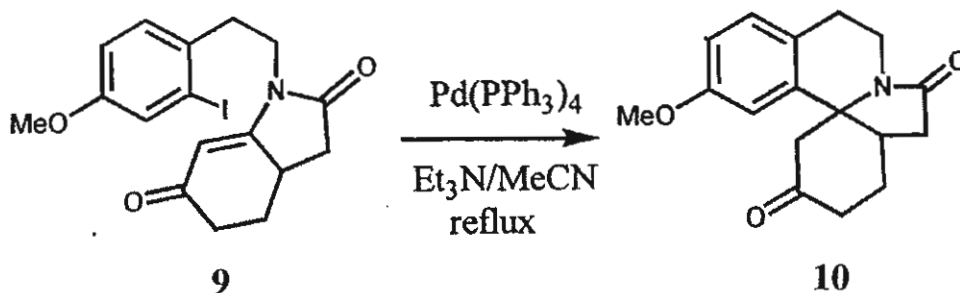
We are also interested in a study towards the synthesis of the erythrina alkaloids³ because they demonstrated a broad range of pharmacological activities and have generated substantial synthetic interests. Therefore, we decided to develop a new and straightforward synthesis of the erythrina alkaloids.

Our methodology involved the use of palladium catalysed cyclization of β -enamido ketone which was achieved as follows.

A mixture of compound **7**, tetrakis(triphenylphosphine) palladium(0) and triethylamine in dry acetonitrile was refluxed for 80 hours. Work-up with aqueous acid and chromatography on silica gel provided 77.3% yield of the desired erythrina compound **8**.

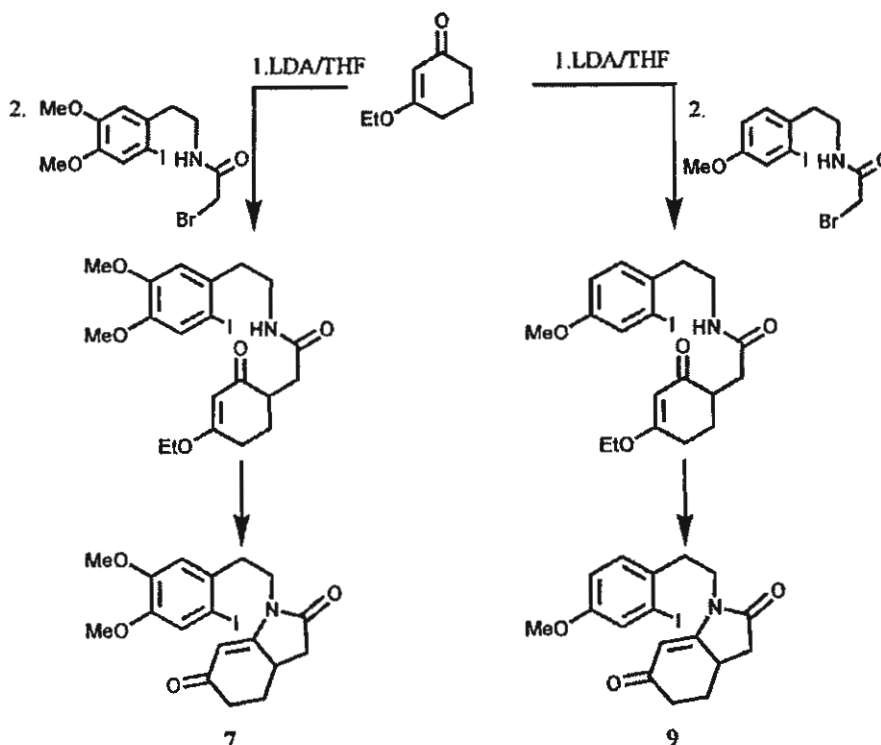


In traditional synthetic route to erythrina alkaloid from β -enamido ketone involved acid-catalysed cyclization promoted by *para* methoxyl or methylenedioxy group of the β -enamido ketone. When there are no activating groups in the target molecule, a solution to this problem would be resolved by using palladium-catalysed cyclization as shown below.

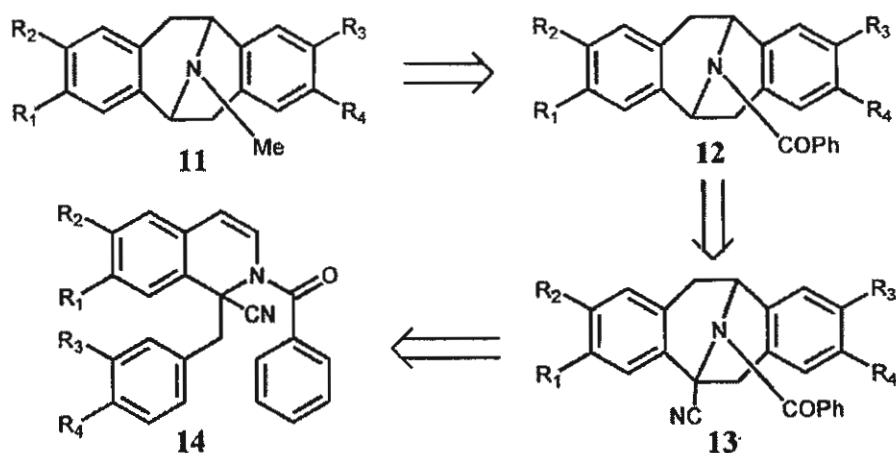


As can be seen in compound **10**, the point of attachment of cyclohexanone to the aromatic nucleus, at the *meta* position, this can not be accomplished using normal acid catalysed cyclization. The procedure of palladium-catalysed cyclization for the conversion of compound **9** to compound **10** was successfully employed. Optimum condition for this reaction was found to be refluxing in dry acetonitrile for 80 hours. Compound **10** was obtained in 62.5% yield.

The key intermediate **7** and **9** can be synthesized by the pathway as shown.



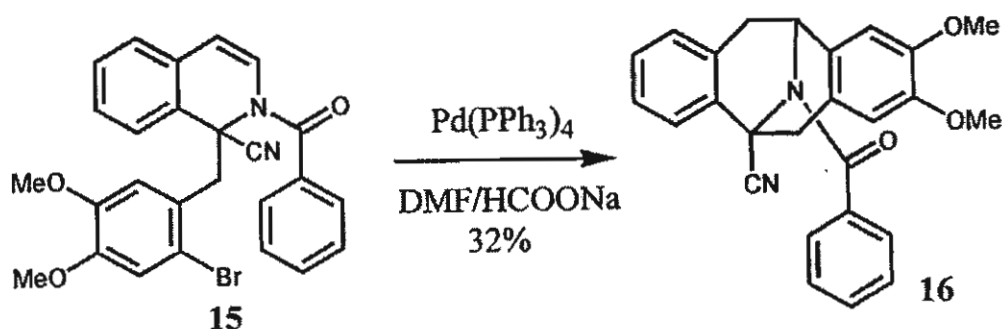
We also planned to develop a new route for the synthesis of pavine alkaloids⁴. Our adopted route was based on the retrosynthesis analysis shown in scheme 2.



Scheme 2

As outline in scheme 2, the benzyloisoquinoline **14** was the key intermediate leading to cyanopavine **13**. Elimination of cyano group of **13** would give the amide **12** which after hydrolysis, then N-methylation could provide the desired pavine alkaloid **11**.

In our investigation, we applied the Heck's reaction to cyclize the benzyloisoquinoline **15**. The optimum condition gave the highest yield of **16** in 32%.



Conclusion

We have devised three new synthetic routes for the synthesis of the three classes of alkaloids : isoindolo-benzazepine, erythrina and pavine alkaloids. The synthetic strategy involves the intramolecular coupling of the key intermediate by tetrakis(triphenylphosphine) palladium(0) exploiting the Heck reaction as the key step.

Acknowledgement : *We are grateful to Thailand Research Fund (TRF) for generous support to our research programme.*

References

1. Cacchi,S.; **Pure&Appl.Chem.** 1990 ; **62**: 713-722
2. Valencia, E., Freyer,A.J. and Shamma,M.; **Tetrahedron Lett.** 1984; **25**(6) : 599-602
3. Shamma,M.; *The Isoquinoline Alkaloids Chemistry and Pharmacology*, Blomquist AT, Wasserman H, New York: Academic Press, 1972
4. Brossi, A., GretheG., Teitel,S., WildmanW.C., and Bailey,D.T., **J.Org.Chem.** 1970 ; **35**: 1100

THE UNUSUAL NMR CHARACTERISTICS OF ISOINDOLOBENZAZEPINE ALKALOIDS

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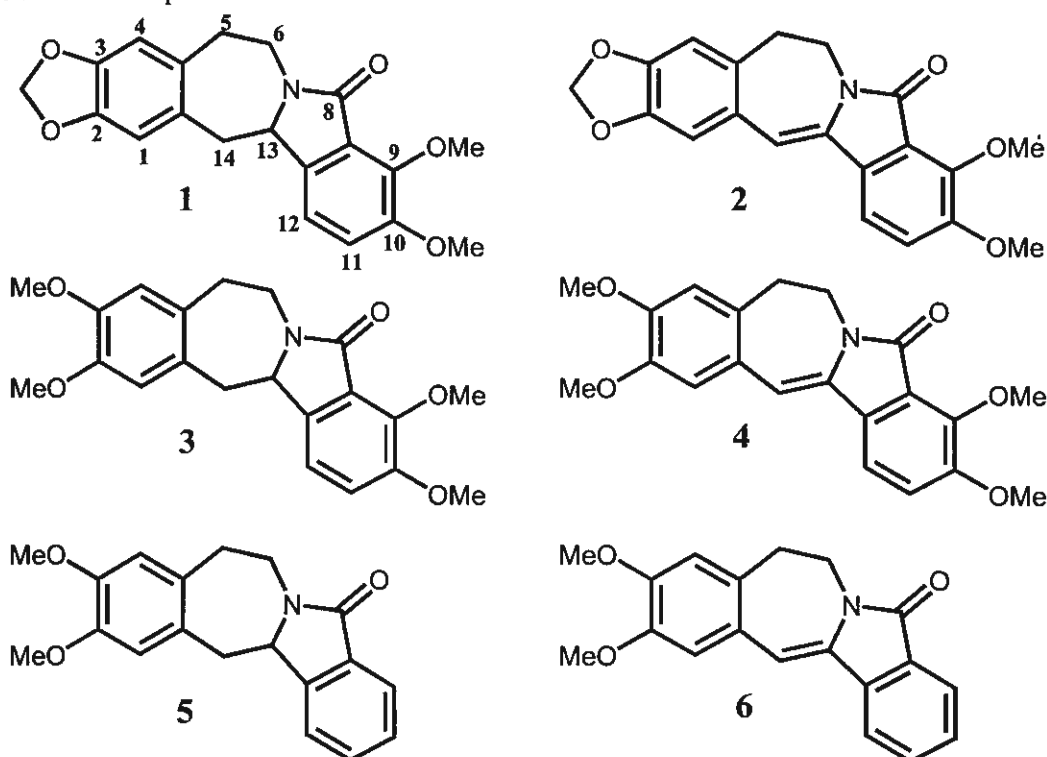
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Introduction

Lennoxamine (compound 1) is an isoindolobenzazepine obtained from *Berberis darwinii* Hook (Berberidaceae)¹. We have successfully synthesized Lennoxamine and also some other isoindolobenzazepine derivatives, including compounds 2, 3, 4, 5² and 6 (fig. 1).

Fig. 1 Isoindolobenzazepine Alkaloids

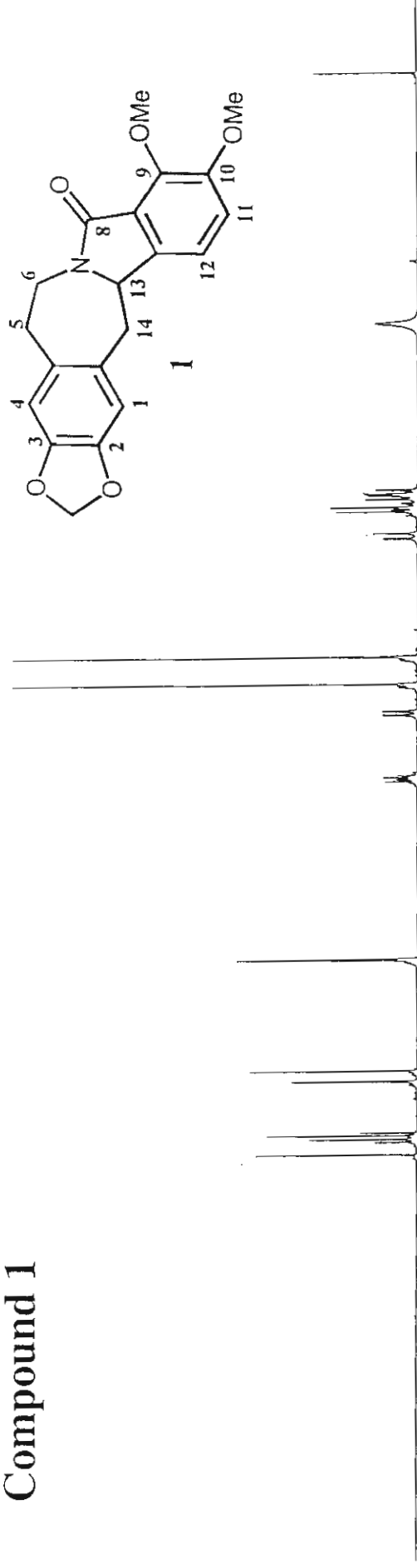


The NMR properties of these compounds have been studied in detail, including measurement of the signals at various temperatures.

NMR Study

All NMR experiments at ambient temperature were carried out on a Bruker AVANCE 400. The ¹H NMR spectra of these isoindolobenzazepine derivatives are shown in figs. 2a-2c.

Compound 1



Compound 2

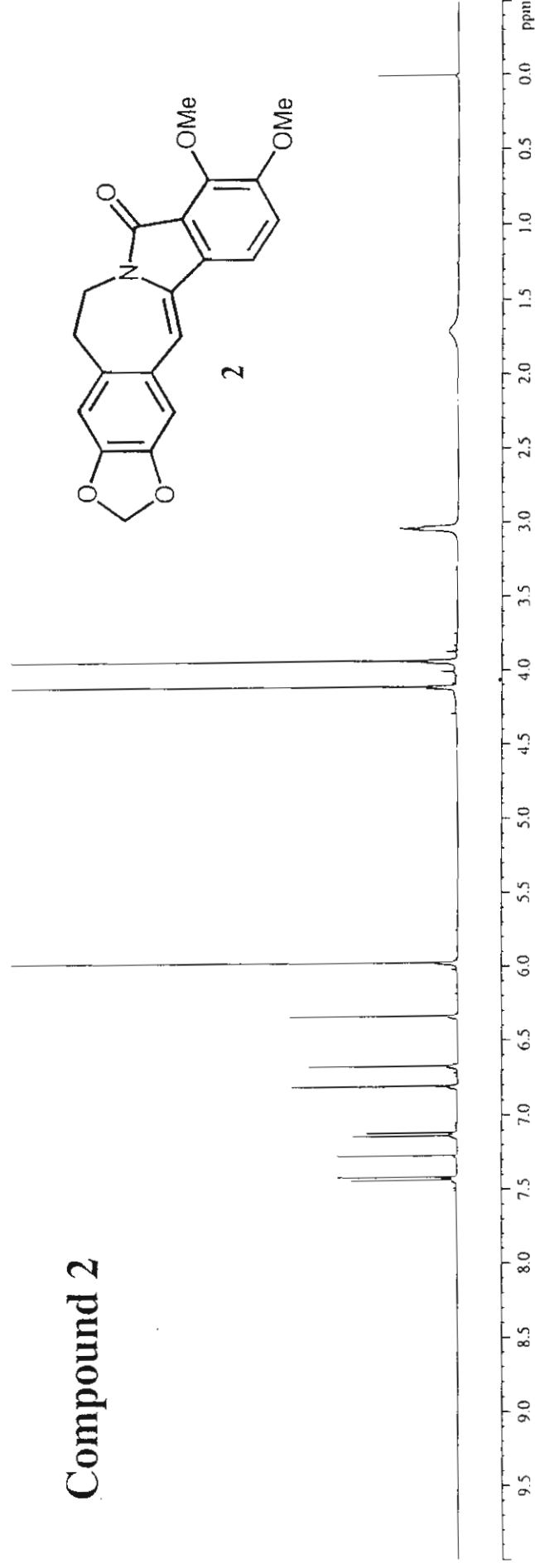


Fig. 2.a ¹H NMR Spectra of Compounds 1 and 2

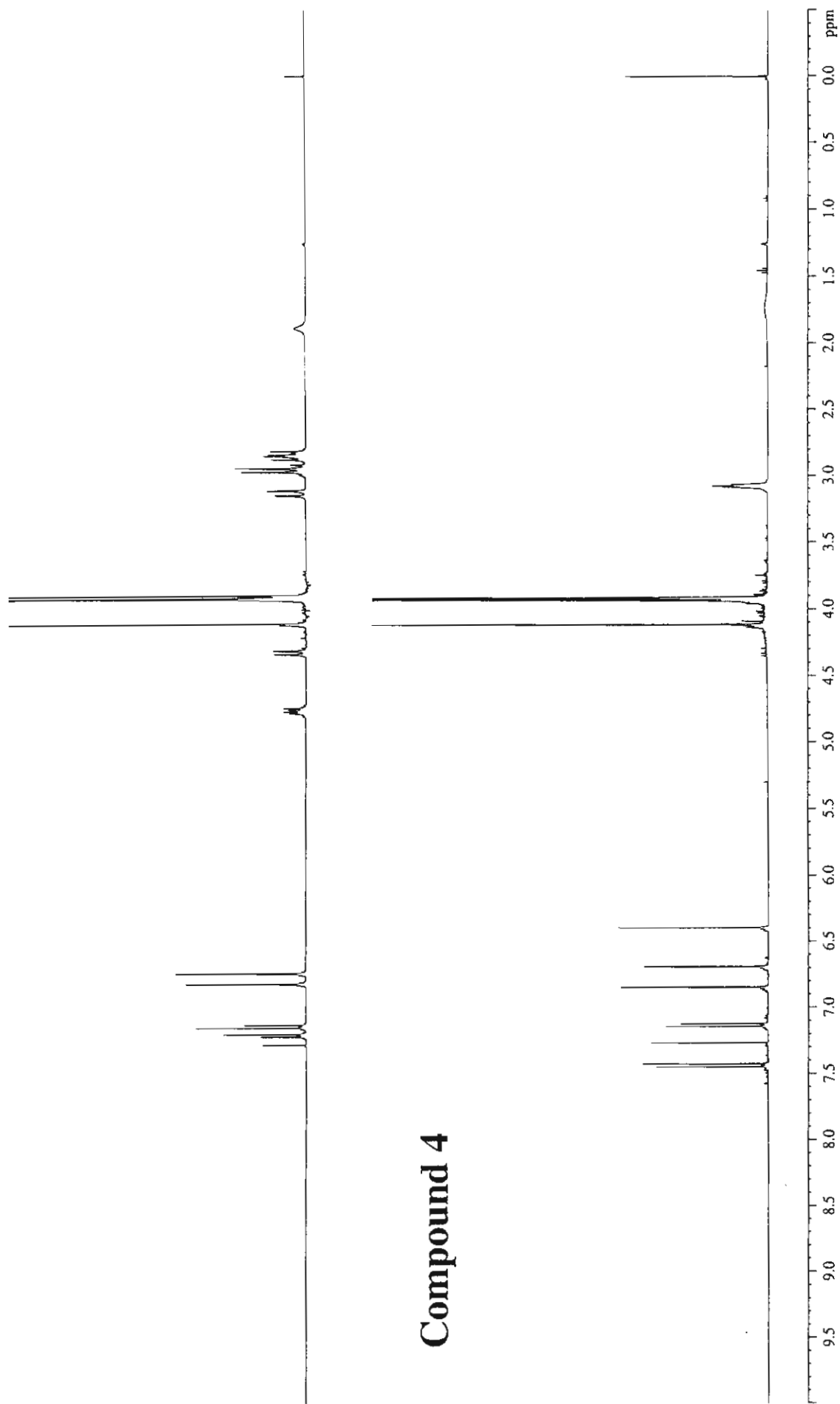
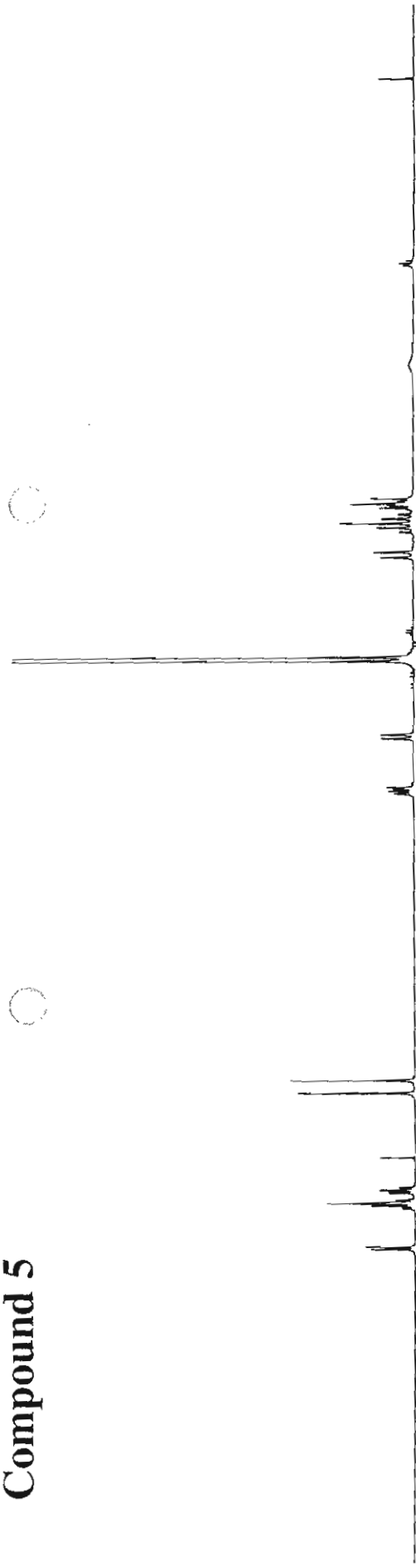


Fig. 2.b H-1 Spectra of Compounds 3 and 4

Compound 5



Compound 6

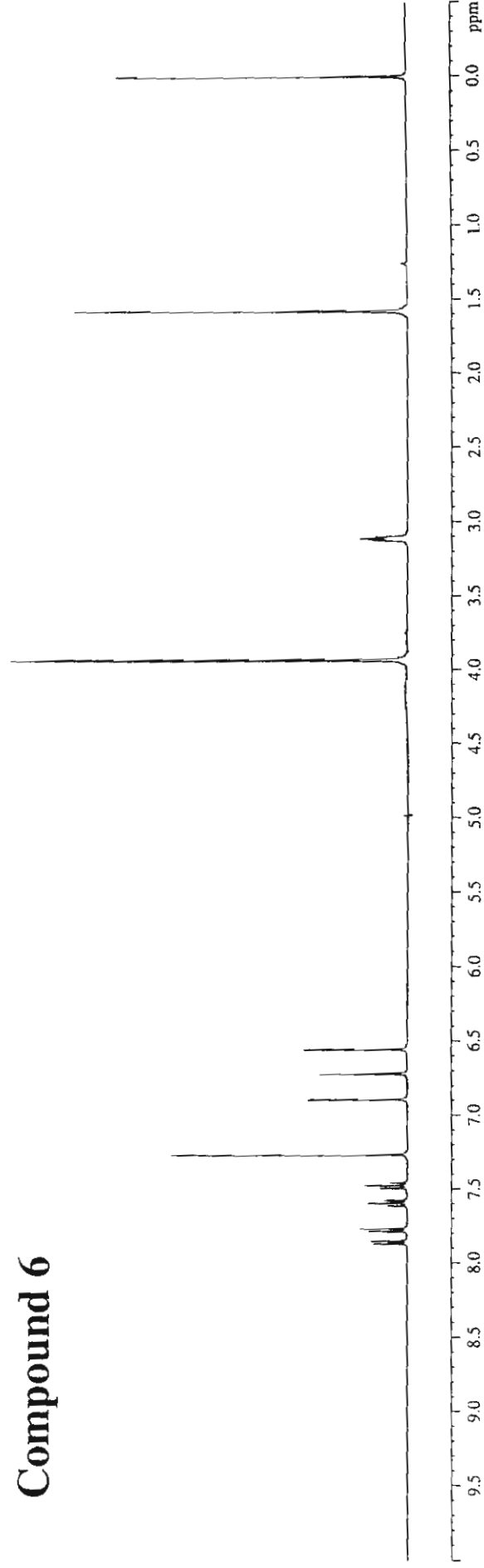


Fig. 2.c H-1 Spectra of Compounds 5 and 6

Very interestingly, the methylene protons alpha to the lactam nitrogen of compounds **2** and **4** are NMR-silent and the proton signal intensity of compound **6** is very low, while the intensities of protons in position 6 of the remaining compounds **1**, **3** and **5** are normal and non-equivalent. Similarly, the methylene protons in position 5 of the NMR-normal compounds (**1**, **3**, **5**) are non-equivalent and the corresponding protons of the NMR-silent compounds (**2**, **4**, **6**) are equivalent. We also found that the NMR-silent phenomena occur in DEPT and heteronuclear correlated spectra whose signals involve with the protons in position 6. The main structural difference between two groups, NMR-normal and NMR silent compounds, is the $\alpha\beta$ -single and double bonds (positions 13 and 14) to the lactam nitrogen.

As the methylene proton peak of compound **6** exists with low intensity, we planned to study all of the NMR-silent compounds under various temperature. We found that the higher the temperature, the higher intensity of the methylene proton peak was observed as shown in fig. 3. The methylene protons in positions 5 and 6 become non-equivalent at lower temperature, so the separated methylene proton peaks of the compounds **6**, **2** and **4** are presented in figs. 3-5, respectively. In fig. 3, the ^1H NMR spectra of compound **6** with 3 various temperatures, -60 , 25°C (in CDCl_3) and 120°C (in DMSO-d_6), are illustrated. The ^1H NMR spectra of compound **2** are presented with varying temperatures of -60 and 25°C in fig. 4, while the spectra of the compound **4** with 3 various temperatures, -60 , 25 and 60°C in fig. 5.

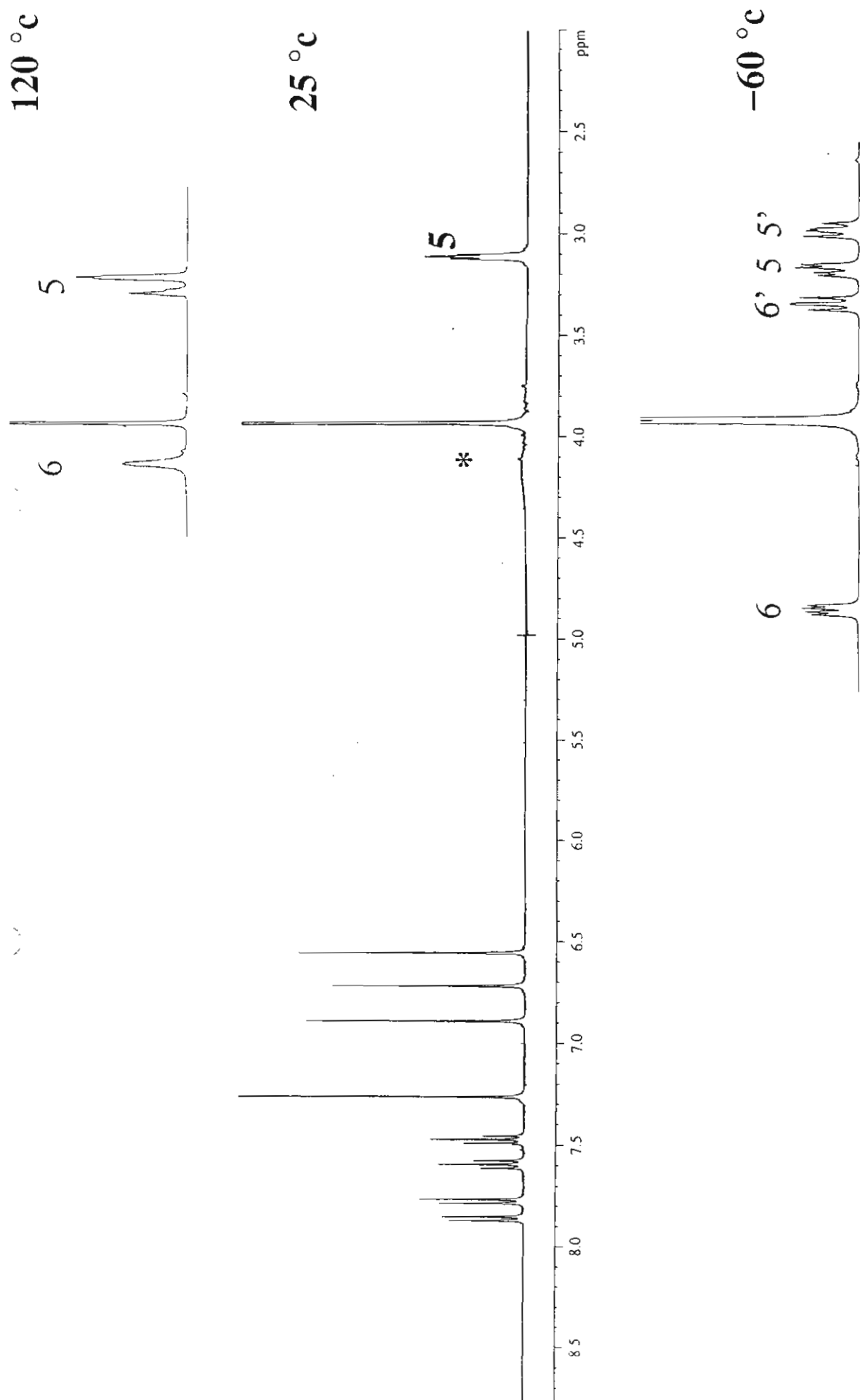


Fig. 3 ^1H -1 Spectra of Compound 6 at -60°C , 25°C (in CDCl_3) and 120°C (in $\text{DMSO}-d_6$)

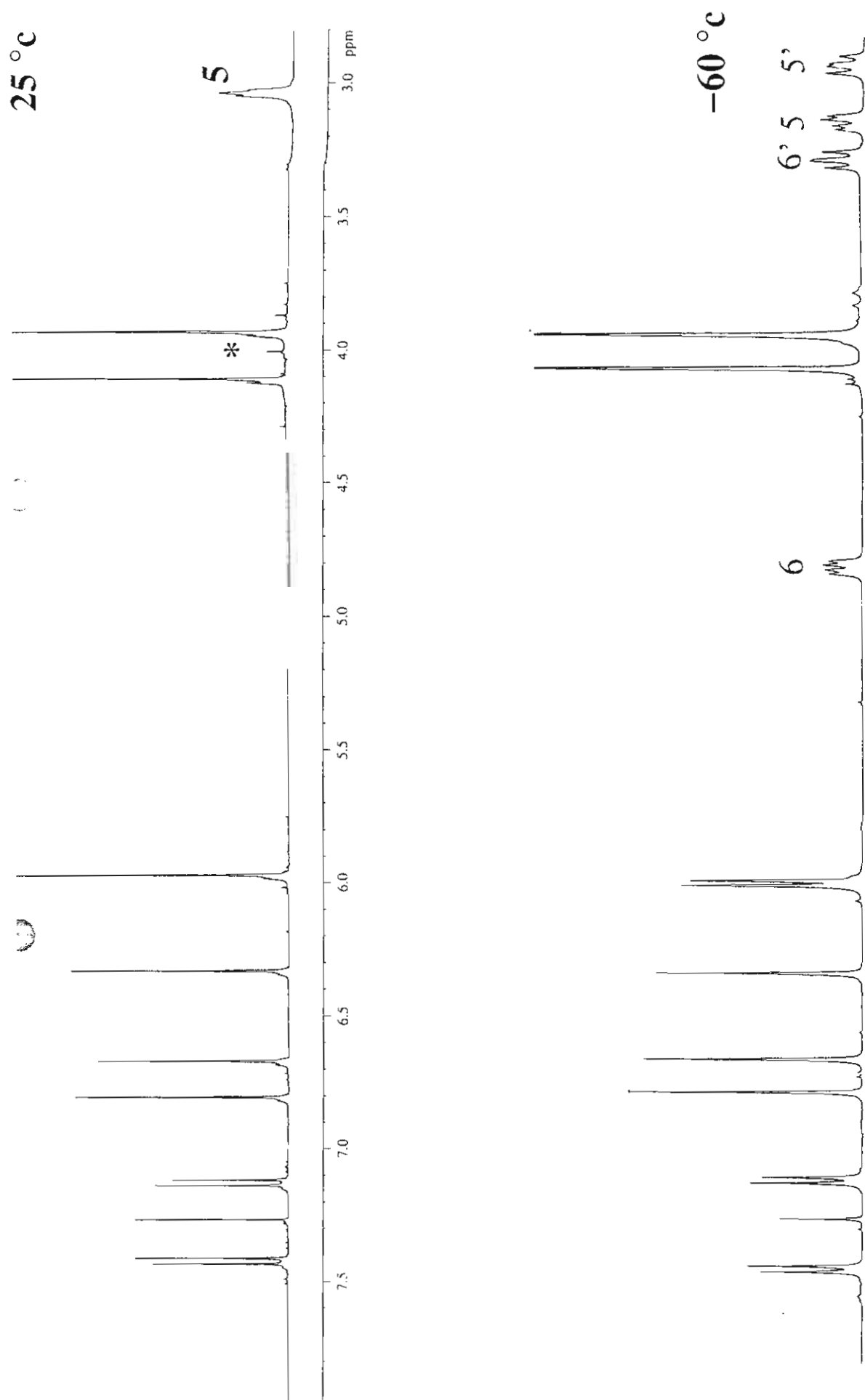


Fig. 4 ¹H NMR Spectra of Compound 2 at -60, 25 °C (in CDCl₃)

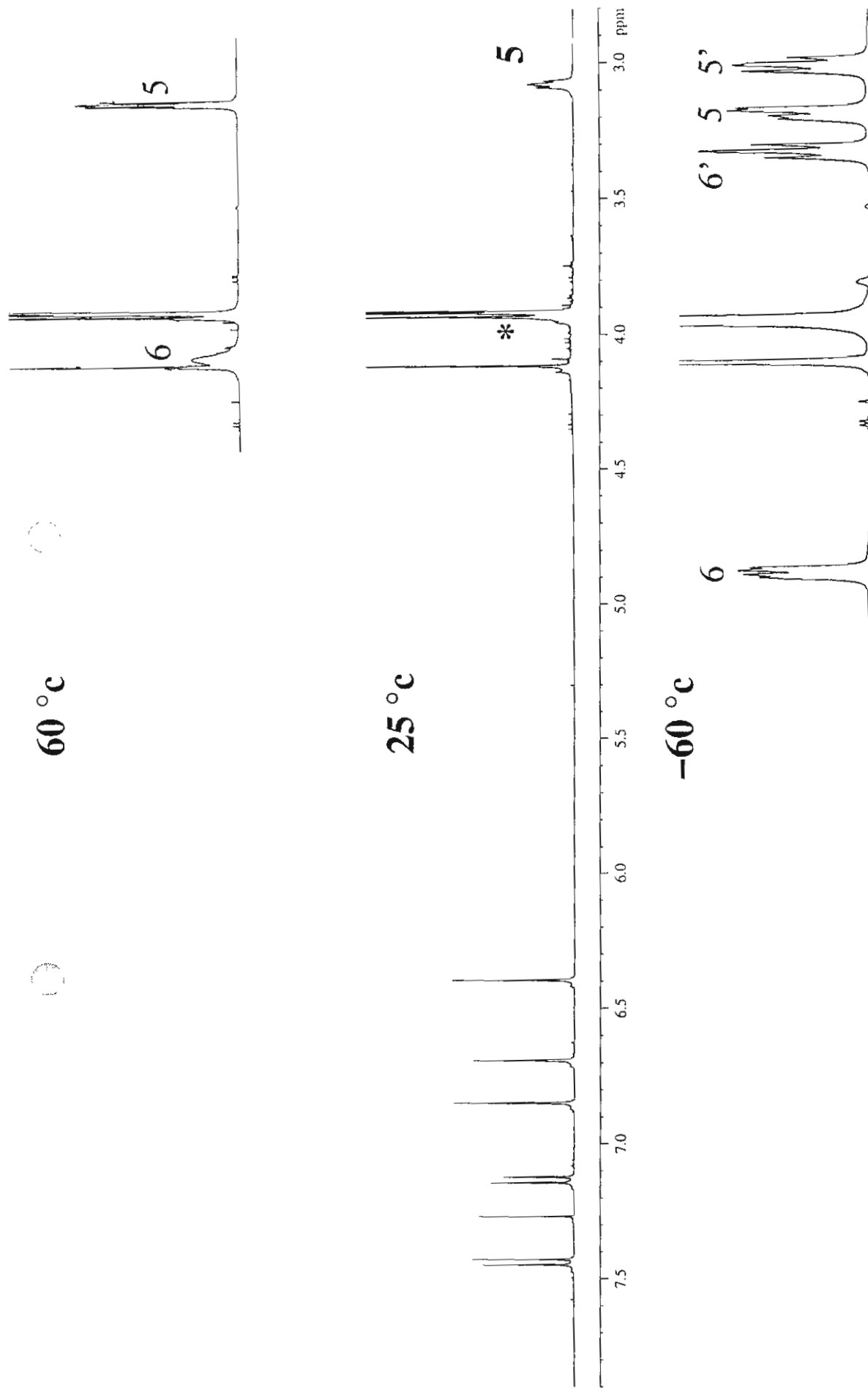


Fig. 5 ^1H -1 Spectra of Compound 4 at -60, 25 and 60 °C (in CDCl_3)

Discussion

The ^1H NMR spectra under various temperature of the isoindolobenzazepine alkaloids with saturated 7-membered ring (compounds **1**, **3** and **5**) show non-equivalent methylene protons in both positions 5 and 6; however, the corresponding compounds with unsaturated bond between positions 13 and 14 (compounds **2**, **4** and **6**) show non-equivalent methylene protons in both positions 5 and 6 at low temperature (-60°C). This means that some atoms, especially position 6, in the rigid unsaturated 7-membered ring flip to and fro rapidly at ambient temperature in order to reduce energy exerted from the steric effect; therefore, the methylene proton peak in position 6 is NMR-silent. The two methylene protons show two separated peaks at low temperature because they were frozen.

Conclusions

This phenomenon is a model to study NMR signals from two separated peaks, two broad peaks, no intensity (NMR-silent) peak, one broadly overlapped peak to one sharp peak with varying temperature from low to high. The NMR-silent evidence is rarely observed at ambient temperature. Further study of the other effects is under investigation.

Acknowledgements

Financial Support from Thailand Research Fund (TRF) is gratefully acknowledged. We would like to thank the Varian, Darmstadt, Germany and also Bruker South-east Asia, Thailand for some NMR experiments at low and high temperature.

References

1. Valencia, E.; Freyer, A.J. and Shamma, M.; *Tet.Lett.* 1984, 25(6), 599-602.
2. Ruchirawat, S.; Lertwanawatana, W.; Thianpatanagul, S.; Cashaw, J.L. and Davis, V.E.; *Tet.Lett.* 1984, 25(32), 3485-8.

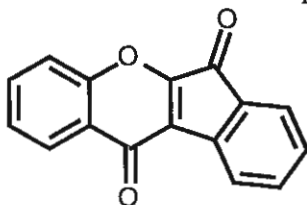
Efficient Synthesis Of The Tetracyclic Isoflavone: Wrightiadione

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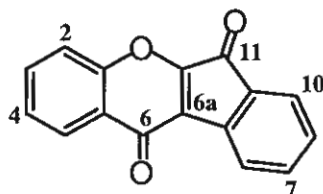
We wish to report the first synthesis of tetracyclic isoflavone wrightiadione. This compound contains the unique isoflavone ring system which was isolated from, *Wrightia tomentosa*, a Thai medicinal plant. Two approaches to the synthesis of this isoflavone will be presented.



Financial support from Thailand Research Fund (TRF) is gratefully acknowledged.

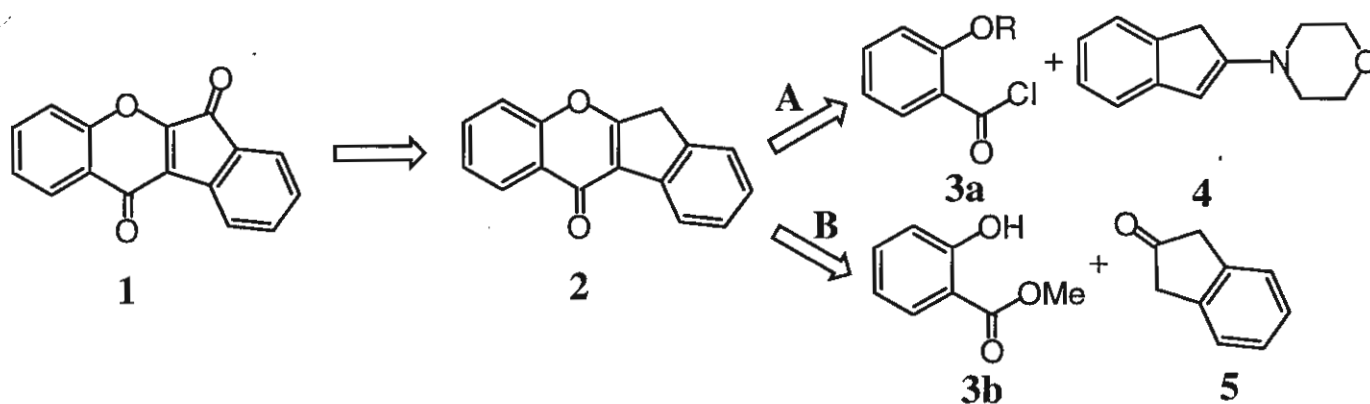
Introduction

Wrightiadione **1**, a novel isoflavone, was isolated from the bark of *Wrightia tomentosa* which has been used as medicinal plant in Thailand.¹ This compound contains the tetracyclic ring system which represents the first tetracyclic isoflavone and it is a unique natural product.



Wrightiadione

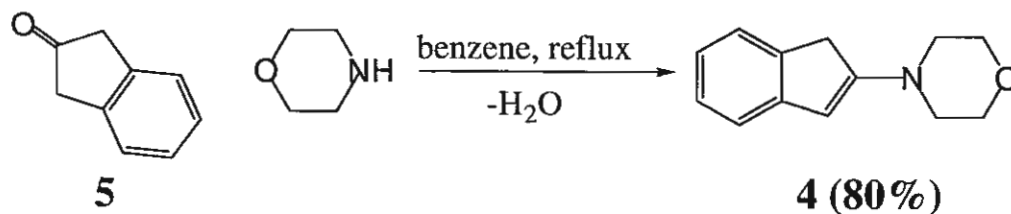
Retrosynthesis



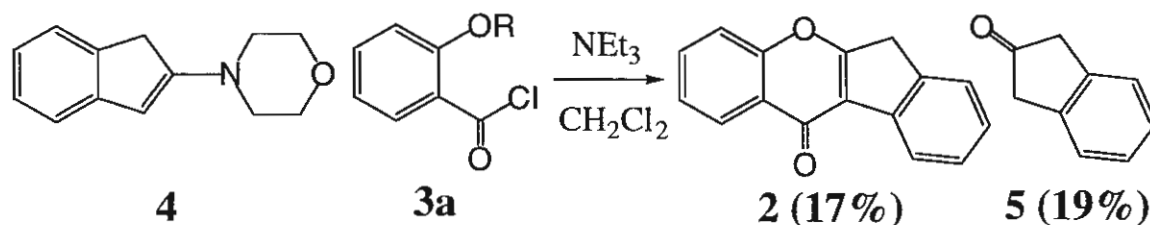
Retrosynthesis of compound **1** suggested isoflavone **2** as key intermediate as methylene position of this compound **2** could be oxidised to target molecule **1**. Compound **2** could be prepared from the acylation of acid chloride **3a** with 2-morpholine-2-indanone **4** by classical method of isoflavone synthesis in route A.² Another route, condensation of methyl salicylate **3b** with 2-indanone **5** by LDA/THF, -78°C could give isoflavone **2** in route B.³

Route A

2-Morpholine-2-indanone **4** was prepared from the reaction of 2-indanone **5** and morpholine in good yield (80%). (eq 1) However, on treatment of acid chloride **3a** with 2-morpholine-2-indanone **4** in the presence of triethylamine, isoflavone **2** was obtained in poor yield (17%) and 2-indanone was also obtained. (eq 2)



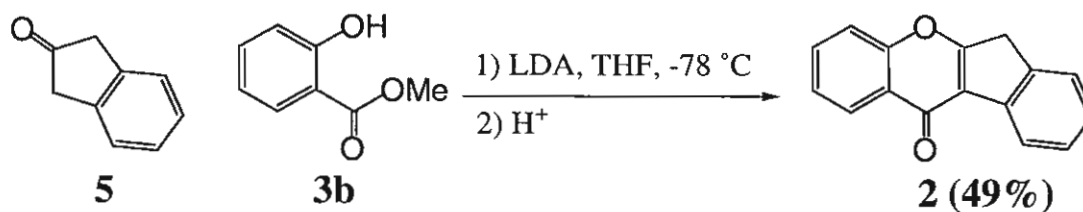
(eq 1)



(eq 2)

Route B

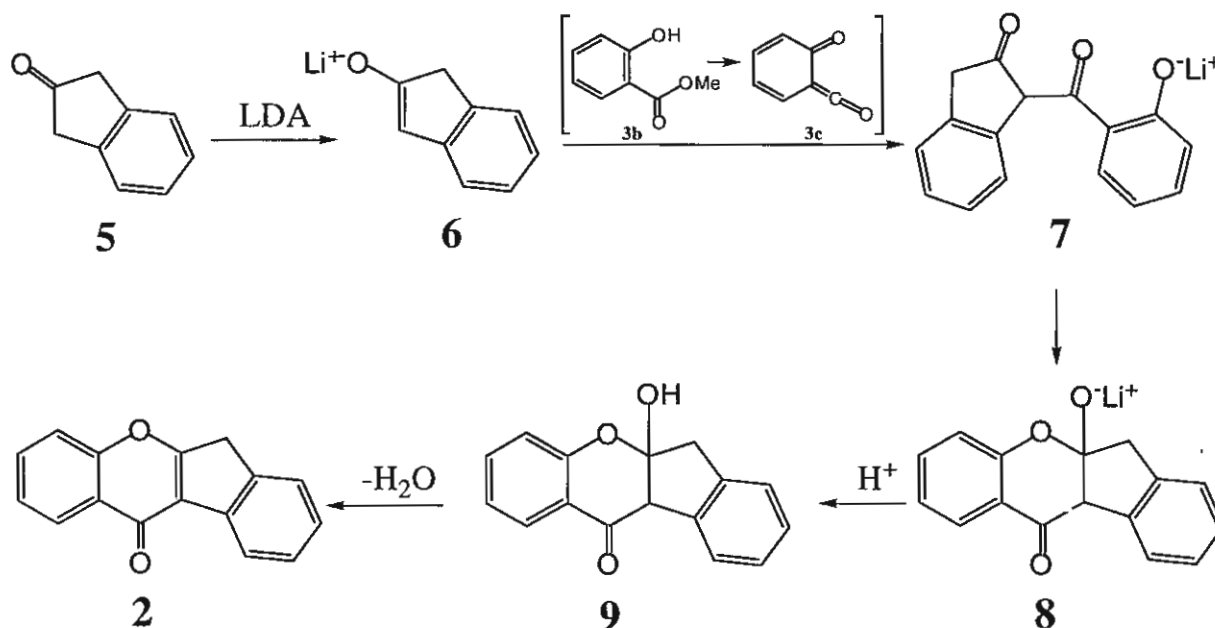
Isoflavone **2** was synthesised from the reaction of 2-indanone **5** and unprotected methyl salicylate **3b** by using LDA/THF at -78 °C as the base in moderate yield (49 %).



(eq 3)

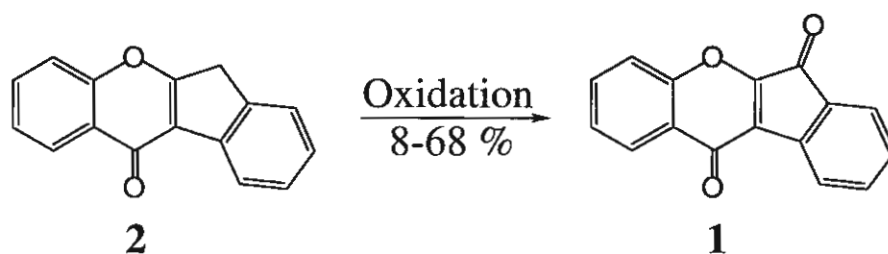
Mechanism

The enolate **6** was formed and reacted with methyl salicylate **3b** to give intermediate **7**. It is most likely that the reactivity of methyl salicylate as an acylating agent is due to the formation of the reactive intermediate **3c**. Cyclisation of intermediate **7** could lead to compound **9** *via* intermediate **8**. Dehydration of compound **9** produced the required **2** as shown in **Scheme 1**.



Oxidation step

In order to obtain the final product, the key benzylic oxidation had to be performed. It is a well known process for the benzylic oxidation to the corresponding carbonyl group. Many oxidation procedures for the preparation of wrightiadione **1** have been accomplished in varied yields as shown in Table 1. (eq 4)



The mass spectral fragmentation of wrightiadione **1**, gave the parent peak for $C_{16}H_8O_3$ [m/z 248 (M^+ , 100)] as the base peak and the fragment corresponding to losing CO molecule at m/z 220 (24), $C_{15}H_8O_2$.

The IR spectrum of compound **1** showed the absorptions at 1727 cm^{-1} and 1641 cm^{-1} corresponding to the carbonyl groups of five-membered ring and six-membered ring of compound **1**, respectively. The correlation of protons and carbons of compound **1** based on 1H NMR, ^{13}C NMR and 1H - ^{13}C ; ^{13}C - 1H COSY were obtained as shown in Table 2. For ^{13}C NMR spectrum, 15 peaks were observed for compound **1**. However, the signal of C-6a was not observed.

The comparison of NMR data of synthetic wrightiadione **1** with natural product revealed the discrepancy of our synthetic compound with natural product.¹ To prove the structure of the synthetic compound, X-ray crystallography of compound **1** was used to confirm. The ORTEP view is shown in **Figure 1**. [The X-ray crystallographic analysis was kindly carried out by Dr. Palangpon Kongseri of Mahidol University]

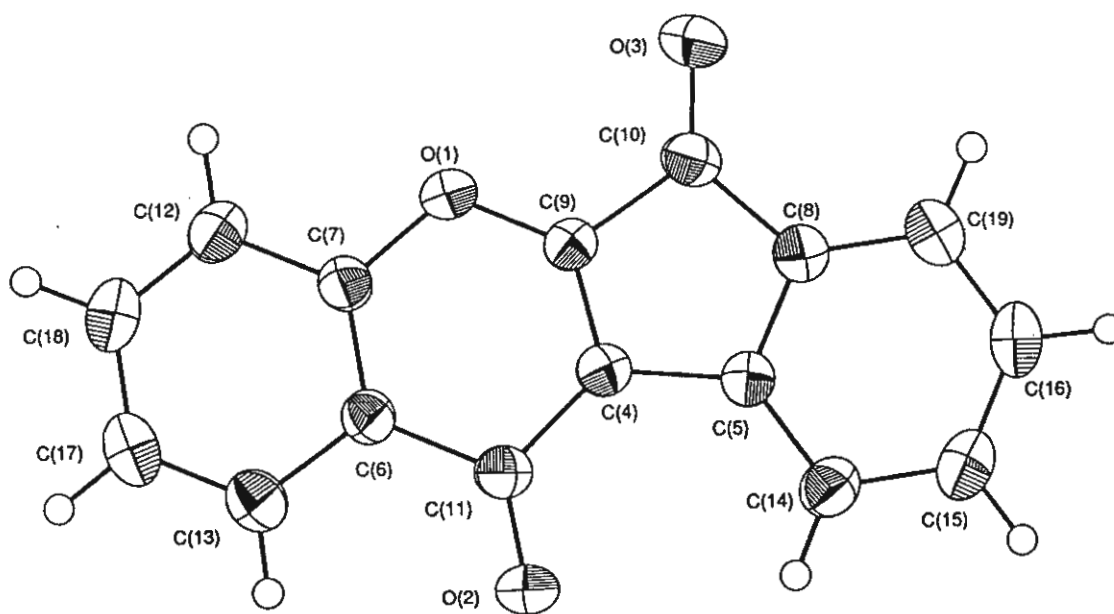


Figure 1. ORTEP view of the molecule with labeling of non hydrogen atoms

Table 1 Yield of compound **1** from various oxidation methods

Entry	Conditions
1	PDC, celite 535, benzene, reflux ⁴
2	PCC, celite 535, benzene, reflux ⁴
3	SeO ₂ , EtOH, H ₂ O, reflux ^{5,6}
4	CuBr ₂ , EtOAc, reflux ^{7,8}
5	50% HBr, DMSO, reflux ⁹
6	CAN, MeOH, RT ¹⁰
7	CAN, AcOH, reflux ¹¹
8	PCC, CH ₂ Cl ₂ , reflux ^{12,13}
9	NHPI, MeCN, reflux ¹⁴

Table 2 ^{13}C - ^1H NMR chemical shift assignment for **1**.^a

Carbon	^{13}C NMR	^1H NMR
1a	157.6*	-
2	120.7	7.69 dd (1.1, 8.5)
3	136.1	7.80 ddd (1.6, 7.1, 8.0)
4	127.8	7.54 ddd (1.1, 7.7, 8.0)
5	127.4	8.30 dd (1.6, 8.0)
5a	127.3	-
6	176.7	-
6a	**	-
6b	128.3	-
7	126.1	7.61 bd (7.3)
8	130.1	7.30 ddd (0.9, 7.3, 7.6)
9	137.4	7.53 ddd (1.1, 7.3, 7.6)
10	125.1	7.96 dd (0.9, 7.3)
10a	141.4	-
11	189.7	-
11a	157.3*	-

^a 400 MHz, CDCl_3 . * signals may be interchangeable, **C-6a not observed

Conclusion

In this work, we have accomplished the synthesis of wrightiadione **1** by various oxidation reactions of its key intermediate **2** which was prepared in one step from readily available starting materials. Moreover, we have further confirmed the structure of our synthetic wrightiadione by X-ray crystallography.

Acknowledgment

Financial support from Thailand Research Fund (TRF) is gratefully acknowledged.

References

1. Lin, L.-J.; Topcu, G.; Lotter, H.; Ruangrunsi, N.; Wagner, H.; Pezzuto, J. M.; Cordell, G. A. *Phytochemistry* **1992**, *81*, 4333.
2. Uchiyama, M.; Matsui, M. *Agr. Biol. Chem.* **1967**, *31*, 1490.
3. Morris, J.; Wishka, D. G.; Fang, Y. *Synth. Commun.* **1994**, *24*, 849.
4. Kodukulla, R. P. K.; Trivedi, G. K.; Vora, J. D.; Mathur, H. H. *Synth. Commun.* **1994**, *24*, 819.
5. Corey, E. J.; Schaefer, J. P. *J. Amer. Chem. Soc.* **1960**, *82*, 918.
6. Bockstahler, E. R.; Wright, D. L. *J. Amer. Chem. Soc.* **1949**, *71*, 3760.
7. King, L. C.; Ostrum, G. K. *J. Org. Chem.* **1964**, *29*, 3459.
8. Doifode, K. B.; Marathe, M. G. *J. Org. Chem.* **1964**, *29*, 2025.
9. Floyd, M. B.; Du, M. T.; Fabio, P. F.; Jacob, L. A.; Johnson, B. D. *J. Org. Chem.* **1985**, *50*, 5022.
10. Badea, I.; Cotellet, P.; Catteau, J.-P. *Synth. Commun.* **1994**, *24*, 2011.
11. Trahanovsky, W.; Young, L. B. *J. Org. Chem.* **1966**, *31*, 2033.
12. Parish, E. J.; Chitrakorn, S.; Wei, T.-Y. *Synth. Commun.* **1986**, *16*, 1371.
13. Dauben, W. G.; Lorber, M.; Fullerton, D. F. *J. Org. Chem.* **1969**, *34*, 3587.
14. Ishii, Y.; Nakayama, K.; Takeno, M.; Sakaguchi, S.; Iwahama, T.; Nishiyama, Y. *J. Org. Chem.* **1995**, *60*, 3934.

**INVESTIGATION OF *HYDNOPHYTUM FORMICARUM* JACK. AND
POLYALTHIA CERASOIDES (ROXB.) BENTH. EX BEDD.**

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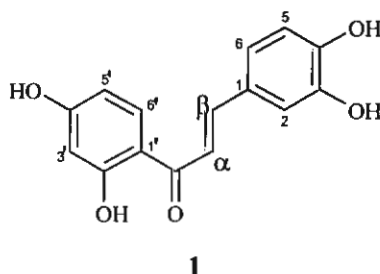
Introduction

Hydnophytum formicarum Jack., a Thai medicinal plant whose tuber possesses cardiovascular effect, antiparasitic action, antiinflammatory effect on gout as well as relief of skin rash, legache.¹ In addition, it has also been used for treatment of hepatitis (Malaysia) and headache (Malaysia-Java)².

Hydnophytum formicarum Jack. (Hua roi ruu) is commonly found in the East and South of Thailand, South-East Asia, Malaysia, Philippines, Indonesia, Papua New Guinea, Pacific Islands³, the Andamans, Tenas-serim, Cochin-China, Peninsula on the coast and in the mountains⁴.

Common name of *Hydnophytum formicarum* Jack. is an ant plant⁵. Names in Thai are varied from province to province, for example, kra chao pheemot (Surat thani), daa-luu-puu-taa-li-maa (Malay-Pattani), pum pao or pum pha (Trat), roi ruu (Pattani), hua roi ruu (Central)⁶. In Malaysia and Java *Hydnophytum formicarum* Jack is known as kepala berok or baboon's head⁵. In this study isolation and purification of some compounds from crude ethyl acetate is reported.

Result and Discussion



Structure of compound **1** was elucidated by IR, ^1H NMR, ^{13}C NMR and Mass spectra. Upon careful analysis of spectral data, compound **1** was shown to be butein.

Recently, butein (3,4,2',4'-tetrahydroxychalcone) has been reported to exhibit an anti-proliferative action on human tumor⁷, anti-oxidant effect⁸, endothelium-dependent relaxation of rat aorta⁹. In addition, butein show specific protein tyrosine kinase inhibitor¹⁰, inhibition of the efflux of glutathione S-conjugate, glutathione reductase¹¹.

In our study, compound **1** was tested on HuCCA-1 and KB cell lines. The results show that IC_{50} of compound **1** is greater than 10 $\mu\text{g/mL}$.

Experimental

Plant materials

Tubers of *Hydnophytum formicarum* Jack. were purchased from traditional drug store "Chao krom pur" Wat Sampluem, Samphanthawong, Bangkok.

Extraction and Isolation

The air-dried tubers of *Hydnophytum formicarum* Jack (approximately 20 cm diameter; 10 kg) were ground and extracted with n-hexane (7 days x 3), followed by filtration. The filtrate fractions were combined and evaporated to dryness under reduced pressure to obtain crude hexane extract. Similarly, the extraction was carried out using dichloromethane and ethyl acetate respectively. Crude ethyl acetate extract was isolated by silica gel column chromatography, elution by hexane-acetone, gradient solvent (9:1, 8:2, 7:3). The appropriate fractions were combined based on TLC monitoring.

Further purification of the obtained fractions were carried out by repeated silica gel column and/or preparative TLC together with crystallization to give compound **1** (18 mg).

Crystal : orange yellow needles (methanol), m.p. 213-215 °C

FTIR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3546, 3295, 1638

^1H NMR (CD_3OD , 400 MHz) 7.81 d ($J = 8.9$), 7.70 d ($J=15.3$), 7.40 d ($J=15.3$), 7.15 d ($J=1.8$), 7.05 dd ($J= 8.2, 1.8$), 6.82 d ($J=8.2$), 6.40 dd ($J=8.9, 2.4$), 6.33 d ($J=2.3$)

^{13}C NMR (CD_3OD , 100 MHz) 191.87, 165.49, 164.38, 147.87, 144.83, 144.61, 131.56, 126.77, 122.19, 116.86, 116.80, 114.28, 113.21, 107.86, 102.68

EI-MS m/z (%rel.int.) :

272 ($[\text{M}]^+$, 60), 255 (9), 163 (30), 150 (30), 137 (100), 123 (12), 110 (18)

References

1. Prommee, P. and et.al. (1988). *Thai traditional medicine*. Bangkok : Mahachulalongkon Publishing, p. 51. (Thai)
2. Beckstrom-Sternberg, S.M., Duke, J.A., and Wain, K.K. (1994, July). "*The Ethnobotany Data base*". (Online). <http://probe.nal.usda.gov:8300/cgi-bin/webace?db=ethnobotdb&class=Taxon&object=Hydnophytum+formicarium>
3. Huxley, C.R. and Jebb, M.H.P. (1991). 'The Tuberous epiphytes of the rubiaceae I: A new subtribe the hydnophytinae'. *Blumea*. 36 : 1-20.
4. Suvatti, C. (1978). *Flora of Thailand*. Bangkok : Kurusapha Ladprao, p.1138.
5. Morse, C. (1999, 23 August). "Botanical Collections". (Online). http://florawww.eeb.uconn.edu/acc_num/19800652.
6. Smitinund, T. (editor). (1980). *Thai plant names (botanical names-vernacular names)*. second edition, Bangkok : Funny publishing, p.183-184.
7. Yit, C.C. and N.P. Das. (1994). Cytotoxic effect of butein on human colon adenocarcinoma cell proliferation, *Cancer Lett.* 82, 65.

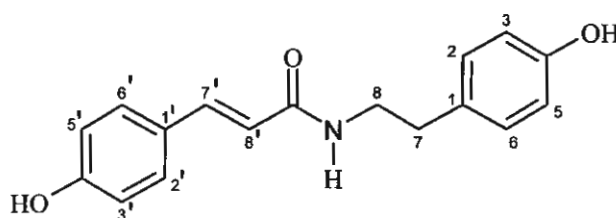
8. Cheng, Z.J. (1998). Antioxidant properties of butein isolated from *Dalbergia odorifera*. *Biochim Biophys Acta*. 1392, 291-299.
9. Yu, S.M., Cheng Z.J., Kuo, S.C. (1995). Endothelium-dependent relaxation of rat aorta by butein, a novel cyclic AMP-specific phosphodiesterase inhibitor. *Eur J Pharmacol*. 280, 69-77.
10. Yang, E.B. and other. (1998). Butein, a Specific Protein Tyrosine Kinase Inhibitor. *Biochem Biophys Res Commun*. 245 : 2, 435-438.

Introduction

Polyalthia cerasoides (Roxb.) Benth ex Bedd., Thai traditional medicine exhibits antituberculosis and antiinflammatory activities¹.

Common name of *Polyalthia cerasoides* (Roxb.) Benth ex Bedd. is saban-nga paa (north), kaa sam seek (Chiangmai), mai luang (Lampang), saypolsa (Kaliang, Maehongson), kaehang (Rachaburi) kajian (Chonburi), mokdong (Rayong), saiden (Konkaen)².

Result and Discussion



2

The structure of compound **2** was elucidated by IR, ¹H NMR, ¹³C NMR and Mass spectra.

After extensive column chromatography of crude ethyl acetate extracts, pure compound **2** was obtained as well as other three compounds for which their structures were being elucidated. Based on IR, ¹H NMR, ¹³C NMR and MS spectral data, structure

of compound **2** was deduced to be paprazine³ alkaloid. Compound **2** has been tested using HuCCA-1 and KB cell lines.

Experimental

Plant materials

Stem bark of *Polyalthia cerasoides* (Roxb.) Benth. Ex Bedd. were purchased from traditional drug store "Chao krom pur" Wat Sampluem, Samphanthawong, Bangkok.

Extraction and Isolation

The air-dried stem bark of *Polyalthia cerasoides* (Roxb.) Benth. Ex Bedd. (5 kg) were ground and extracted with *n*-hexane (7 days x 3), followed by filtration. The filtrate fractions were combined and evaporated to dryness under reduced pressure to obtain crude hexane extract. Similarly, the extraction was carried out using dichloromethane and ethyl acetate respectively. Crude ethyl acetate extract was isolated by silica gel column chromatography, elution by dichloromethane-methanol, gradient solvent (98:2, 96:4, 94:6). The appropriate fractions were combined based on TLC monitoring.

Further purification of the obtained fractions were carried out by repeated silica gel column and/or preparative TLC together with crystallization to afford compound **2** (15 mg).

Crystal : white amorphous solid (methanol), m.p.250-251.5°C

FTIR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3434, 3310, 1663, 1603

¹H-NMR (CD₃OD, 400 MHz) 7.44 d (J=15.7), 7.39 d (J=8.6), 7.04 d (J=8.5), 6.78 d (J=8.6), 6.72 d (J=8.5), 6.37 d (J=15.7), 3.45 dd, 2.74 dd

¹³C-NMR (CD₃OD, 100 MHz) 169.13, 141.69, 130.45, 130.63, 116.62, 116.17, 118.31, 42.44, 35.70

EI-MS m/z (%rel.int.) :

283 ([M]⁺, 8), 164 (72), 147 (100), 120 (55), 119 (25), 107 (29), 91 (26)

References

1. Prommee, P. et.al. (1988). *Thai traditional medicine*. Bangkok : Mahachulalongkon Publishing, p. 50. (Thai)
2. Smitinund, T. (editor). (1980). *Thai plant names (botanical names-vernacular names)*. second edition, Bangkok : Funny publishing, p. 270.
3. Atta-ur-Rahman et al. (1992). Alkaloids of *Fumaria indica*. *Phytochemistry*. 31 : 8, 2869-2872.

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CHEMICAL INVESTIGATION OF *MAMMEA SIAMENSIS*

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Department of Chemistry, Faculty of Science, Mahidol University³

Introduction

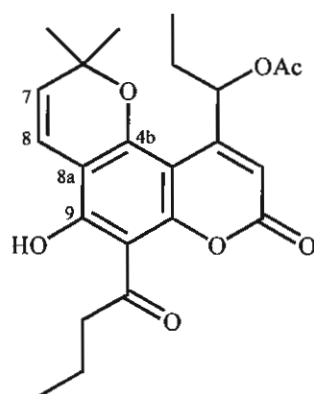
Mammea siamensis, previously known as *Ochrocarpus siamensis*, a plant belongs to the Guttiferae and belongs to the tribe Calophylleae of the subfamily Calophylloideae, is widely distributed in Thailand, Myanmar, Laos, Cambodia and Vietnam. The Thai name for the plant is 'sarapee'. Plant of this subfamily are known to be rich sources of xanthenes, triterpenes, steroids, chromenes and coumarins. The flower of *Mammea* is a well known traditional Thai medicine, especially used as a cardiac stimulant. In general, plant materials are extracted by a systematic series of extractions by solvents of increasing polarity. Usually, preliminary solvent for extraction of plant material is nonpolar, light petroleum ether or hexane, then diethyl ether, dichloromethane or chloroform and finally with ethanol or methanol. Each extract must then be separated into its individual compound for identification. Chromatography is a separation technique of a great resolving power and considerable complexity. The chromatographic methods, column processes are most useful for the separation of pure compounds from mixtures. Other methods, especially used include HPLC, PTLC, GC-MS and fractional crystallization.

Results and Discussion

Dried flowers of *Mammea siamensis* were purchased from a local traditional drug store in Bangkok, Thailand. Extensive column and preparative layer chromatography of hexane extract of the flower of *Mammea siamensis* afforded two novel compounds, mammea

E/BC cyclo D (1) and mammea E/BA cyclo D (2) together with two known compounds, mammea B/AC cyclo D (3)¹ and mammea A/AC cyclo D (4)². In this presentation, we describe the structural determination of compounds 1 and 2. Their structures were elucidated by spectroscopic methods (DEPT, COSY, HETCOR and COLOC) and chemical transformations.

Mammea E/BC cyclo D (1)

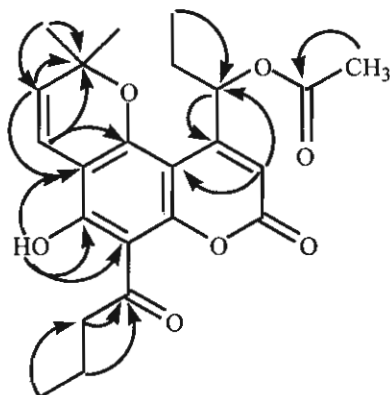


Mammea E/BC cyclo D (1) was obtained as a yellow solid, mp 139.4–140°C, and was found to be optically active ($[\alpha]_D = -48.79^\circ$).

The IR spectrum showed the carbonyl of aryl ketone absorption bands at 1644 and carbonyl of esters at 1732 cm^{-1} .

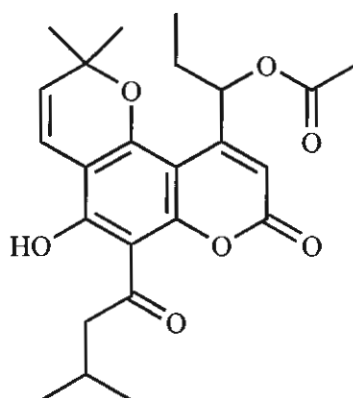
The high-resolution FABMS (positive mode) of 1 showed an accurate $[M+H]^+$ ion peak at m/z 415.1761 in accordance with the molecular formula $\text{C}_{23}\text{H}_{26}\text{O}_7$, which was further supported by the ^{13}C NMR and various DEPT spectral data.

The positions of the butyryl substituent and pyran ring were confirmed by acetylation which resulted in the appropriate NMR downfield shift of $\Delta\delta$ 0.11 for H-7 and highfield shift of $\Delta\delta$ 0.40 for H-8³. The diamagnetic shift of the H-8 resonance required its placement *peri* to the 9-OAc group, thereby locating the chromene ring between C-4b and C-8a of compound 1. Furthermore, the connectivities were supported by the COLOC spectrum as shown



Long-Rang correlations in COLOC of compound **1**.

Mammea E/BA cyclo D (**2**)



Mammea E/BA cyclo D (**2**) was isolated as a yellow semi-solid which was shown to be optically active ($[\alpha]_D = -68.571$)

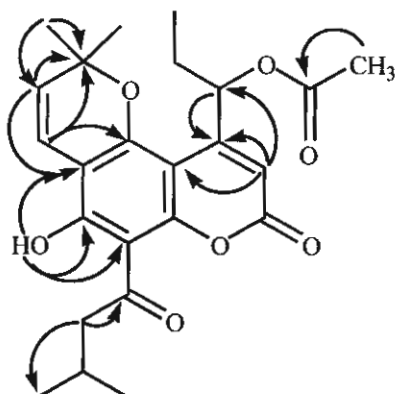
The IR spectrum showed the absorption band corresponding to the carbonyl groups of ester and aryl ketone at 1732 and 1645 cm^{-1} respectively.

The molecular formula $\text{C}_{24}\text{H}_{28}\text{O}_7$ was disclosed on the basis of high resolution mass spectrometry.

The NMR spectrum of **2** is almost identical with spectrum of **1** except for the substituent attached to C-8 of the coumarin nucleus (C-12a in mammea derivative). Unlike the ^1H NMR spectra of **1**, the spectrum of **2** exhibited signals at δ 2.27 (H-10, 9 lines, $J = 6.6$ Hz), 3.12 and 3.15 (H-11 and H-11', dd , $J = 15.5, 6.6$ Hz), 1.03 (H-18, 3H, d , $J = 6.7$ Hz) and 1.026 (H-19, 3H, d , $J = 6.7$ Hz) indicating the presence of 3-methylbutyryl group attached to C-8 of the coumarin nucleus instead of butyryl

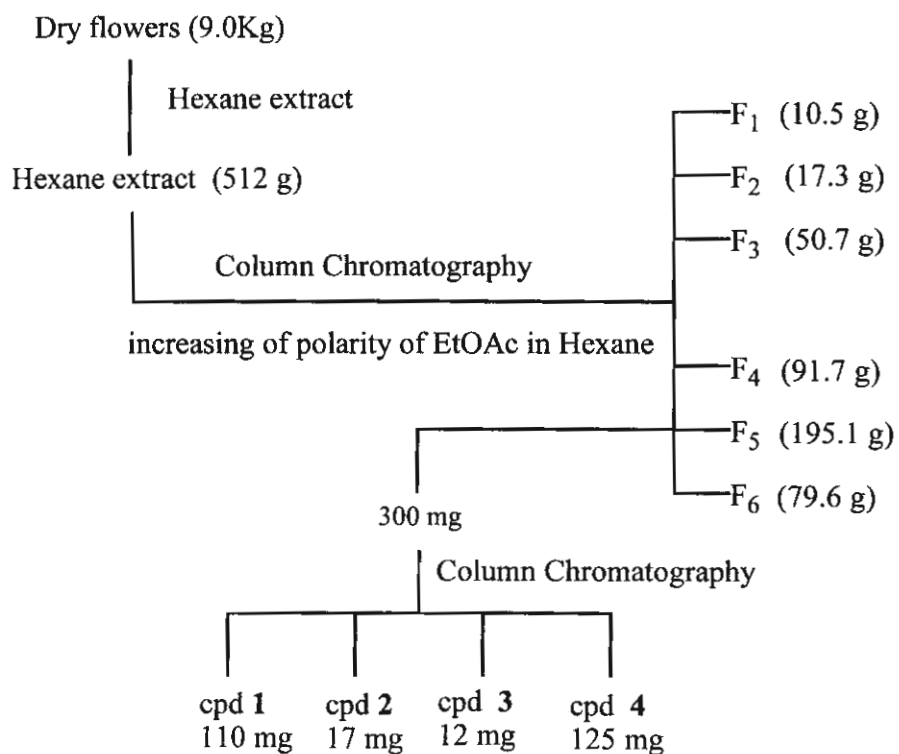
group. Comparison of the molecular formula of compounds **1** and **2** suggested an additional 14 unit for compound **2**.

The position of the phenolic group at C-9 was established by ^1H - ^{13}C correlations long-rang coupling (COLOC) of the phenolic proton (OH-9) to C-9, C-8a and C-12a as shown



Long-Rang correlations in COLOC of compound **2**.

Extraction and Isolation



References

1. Games DE., *Tetrahedron Lett.*, **1972**, *31*, 3187-3190.
2. Thebtaranonth C.; Imraporn S.; Padungkul N., *Phytochemistry*, **1981**, *20*(9), 2305-2306.
3. Arnon A.; Cardillo G.; Merlini L.; Mondelli R., *Tetrahedron Lett.*, **1967**, *43*, 4201-4206.

TWO NEW COUMARINS FROM THE TWIGS OF *MAMMEA SIAMENSIS*

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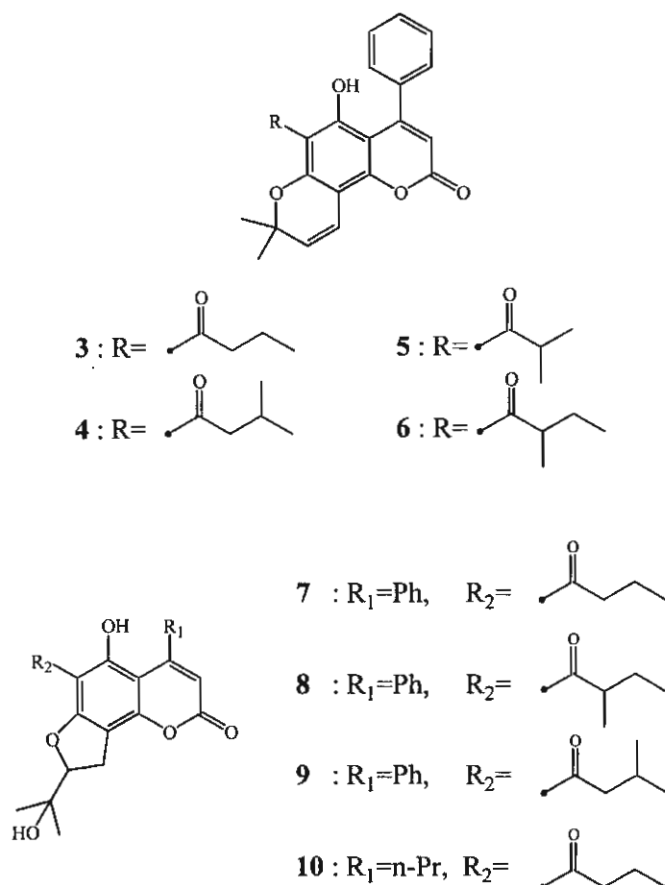
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Introduction

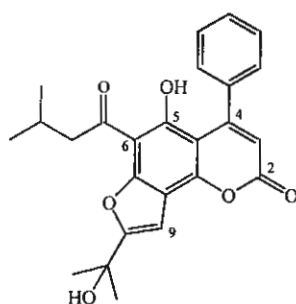
Mammea siamensis (Miq.) T. Anders is a member of tribe Calophylleae, subfamily Calophylloideae, family Guttiferae, which is native to Thailand, Myanmar, Laos, Cambodia and Vietnam. It has elliptic, smooth, dark green, leathery leaves. The flowers are white with numerous yellow stamens. The fruit is green and spherical, about one inch in diameter. The Thai name for the plant is 'sarapee'. The flowers have been used in the traditional medicine as a heart tonic. Previous phytochemical investigations of the flowers, twigs and leaves have shown the presence of coumarines¹, xanthenes² and proanthocyanidine polymers³ in this plant.

Results and Discussion

The hexane extract of the dried twigs of *M. siamensis* was fractionated by column chromatography and the fractions were further purified by preparative TLC and semipreparative HPLC to yield coumarins **1-10**. Two of them, coumarins **1** and **2** are novel compounds. Coumarins **3-10** were known compounds and were identified by spectroscopic data as well as comparison with the published data as mammeas A/AC cyclo D (**3**), A/AA cyclo D (**4**), A/AD cyclo D (**5**), A/AB cyclo D (**6**), A/AC cyclo F (**7**), A/AB cyclo F (**8**), A/AA cyclo F (**9**) and B/AC cyclo F (**10**), respectively. We describe here the isolation and structure elucidation of compounds **1** and **2** by spectroscopic methods, in particular by ¹H NMR and 2D NMR.



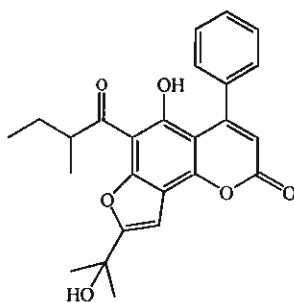
5-hydroxy-8-(1-hydroxy-1-methylethyl)-6-(3-methyl-1-oxobutyl)-4-phenyl-2H-furo[2,3-h]-1-benzopyran-2-one, Compound 1



Compound 1 was isolated as yellowish green needles, m.p. 156-159 °C. The molecular formula of compound 1 was determined to be C₂₅H₂₄O₆ from the [M+H]⁺ at *m/z* 421.1655 in the high resolution FAB-mass spectrum. The IR spectrum suggested the presence of δ-lactone (1728 cm⁻¹) and chelated acyl (1611 cm⁻¹) groups while the maximum absorption of UV spectrum appeared at 290 and 366 nm.

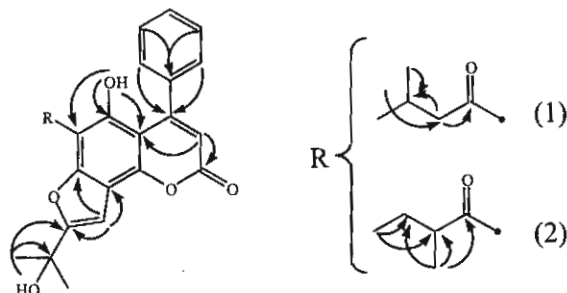
The 400 MHz ^1H NMR of compound **1** exhibited a proton singlet at δ 6.13 which was the characteristic of 3-olefinic proton in the coumarin structure, indicating that C-4 was substituted by phenyl group. The downfield ^1H NMR peak at δ 14.57 suggesting a strongly intramolecular hydrogen bonded phenolic proton (5-OH) located at the *peri* position of a carbonyl group. The COLOC spectrum was also useful for determining the position of the hydroxyl group in the molecule. Cross peak in the COLOC spectrum between C-4a (δ 104.65) and phenolic proton, and between C-4a and H-3 confirmed the position of the hydroxyl group. The nature of substituent was deduced as 3-methylbutyryl side chain from the signals at δ 3.11 (2H, *d*, $J = 6.7$ Hz), 2.28 (1H, *m*, $J = 6.7$ Hz) and 1.04 (6H, *d*, $J = 6.7$ Hz). The ^1H NMR spectrum further showed the presence of hydroxyisopropylfuran at δ 6.96 (1H, *s*) and one singlet corresponding to methyl group on a carbinol carbon at δ 1.74 (2 \times CH₃, *s*) which provided an additional proof for the structure of the isopropyl group.

5-hydroxy-8-(1-hydroxy-1-methylethyl)-6-(2-methyl-1-oxobutyl)-4-phenyl-2*H*-furo[2,3-*h*]-1-benzopyran-2-one, compound 2



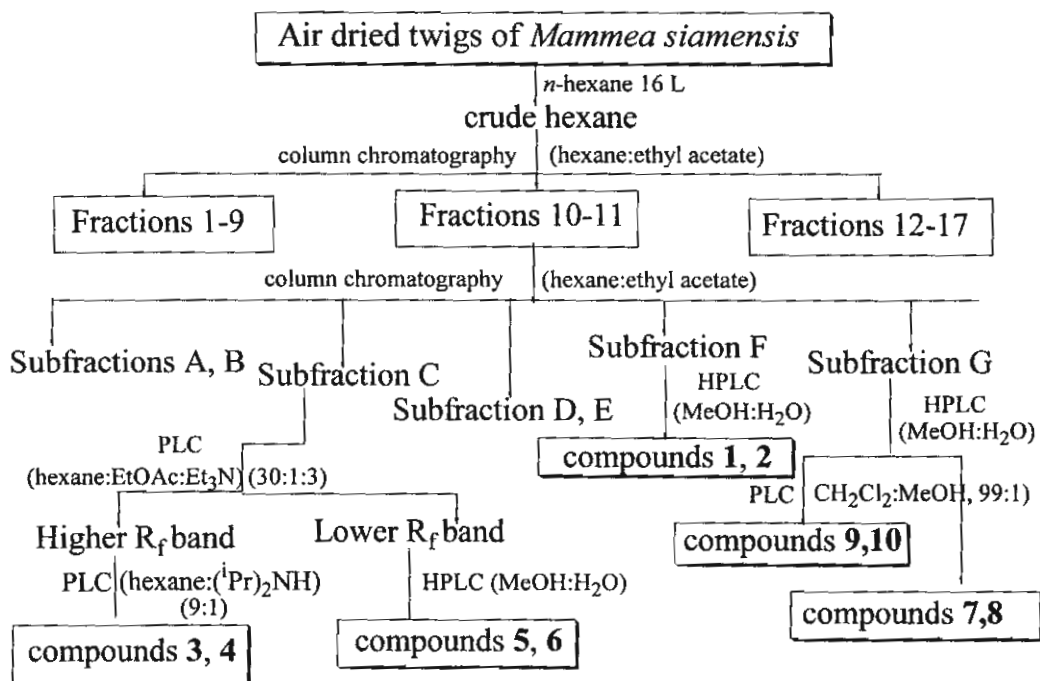
Compound **2** was obtained as yellow needles of m.p. 107-110 °C and was found to be an optically active compound, $[\alpha]_D^{27} +1.43^\circ$ (*c* 0.28, CHCl₃). Compound **2** showed similar patterns to those of **1** in its UV, IR and NMR spectrum, suggesting that they were similar in their structures. The molecular formula was determined to be C₂₅H₂₄O₆ by HRFABMS (*m/z* 421.1646, [M+H]⁺) and was the same as that of **1**. However, compound **2** differs from **1** in the 6-acyl side chain. Instead of the 3-methylbutyryl signals in compound **1**, the signals for 2-methylbutyryl were

observed in compound **2** [δ 3.79 (1H, *sext*, $J = 6.7$ Hz), 1.91 (1H, *m*), 1.52 (1H, *m*), 0.98 (3H, *t*, $J = 7.4$ Hz) and 1.25 (3H, *d*, $J = 6.7$ Hz)].



Main COLOC correlations of compounds **1** and **2**

Extraction and Isolation



Separation of all isolated compounds from *Mammea siamensis*.

References

1. Thebtaranonth C, Imraporn S, Padungkul N. *Phytochemistry* **1981**, 20(9), 2305-6.
2. Poobrasert O, Constant LH, Beecher CWW, Farnsworth NR, Kinghorn AD, Pezzuto JM, Cordell GA, Santisuk T, Reutrakul V. *Phytochemistry*, **1998**, 47(8), 1661-3.
3. Balza F, Abramowski Z, Towers GHN, Wiriyaichitra P. *Phytochemistry*, **1989**, 28(7), 1827-30.

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REINVESTIGATION OF *DERRIS RETICULATA*

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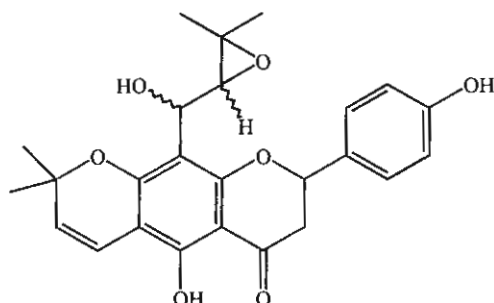
Introduction

Derris reticulata Benth. (Leguminosae) is a Thai medicinal plant used for the relief of thirst and as an expectorant. We have recently investigated this plant in detail and from the dichloromethane extract of dry powdered stems of this plant, we have now isolated and identified two new pyranoflavanones, 1'''-hydroxy-2''',3'''-epoxylupinifolin (1) and 2''',3'''-dihydroxylupinifolin (2), along with lupinifolin (3), 2''',3'''-epoxylupinifolin (4) and dereticlatin (5). Compounds 3, 4 and 5 were isolated from this plant for the first time. In this presentation, we describe the structural determination of compounds 1 and 2. The structures were determined by spectroscopic methods (DEPT, 2D-COSY, HMQC and/or HETCOR and HMBC) as well as by chemical transformations.

Results and Discussion

The dichloromethane extract of stems of *Derris reticulata* was subjected to vacuum liquid chromatography on silica gel to give 8 frs. Fr₆ was separated using silica gel PLC to obtain two bands, band I (compound 1) and band II. Band II was repeatedly separated by HPLC using Luna C8 column affording compound 2

1'''-hydroxy-2''',3'''-epoxylupinifolin (1)



FABMS(positive mode, rel. int.) 439([M+H]⁺, 40), 421(M+H-H₂O)⁺, 100)

CIMS(rel. int.) 439([M+H]⁺, 0.2), 420([M-H₂O]⁺, 11), 405(M+H-H₂O-CH₃)⁺, 32), 285(100).

The IR spectrum of **1** showed strong O-H absorption at 3386 cm⁻¹.

The presence of extra hydroxyl group at the benzylic position was indicated in the proton as well as ¹³C NMR at 5.47(5.46)[*d*, *J* = 7.2 Hz] and 66.42(66.37) respectively as compared with 2''',3'''-epoxylupinifolin(**4**)¹.

The structure of this compound was further confirmed by various spectroscopic methods including the correlations of the ¹³C-¹H and ¹H-¹H in HMBC and COSY respectively as shown in figure 1.

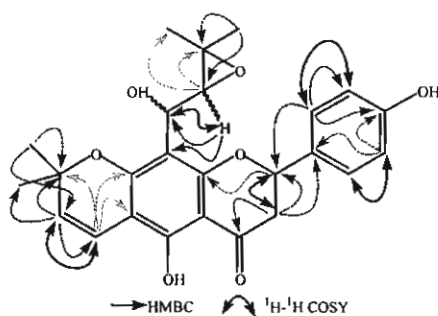
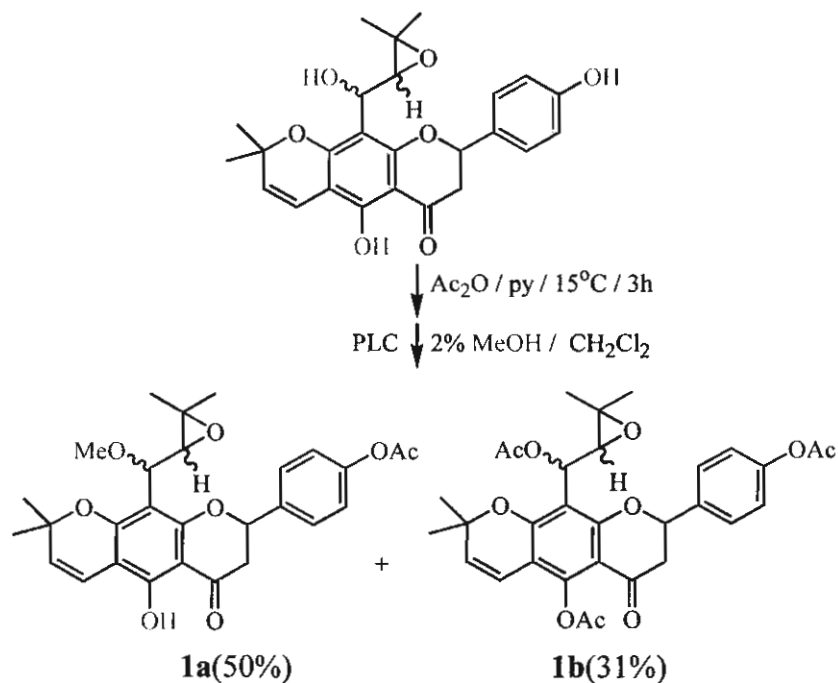


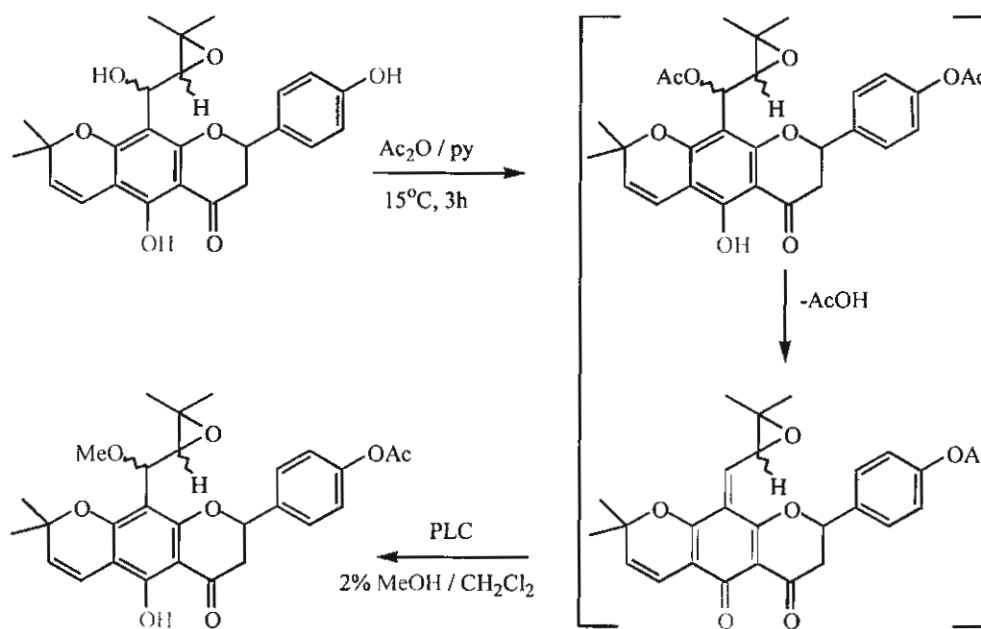
Figure 1 Summary of important connectivities observed in compound **1** by HMBC and ¹H-¹H COSY

The presence of one or more hydroxyl groups was confirmed by acetylation of **1** which afforded triacetate derivative **1b** as shown in scheme 1



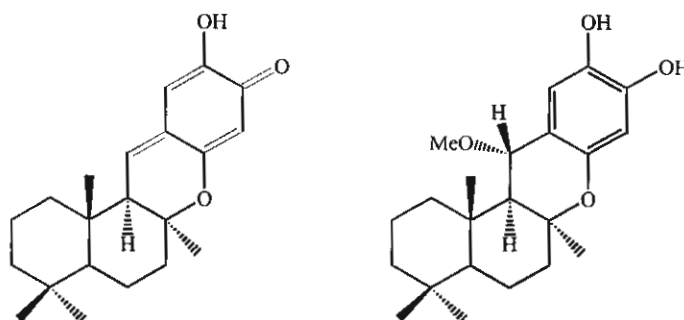
Scheme 1

The formation of the abnormal methoxy derivative under this acetylation condition can be explained by the mechanism shown in scheme 2. Acetylation of the hydroxyl group led to the acetoxy derivative. Loss of the acetic acid with the help of the hydroxyl group led to the quinone methide intermediate which could then react with methanol to give the methoxy compound. Apparently the compound was formed during PLC purification when methanol was used as developing solvent.

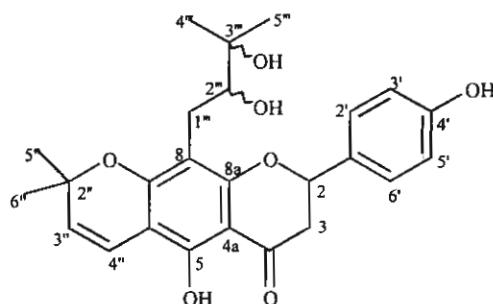


Scheme 2

Interestingly, the methanol adduct was very recently isolated as an artifact resulting from the addition of methanol on quinone methide.²



2''',3'''-dihydroxylupinifolin (2),



EIMS(rel. int.) 440(M^+ , 50), 425($[M-CH_3]^+$, 70), 287(28)

The IR spectrum of **1** showed strong O-H absorption at 3390 cm^{-1} .

The structure was supported by the study of HMBC correlations of various atoms as shown in figure 2.

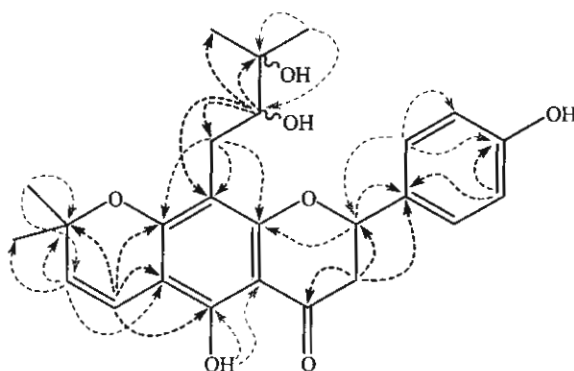
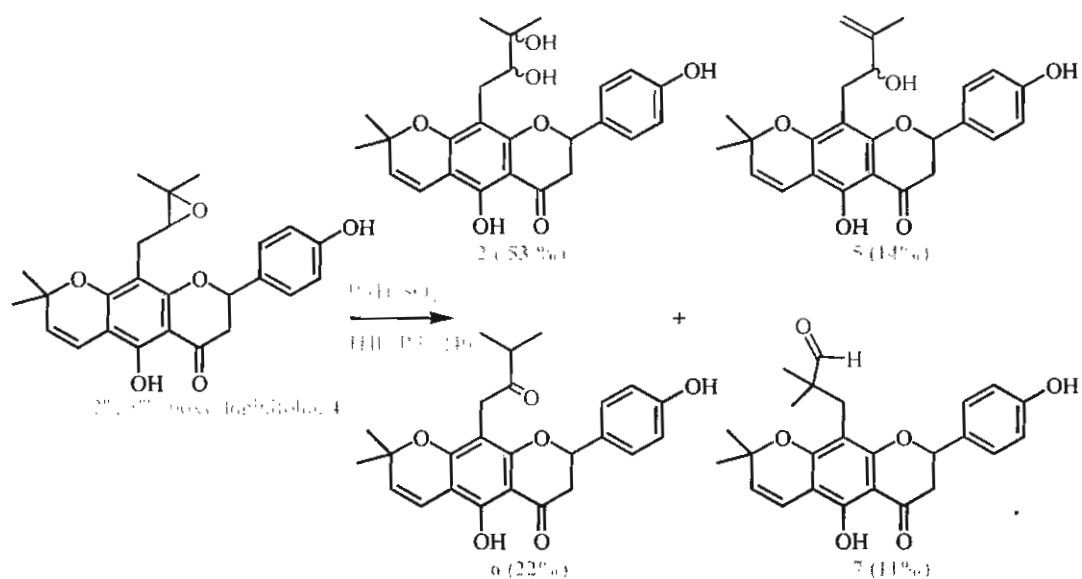


Figure 2 HMBC correlations of diol

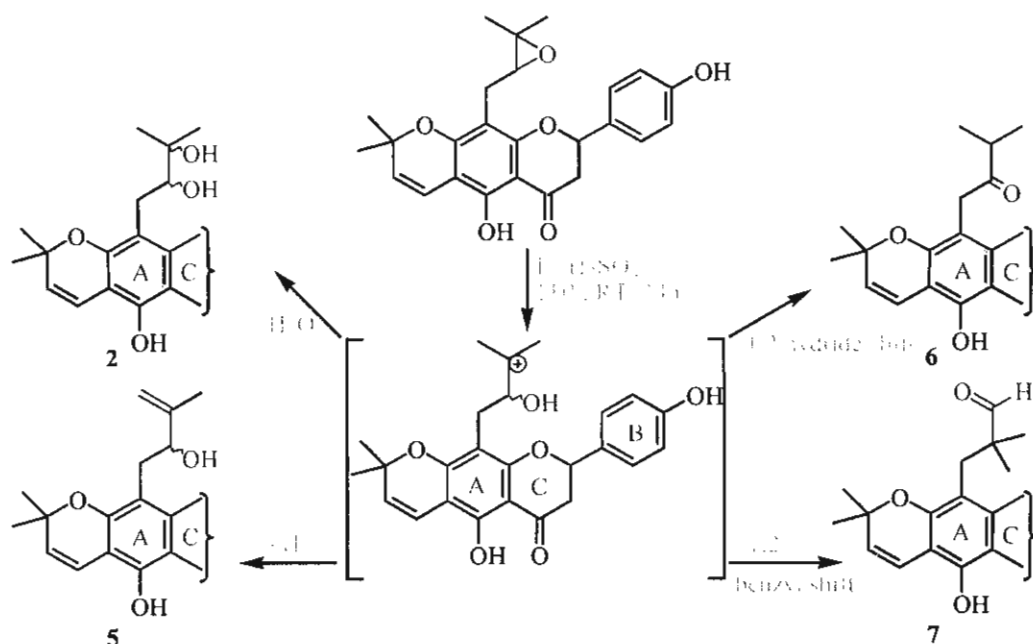
The structure of this compound was further confirmed by reaction of epoxylupinifolin with 1% sulfuric acid in THF at room temperature for 24 h. From this reaction, four products were isolated as shown in scheme 3.



Scheme 3

The diol (**2**) was isolated as the main product in 53% yield, dereticulatin (**5**) was obtained in 14% yield, the ketone and aldehyde derivatives were isolated in 22 and 11 % yields respectively.

We proposed the mechanisms of the formation of these compounds as shown in scheme 4.



Scheme 4

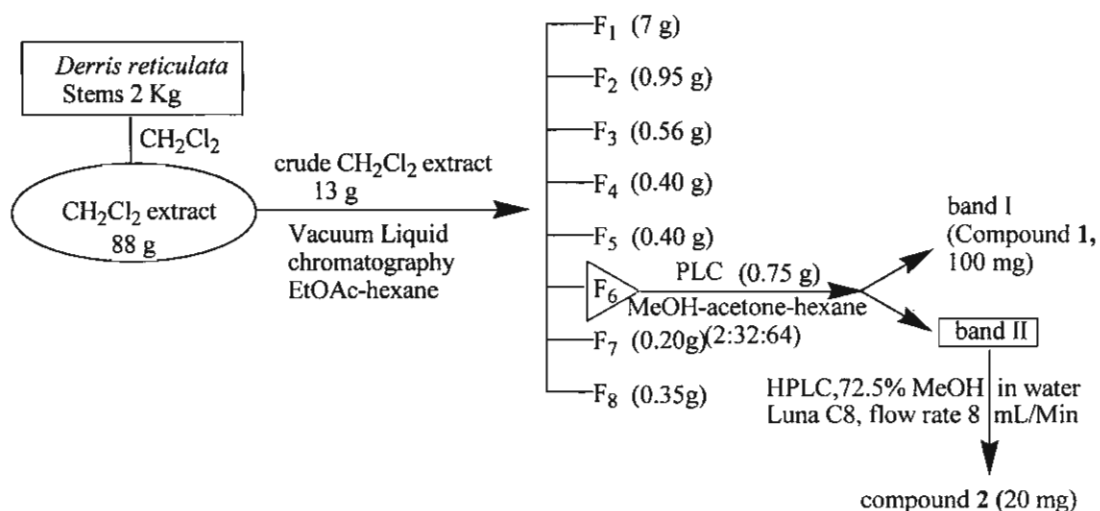
Opening of the epoxide ring gives the stable carbonium ion, addition of water to this carbonium ion provides the diol compound **2**.

Loss of proton from the methyl group can generate the dereticulatin **5**.

1,2-Hydride shift and 1,2-benzyl shift produce the ketone and aldehyde intermediates respectively.

The above finding is very interesting and it gives the insights into the biosynthesis of these C-5 side chains.

Extraction and Isolation



References

1. Chulabhorn Mahidol, Hunsa Prawat, Somsak Ruchirawat, Kittisak Lihkitwitayawuid, Long-Ze Lin and Geoffrey A. Cordell, *Phytochemistry*, 1997, **45**, 825-829.
2. Marie-Line Bourguet-Kondracki, Fabrice Lacombe and Michele Guyot, *J. Nat. Prod.*, 1999, **62**, 1304-1305.

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การเสนอผลงานในที่ประชุมวิชาการในประเทศ

องค์ประกอบทางเคมีจากสารสกัดเอทิลแอซิเตตของหัวร้อยรู
(*HYDNOPHYTUM FORMICARUM* JACK.)
CONSTITUENTS FROM ETHYL ACETATE EXTRACTS OF
HYDNOPHYTUM FORMICARUM JACK.

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บทคัดย่อ

หัวร้อยรูเป็นพืชสมุนไพรไทยที่ใช้กันในอดีตจนถึงปัจจุบัน มีสรรพคุณต่าง ๆ เช่น บำรุงหัวใจ ขับพยาธิ แก้อักเสบ เมื่อนำส่วนหัวของต้นหัวร้อยรูมาสกัดด้วยเอทิลแอซิเตต และนำไปแยกด้วยคอลัมน์โครมาโทกราฟี ได้สารบริสุทธิ์ 4 ชนิด ได้แก่ isoliquiritigenin(1), protocatechualdehyde(2), butin(3), และ butein(4) วิเคราะห์โครงสร้างของสารเหล่านี้โดยใช้ข้อมูลทางสเปกโทรสโกปี (COSY, HMQC และ HMBC)

Abstract

Hydnophytum formicarum Jack., a Thai medicinal plant has long been used as traditional medicine, possesses cardiac effect, antiparasitic and anti-inflammatory activities. Its tubers crude ethyl acetate extracts were isolated by column chromatography giving four pure compounds of isoliquiritigenin(1), protocatechualdehyde(2), butin(3) and butein(4). Structures of these compounds were elucidated on the basis of spectral analysis, including COSY, HMQC and HMBC.

Introduction

Hydnophytum formicarum Jack., a medicinal plant whose tuber possessed cardiovascular effect, antiparasitic action, antiinflammatory effect on gout as well as relief of skin rash, legache and neurotonic. In addition, it has also been used for treatment of hepatitis (Malaysia), headache (Malaysia-Java) and liver complaints (Philippines).

Besides being obtained from natural sources, tubers of *Hydnophytum formicarum* Jack. have been produced by tissue culture and investigated for alkaloids content. The results have shown that tubers of tissue culture produce more alkaloid than the natural one.

However, our previous chemical investigation of *Hydnophytum formicarum* Jack. has shown that β -sitosterol and ketone compound, $C_{18}H_{16}O_2$, were found in crude hexane and crude chloroform respectively.

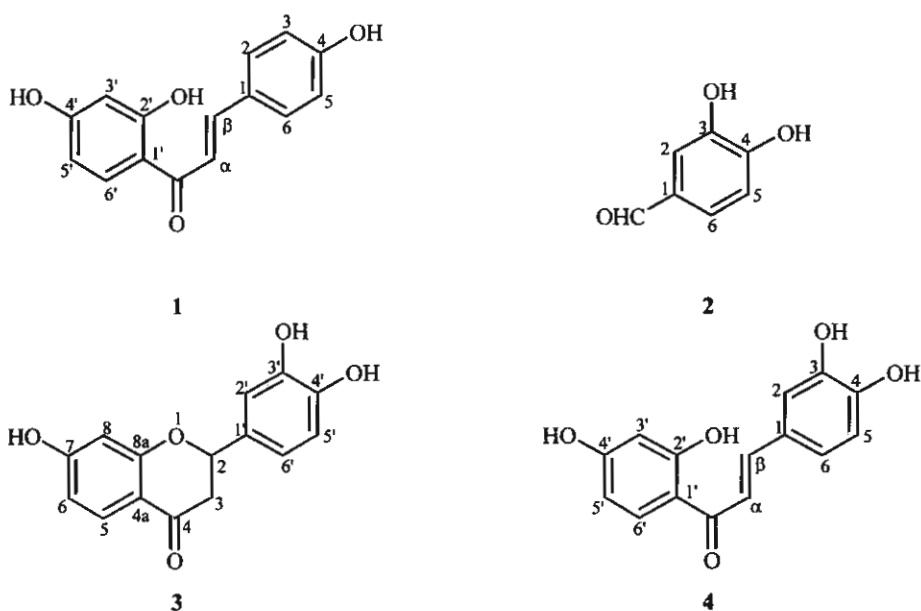
This study we aim to isolate and elucidate structure of some pure compounds using spectroscopic methods.

Experimental

Plant materials were purchased from traditional drug store "Chao krom pur" Wat Sampluem, Samphanthawong, Bangkok. Crude ethyl acetate extract (59 g) of tuber of *Hydnophytum formicarum* Jack. was isolated by silica gel column chromatography using gradient elution of hexane-acetone and finally enriched with methanol. Three selected main fractions were separately isolated and purified by repeated silica gel column and preparative TLC including recrystallization to give isoliquiritigenin **1** (from 5% methanol-dichloromethane), protocatechualdehyde **2** (from 3% methanol-dichloromethane), butin **3** and butein **4** (from 6% methanol- dichloromethane).

Results and Discussion

The crude ethyl acetate extract (59 g) of *H. formicarum* Jack. was separated by silica gel column using gradient elution of hexane-acetone with increasing polarity. Several crude fractions were collected and examined by ^1H NMR spectroscopy. Thus selected fractions were further purified by silica gel column and preparative TLC to obtain 4 compounds, **1-4**.



Compound 1

Compound **1** was isolated as yellow solid, m.p. 182-183°C. The structure of **1** was elucidated by spectroscopic method. Its molecular formula $\text{C}_{15}\text{H}_{12}\text{O}_4$ was established by EIMS showed the molecular ion at m/z 256. The IR spectrum of **1** exhibited characteristic absorption bands for hydroxy (3514 cm^{-1}), carbonyl (1634 cm^{-1}) functionalities. While the UV spectrum showed a maximum absorption at 369 nm.

The 100 MHz ^{13}C NMR of compound **1** exhibited 13 signals. The DEPT spectra (DEPT-90 and DEPT-135) established the existence of nine methine carbons δ 144.40(CH- β), 131.72(CH-6'), 130.48(CH-2,6), 117.20(CH- α), 115.90(CH-3,5), 108.18(CH-5'), 103.08(CH-3'), six quaternary carbons δ 191.92(CO), 165.71(C-4'), 164.36(C-2'), 159.50(C-4), 126.58(C-1), 113.61(C-1').

The 400 MHz ^1H NMR of compound **1** showed three protons belonging to 1,2,4-trisubstituted benzene ring system (ABX system) at δ 7.74 (1H, *d*, $J = 8.8$ Hz, H-6'), 6.36 (1H, *dd*, $J = 8.8, 2.4$ Hz, H-5'), 6.31 (1H, *d*, $J = 2.4$ Hz, H-3'). Two doublets at δ 7.36 ($J = 15.4$ Hz) and 7.75 ($J = 15.4$ Hz) were observed suggesting the presence of double bond between C- α and C- β . In addition, four protons belonging to 1,4-disubstituted benzene ring system (AA'BB' system) at δ 7.47 (2H, *d*, $J = 8.6$ Hz, H-2,6), 6.80 (2H, *d*, $J = 8.6$ Hz, H-3,5) were noted. Correlations of these protons were observed in COSY spectral data. Connectivity of C-H bond was shown in HMQC NMR spectral data.

Based on the spectral data of compound **1** and comparison of its ^1H and ^{13}C NMR with isoliquiritigenin, structure of **1** was identified to be isoliquiritigenin.

Compound 2

Compound **2** was isolated as brown crystal, m.p. 153-154°C. Its molecular formula $\text{C}_7\text{H}_6\text{O}_3$ was established by EIMS at m/z . The IR spectrum of **2** exhibited characteristic absorption bands at 3326 cm^{-1} (OH), 1647 cm^{-1} (C=O), 1595 cm^{-1} (aromatic), 1168 cm^{-1} (C-O). While the UV spectrum showed maximum absorption at 232, 278, 312 nm.

The 100 MHz ^{13}C NMR of compound **2** exhibited 7 signals. The DEPT spectra (DEPT-90 and DEPT-135) established the existence of four methine carbons δ 191.93 (CHO), 125.83 (CH-6), 114.90 (CH-5), 113.97 (CH-2), three quaternary carbons δ 151.74 (C-4), 145.18 (C-3), 129.29 (C-1).

The 400 MHz ^1H NMR of compound **2** showed one proton low field singlet at δ 9.63 and was assigned to be aldehyde (CHO). In addition, three protons belonging to 1,2,4-trisubstituted benzene ring system (ABX system) was observed at δ 7.25 (1H, *br s*, H-2), 7.23 (1H, *br d*, H-6), 6.87 (1H, *d*, $J = 8.0$ Hz, H-5). This assignment was confirmed by ^1H - ^1H correlation in COSY spectral data including the correlation between H-2 (δ 7.25) with C-2 (δ 113.97) and H-6 (δ 7.23) with C-6 (δ 125.83) in HMQC

experiment. In addition, the connectivity of C-H through two or three bond was observed in HMBC spectra.

By careful analysis of the obtained spectral data of **2** and comparison of ^1H and ^{13}C NMR including the melting point, thus, compound **2** was determined to be protocatechualdehyde.

Compound 3

Compound **3** was isolated as yellow solid, m.p. 204-205°C. The structure of **3** was determined by spectroscopic method. Its molecular formula $\text{C}_{15}\text{H}_{12}\text{O}_5$ was established by EIMS showing molecular ion at m/z 272. The IR spectrum of **3** show characteristic absorption bands for hydroxy ($3491, 3371\text{ cm}^{-1}$), carbonyl (1666 cm^{-1}) and C-O ($1327, 1287, 1171\text{ cm}^{-1}$) moieties. The UV spectrum showed maximum absorption at 233, 278, 311 nm.

The 100 MHz ^{13}C NMR of compound **3** exhibited 15 signals. The DEPT spectra (DEPT-90 and DEPT-135) established the existence of one methylene carbon δ 43.62(CH₂-3), seven methine carbons δ 128.74 (CH-5), 118.15(CH-6'), 114.98(CH-5'), 113.21(CH-2'), 110.65(CH-6), 102.77(CH-8), 79.45(CH-2), seven quaternary carbons δ 192.07(CO), 164.86(C-7), 163.77(C-8a), 144.96(C-3'), 144.59(C-4'), 130.31(C-1'), 113.64(C-4a).

The 400 MHz ^1H NMR of compound **3** showed six protons belonging to two sets of 1,2,4-trisubstitued benzene ring systems (ABX system) at δ 7.69 (1H, *d*, $J = 8.7$ Hz, H-5), 6.44 (1H, *dd*, $J = 8.7, 2.2$ Hz, H-6), 6.32 (1H, *d*, $J = 2.2$ Hz, H-8), and δ 6.87 (1H, *d*, $J = 1.8$ Hz, H-2'), 6.77 (1H, *d*, $J = 8.2$ Hz, H-5'), 6.74 (1H, *dd*, $J = 8.2, 1.8$ Hz, H-6'). In addition, one methine proton at 5.22 (1H, *dd*, $J = 13.2, 2.8$ Hz, H-2) coupled with two methylene protons at 2.94 (1H, *dd*, $J = 13.2, 17.0$ Hz, H-3a) and 2.65 (1H, *dd*, $J = 17.0, 2.8$ Hz, H-3b) was observed. The large coupling constant ($J = 13.2$ Hz) was observed for vicinal H-2 and H-3a. Two geminal protons H-3a and H-3b was coupled

showing J value of 17.0 Hz. Comparison with the reported value suggesting that H-3a oriented at equatorial position.

In COSY experiment, H-2 at δ 5.22 correlated with H-3a (δ 2.94) and H-3b (δ 2.65). This suggested that H-2 attaching to chiral C-2 atom. Assignment of direct C-H connectivity was well correlated as observed in HMQC spectral data.

HMBC spectral data showed C-H connectivity through two or three bond. In case of C-2 at δ 79.45 which connected with H-3a (δ 2.94) through two bond.

According to the available spectral data of compound **3** and comparison of its ^1H NMR with butin, thus, we concluded that compound **3** was butin. The specific rotation of compound **3** was measured and found to be -87° . It was suggested that **3** was (-)-butin.

Compound 4

Compound **4** was isolated as orange-yellowish needles, m.p. 211-212°C. EIMS showed molecular ion at m/z 272 for its molecular formula $\text{C}_{15}\text{H}_{12}\text{O}_5$. The IR spectrum of **4** exhibited characteristic absorption bands for hydroxy ($3546, 3471\text{ cm}^{-1}$), carbonyl (1638 cm^{-1}) and C-O (1234 cm^{-1}) functionalities. While the UV spectrum showed maximum absorption at 262, 380 nm.

The 100 MHz ^{13}C NMR, DEPT-90 and DEPT-135 of compound **4** exhibited 15 signals of eight methine carbons [δ 102.66(C-3'), 107.86(C-5'), 114.28(C-2), 115.10(C-5), 116.86(C- α), 122.19(C-6), 131.56(C-6'), 144.61(C- β)], seven quaternary carbons [δ 113.21(C-1'), 126.78(C-1), 144.83(C-4), 147.87(C-3), 164.38(C-4'), 165.49(C-2'), 191.87(CO)].

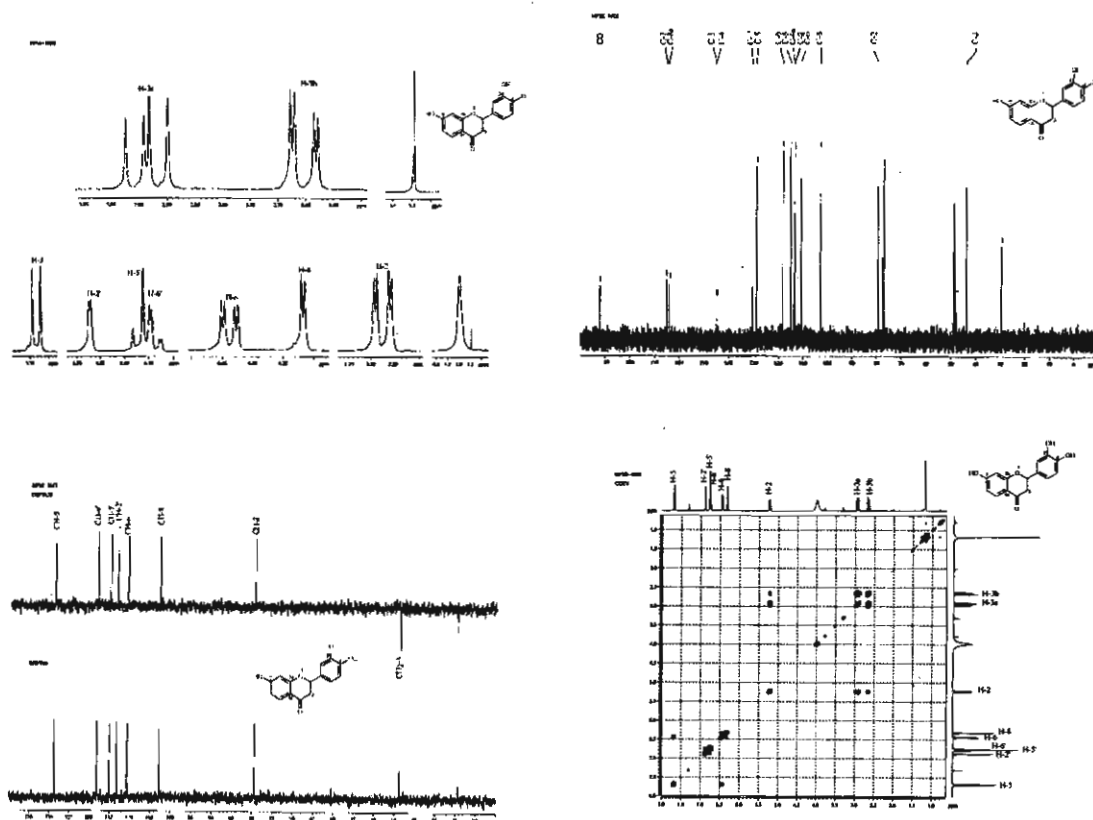
The 400 MHz ^1H NMR of compound **4** showed six protons belonging to the two sets of 1,2,4-trisubstituted benzene ring system (ABX system) at δ 7.15 (1H, d , $J = 1.8$ Hz, H-2), 7.05 (1H, dd , $J = 8.2, 1.8$ Hz, H-6) 6.82 (1H, d , $J = 8.2$ Hz, H-5) and δ 7.81 (1H, d , $J = 8.9$ Hz, H-6') 6.40 (1H, dd , $J = 8.9, 2.4$ Hz, H-5') 6.33 (1H, d , $J = 2.4$ Hz). Correlation of these ring protons was noted as shown in COSY spectral data. Two

doublets at δ 7.40 and 7.70 ($J = 15.3$ Hz) were observed suggesting the presence of trans-double bond between C- α and C- β . This assignment was confirmed by the three bond correlation between C-6 (δ 122.19), H- β and C-2 (δ 114.28), H- β in HMBC experiment. The direct C-H connectivity of compound **4** was observed.

Based on the spectral data of compound **4** and comparison of its ^1H NMR with butein, therefore, structure of **4** was concluded to be butein which its m.p. was reported to be 214°C.

Conclusion

In this study we have isolated four compounds (**1-4**) from crude ethyl acetate. Although these compounds were previously isolated from various plants, but they have been first isolated from *Hydnophytum formicarum* Jack. in our laboratory. In addition, 1D and 2D-NMR spectral data of these compounds have been established.



Acknowledgement

We are grateful to the Thailand Research Fund (TRF) for the generous support of our research programme.

Reference

Khumsap, S., Prachayasittikul, S., Chainakul, S. and Tambunchong, C. **Some constituents from *Hydnophytum formicarum* Jack.** Presented at 18th Conference on Science and Technology, October 27-29, 1992, Abstr. B-085, p-456.

บรรยายพิเศษทางวิชาการ

Chulabhorn Research Institute
Special Lecture

" Synthetic Implications in Natural Products Chemistry "

by

Professor G. Adam
Department of Natural Products Chemistry
Institute of Plant Biochemistry
Germany

10 : 00 am

on

October 6, 1998

at

Conference room, 6th Floor
General Building

Laboratory of Medicinal Chemistry
Tel. 5740622-33 Ext. 1608
Fax 574-2027

Chulabhorn Research Institute
Special Lecture

"Bioactive Natural Products from Plants"

by

Professor Dr. Wolfgang Kraus
Department of Chemistry
University of Hohenheim
Stuttgart, Germany

2:00 - 3:00 pm

on

October 22, 1998

at

Conference room, 6th Floor
General Building

Laboratory of Medicinal Chemistry
Tel. 5740622-33 Ext. 1608
Fax 574-2027

Chulabhorn Research Institute
Special Lecture

" Anionically Induced Domino Reactions : Some Recent Results "

by

*Professor Dietrich Spitzner
Department of Chemistry
University of Hohenheim
Stuttgart, Germany*

3:00 - 4:00 pm.

on

October 22, 1998

at

*Conference room, 6th Floor
General Building*

*Laboratory of Medicinal Chemistry
Tel. 5740622-33 Ext. 1608
Fax 574-2027*

Chulabhorn Research Institute
Special Lecture

*" Novel Generation of 1,3-Dipoles Utilizing Group-14 Metals
and Its Application to Heterocyclic Synthesis "*

by

*Professor Dr. Mitsuo Komatsu
Department of Applied Chemistry
Faculty of Engineering, Osaka University
JAPAN*

2:00 - 3:00 pm

on

November 10, 1998

at

*Conference room, 6th Floor
General Building*

Chulabhorn Research Institute
Special Lecture

"Asymmetric Total Synthesis of Taxol"

by

Professor T. Mukaiyama
Science University of Tokyo
JAPAN

2:00 - 3:30 pm

on

November 18, 1998

at

Conference room, 6th Floor
General Building

Chulabhorn Research Institute
Special Lecture

*" Combinatorial Biosynthesis : The Biosynthesis of Erythromycin
and Rapamycin "*

by

*Dr. James Staunton F.R.S.
Department of Chemistry
University of Cambridge
ENGLAND*

2:00 - 3:00 pm

on

January 18, 1999

at

*Conference room, 6th Floor
General Building*

สถาบันวิจัยจุฬาภรณ์

ขอเชิญร่วมฟังบรรยายพิเศษ

"Some Aspects of Solid Phase Organic Synthesis"

โดย

ดร. ธีรยุทธ อีได้อัย

ภาควิชาเคมี คณะวิทยาศาสตร์

จุฬาลงกรณ์มหาวิทยาลัย

เวลา 13.30 น.

วันพฤหัสบดีที่ 4 มีนาคม 2542

ณ ห้องประชุม ชั้น 6 อาคารวิจัย

ห้องปฏิบัติการภาควิชาเคมี

โทร. 574-0622-33 ต่อ 1608

โทรสาร 574-2027

Chulabhorn Research Institute
Special Lecture

" Molecular Genetics of Isoquinoline Alkaloid Biosynthesis "

by

Prof. Dr. Toni Kutschan
Institute of Pharmaceutical Science
University of Munich
GERMANY

2:00 - 3:00 pm

on

April 7, 1999

at

Conference room, 6th Floor
General Building

Laboratory of Medicinal Chemistry
Tel. 5740622-33 Ext. 1608
Fax 574-2027

Chulabhorn Research Institute
Special Lecture

" Benzyne Zirconocene Complexes in Organic Synthesis "

by

Dr. Roderick W. Bates
Department of Chemistry
Faculty of Sciences
Chulalongkorn University
Thailand

10:00 am

on

April 8, 1999

at

Conference room, 6th Floor
Research Building

Laboratory of Medicinal Chemistry
Tel. 5740622-33 Ext. 1608
Fax 574-2027

สถาบันวิจัยจุฬาภรณ์

ขอเชิญร่วมฟังบรรยายพิเศษ

" *Synthetic Studies on (5*R*)-ABC Segment
of Ciguatoxin* "

โดย

ดร. รุ่งนภา แซ่เอ็ง

เวลา 10.00 น.

ณ ห้องสมุดที่ 6 พฤษภาคม 2542

ณ ห้องประชุม ชั้น 6 อาคารวิจัย

ห้องปฏิบัติการเภสัชเคมี

โทร. 574-0622-33 ต่อ 1608

โทรสาร 574-2027

สถาบันวิจัยจุฬาภรณ์

ขอเชิญร่วมฟังบรรยายพิเศษ

*"Glycosidation on solid support glycosyl donor
linked with a sulfonate traceless linker"*

โดย

อาจารย์ บุญเอก ยิ่งยงณรงค์กุล

ภาควิชาเคมี คณะวิทยาศาสตร์

มหาวิทยาลัยรามคำแหง

เวลา 10.00 น.

วันพฤหัสบดีที่ 6 มกราคม 2543

ณ ห้องประชุม ชั้น 6 อาคารวิจัย

สถาบันวิจัยจุฬาภรณ์

ห้องปฏิบัติการเภสัชเคมี

โทร. 574-0622-33 ต่อ 1608

โทรสาร 574-2027

๗๑ เจริญรัตน์ วัฒนพงษ์บรรณารักษ์พิเศษ

৩৫

ดร. เกียรติพงษ์ ทองพันธุ์

ภาคใต้เคมี คณะวิทยาศาสตร์

มหาเวทย์พิฆาต

6667 10.00 %.

อันพฤษ์สมบัติที่ 2 มีนาคม 2543

ณ. ห้องประชุม ชั้น 6 อาคารวิจัย

สหพันธ์วิจัยอุทกศาสตร์

ห้องปฏิบัติการวัสดุเคมี

โทร. 574-0622-33 ต่อ 1608

โทรสาร 574-2027

Chulabhorn Research Institute
Special Lecture

*" A Biorational Approach to New Antibacterial Leads :
Importance of The Nitrogen Heterocycles. "*

by

*Prof. John Bremner
Department of Chemistry
University of Wollongong
AUSTRALIA*

2:00 - 3:00 pm

on

June 20, 2000

at

*Conference room, 6th Floor
General Building*

Chulabhorn Research Institute
Special Lecture

" [2+2]-,[2+3]-and [2+4]-Annulation Reactions of Quinones "

by

Prof. William S. Murphy
Department of Chemistry
University College Cork
IRELAND

2:00 - 3:00 pm

on

June 27, 2000

at

Conference room, 6th Floor
General Building

Laboratory of Medicinal Chemistry
Tel. 5740622-33 Ext. 1608
Fax 574-2027

สถาบันวิจัยจุฬาภรณ์

ขอเชิญร่วมฟังบรรยายพิเศษ

เรื่อง

"Diastereoselective Cyclization of Allene Using Organocobalt Reagent"

และ

"Stereocontrolled Synthesis of Indolizidine Rings"

โดย

นางสาวชัชราภรณ์ สัตย์เจริญ

นางสาวจุฑาทิพย์ บุญสมบัติ

ภาควิชาเคมี คณะวิทยาศาสตร์

จุฬาลงกรณ์มหาวิทยาลัย

เวลา 10.00 น.

วันพฤหัสบดีที่ 14 กันยายน 2543

ณ ห้องประชุม ชั้น 6 อาคารอเนกประสงค์

สถาบันวิจัยจุฬาภรณ์