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

รหัสโครงการ : RTA/01/2540

ชื่อโครงการ : การสังเคราะห์และปฏิกิริยาสารอัลคาลอยด์และสารประเภท Oxygen Heterocycle ต่าง ๆ และการศึกษาเกี่ยวกับสมุนไพรไทยบางชนิด

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ระยะเวลาที่ทำการวิจัย : ธันวาคม 2540 ถึง กุมภาพันธ์ 2544

วัตถุประสงค์ :

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

สำหรับโครงการที่สองเป็นการวิจัยเกี่ยวกับสมุนไพรไทยบางชนิด เช่น ต้นสารภีบ้าน และ ต้นหัวร้อยรู นอกจากการแสวงหาความเป็นเลิศทางวิชาการแล้ว ยังต้องการหาตัวยาจากสมุนไพรเพื่อเป็นการ validate ข้อมูลต่าง ๆ ที่ใช้กันมาและเสริมสร้างให้มีผลิตผลจากภายในประเทศ 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 (^{1}H, ^{13}C) , MS เมื่อพิสูจน์ว่าเป็นสารที่ต้องการแล้ว ก็จะต้องพยายามหากระบวนการต่าง ๆ ที่จะทำให้ได้สารมากที่สุด (highest yield) เพื่อนำไปทำปฏิกิริยาต่อ ๆ ไปจนได้สารที่ต้องการ ในกรณีที่ไม่ได้สารที่ต้องการหรือได้น้อยมาก งานวิจัยก็ต้องปรับเปลี่ยนไปเพื่อให้ได้สารที่ต้องการ

ในการศึกษาปฏิกิริยาของสารต่าง ๆ ก็เช่นเดียวกัน เมื่อสารทำปฏิกิริยากันแล้วก็จะต้องแยกทำให้บริสุทธิ์ หาสูตรโครงสร้างต่าง ๆ หลังจากนั้นก็จะหาคำอธิบายกลไกของปฏิกิริยา

งานวิจัยในด้านสมุนไพรจะประกอบด้วยการเก็บสมุนไพร (collection) หาชื่อต้นไม้ (identification) สกัด (extraction) แยกสารและทำให้บริสุทธิ์ (isolation & purification) หาสูตรโครง

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

ผลที่ได้จากาศึกษาวิจัย : ผลของการวิจัยได้บรรลุวัตถุประสงค์ ดังนี้ คือ

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

การนำไปใช้ประโยชน์ :

การวิจัยนี้ได้ประสบความสำเร็จด้านการสังเคราะห์อัลคาลอยด์และสารประเภท Oxygen Heterocycles รวมถึงการสกัดสารสำคัญจากสมุนไพรไทยบางชนิด และการทดสอบฤทธิ์ทางชีวภาพของผลิตภัณฑ์ที่ได้ ซึ่งผลของการวิจัยนี้ อยู่ในระดับอินทรีย์เคมีพื้นฐาน 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 Heterocycles and Studies of Some Thai Medicinal Plants

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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: ^{1}H 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

รายงานสรุปโครงการวิจัยทุนส่งเสริมกลุ่มวิจัย สาขาเคมี
สำหนักงานกองทุนสนับสนุนการวิจัย
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.

1.6 การสำรวจหาข้อมูลการสังเคราะห์ขั้นพื้นฐานบางส่วนในกระบวนการสังเคราะห์สารต้านเชื้อแบคทีเรียประเภทฟลูโอโรควิโนลีน

Investigation on synthetic process of some antibacterial fluoroquinolones

และโครงการหลัง แบ่งเป็นโครงการย่อย 2 โครงการดังนี้

2. การศึกษาเกี่ยวกับสมุนไพรไทยบางชนิด

Studies of some Thai Medicinal Plants

2.1 การศึกษาทางด้านเคมีและเภสัชวิทยาของสารสกัดจากต้นสารภีบ้าน (*Mammea siamensis*)

Chemical and pharmacological investigations of *Mammea siamensis*

2.2 การแยกและหาโครงสร้างของสารในสารสกัดจากต้นหัวร้อยรูในตัวทำละลาย Chloroform

Isolation and characterization of some constituents from chloroform extracts of *Hydrophytum formicarum* Jack.

หัวหน้าโครงการวิจัย คือ รศ. ดร. สมศักดิ์ รุจิรวัฒน์ ซึ่งได้รับคัดเลือกให้เป็นเมธีวิจัยอาวุโส สาขาเคมี ประจำปี 2540 จากสำนักงานกองทุนสนับสนุนการวิจัย

มีผู้ร่วมโครงการทั้งหมดจาก 2 สถาบัน คือ

1. สถาบันวิจัยจุฬาภรณ์
2. สถาบันเทคโนโลยีราชมงคล

และ 5 มหาวิทยาลัย คือ

1. มหาวิทยาลัยมหิดล
2. มหาวิทยาลัยเทคโนโลยีพระจอมเกล้า ธนบุรี
3. มหาวิทยาลัยศรีนครินทรวิโรฒ
4. มหาวิทยาลัยรามคำแหง
5. มหาวิทยาลัยเกษตรศาสตร์

จำนวนทั้งสิ้น 16 คน ดังรายละเอียดต่อไปนี้

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

3.	รศ. ดร. สุภาลักษณ์ ปรัชญาสิทธิกุล	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ ประสานมิตร
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5.	รศ. ดร. สมยศ สุทธิไวยกิจ	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยรามคำแหง
6.	ผศ. ดร. วนิดา พวกุล	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเทคโนโลยีพระจอมเกล้า ธนบุรี
7.	ผศ. ดร. สุภัทรา โพธิ์พ่วง	ภาควิชาวิศวกรรมเคมี คณะวิศวกรรมศาสตร์ สถาบันเทคโนโลยีราชมงคล
8.	ดร. เพ็ญศรี บุญสวรรค์ส่ง	ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเกษตรศาสตร์
9.	ดร. พูลศักดิ์ สหกิจพิจารณ์	ห้องปฏิบัติการเภสัชเคมี สถาบันวิจัยจุฬาภรณ์
10.	ดร. หรรษา ประวัติ	ห้องปฏิบัติการเภสัชเคมี สถาบันวิจัยจุฬาภรณ์
11.	ดร. ธรรมนูญ มุฑระพัฒน์	ห้องปฏิบัติการเภสัชเคมี สถาบันวิจัยจุฬาภรณ์
12.	ดร. พูลศักดิ์ พลอยประดิษฐ์	ห้องปฏิบัติการเภสัชเคมี สถาบันวิจัยจุฬาภรณ์
13.	นายสมชาย พิสุทธิ์เจริญพงศ์	ห้องปฏิบัติการเภสัชเคมี สถาบันวิจัยจุฬาภรณ์
14.	นายนพพร ทัศนา	ห้องปฏิบัติการเภสัชเคมี สถาบันวิจัยจุฬาภรณ์
15	น.ส. วิไลลักษณ์ ปรัชญาวรากร	ห้องปฏิบัติการเภสัชเคมี สถาบันวิจัยจุฬาภรณ์
16.	นายศานิตย์ ทองเนตร	ห้องปฏิบัติการเภสัชเคมี สถาบันวิจัยจุฬาภรณ์

นอกจากนี้ กลุ่มวิจัยยังได้ผลิตนักศึกษาในระดับปริญญาเอก ปริญญาโท ปริญญาตรี และนักวิจัยอื่น ๆ ที่ร่วมโครงการ ดังนี้

ชื่อ - นามสกุล	ต้นสังกัด		
	ภาควิชา	คณะ	มหาวิทยาลัย/สถาบัน
1. น.ส. ฐิติมา วงศ์จรรยา			สถาบันวิจัยจุฬาภรณ์
2. น.ส. ศิริพร วงษ์บัณฑิต			สถาบันวิจัยจุฬาภรณ์
3. น.ส. วิรงรอง กวีไตรภพ			สถาบันวิจัยจุฬาภรณ์
3. น.ส. ปฏิมา ไพศาล			สถาบันวิจัยจุฬาภรณ์
4. นายธวัชชัย โลกะนัง	เคมี	วิทยาศาสตร์	มหิดล
5. น.ส. วรรณา ผู้อุสาห์	เคมี	วิทยาศาสตร์	มหิดล

ชื่อ-นามสกุล	ต้นสังกัด		
	ภาควิชา	คณะ	มหาวิทยาลัย/สถาบัน
6. น.ส. รัชนก ปิ่นแก้ว	เคมี	วิทยาศาสตร์	มหิดล
7. น.ส. วิยดา จินากลึง	เภสัชเคมี	เภสัชศาสตร์	มหิดล
8. น.ส. วันทนา มงคลวิสุทธิ์	เคมี	วิทยาศาสตร์	รามคำแหง
9. นายประสิทธิ์ บูรพาเรืองแสง	เคมี	วิทยาศาสตร์	ศรีนครินทรวิโรฒ
10. นายอณุชา น้ำสอาด	เคมี	วิทยาศาสตร์	มหิดล
11. น.ส. สุดา จักรทอง	เคมี	วิทยาศาสตร์	มหิดล
12. นายสุพจน์ จันทร์เจริญ	เคมี	วิทยาศาสตร์	มหิดล
13. น.ส. อุมาพร 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. นายสุรชัย ธชีพันธ์	เคมี	วิทยาศาสตร์	เกษตรศาสตร์
18. นางสาวภัทรียา เกษสี	เคมี	วิทยาศาสตร์	เทคโนโลยี พระจอมเกล้าธนบุรี
19. นายคมสันต์ สัจวสันต์	เคมี	วิทยาศาสตร์	เทคโนโลยี พระจอมเกล้าธนบุรี
20. น.ส. ศิรประภา ติกะโกศล	เคมี	วิทยาศาสตร์	เทคโนโลยี พระจอมเกล้าธนบุรี
21. น.ส. วนิดา ล่าชิต	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. เนทิยา ตัณฑชุณห์	เคมี	วิทยาศาตร์	เทคโนโลยี พระจอมเกล้าธนบุรี
26. นายธิติ จันทร์ภิรมย์	เคมี	วิทยาศาสตร์	มหิดล
27. น.ส. พุฒิรัตน์ สารบรรณ	เคมี	วิทยาศาสตร์	ศรีนครินทรวิโรฒ

จากการวิจัยมาเป็นลำดับ ผลงานวิจัยของโครงการต่าง ๆ ได้บรรลุวัตถุประสงค์ตามเป้าหมาย เป็นที่น่าพอใจ ดังรายงานความก้าวหน้าที่นำเสนอไปเรียบร้อยแล้ว โดยผลงานส่วนหนึ่งได้เผยแพร่ในรูปโปสเตอร์และการบรรยายในการประชุมสัมมนาทางวิชาการทั้งในและต่างประเทศ ดังนี้

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ผลงานตีพิมพ์ในวารสารวิชาการในประเทศไทย

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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. ธีรยุทธ 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 Uning Organocobalt Reagent	นางสาววชิราภรณ์ 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

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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 planets 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 ultilized 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

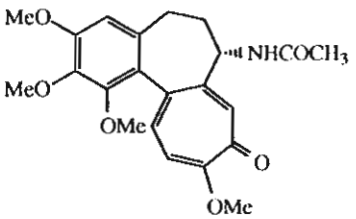
Apart from being an excellent source of anti-infectious drugs (8,13,14) plants are also good source of anticancer agents (7-12). National Cancer Institute (NCI), USA has launched an extensive program for the development of natural products for the treatment of various forms of cancer. Many clinically 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 endevour. 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

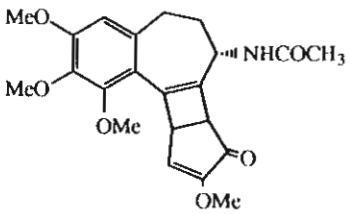
problems, interest in camptothecin intensified once it was discovered that it exhibits a novel mechanism of action by inhibiting the enzyme DNA topoisomerase I. Accordingly, a number of camptothecin analogues have been developed in an attempt to reduce toxicity, optimize efficacy, and improve water solubility 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.

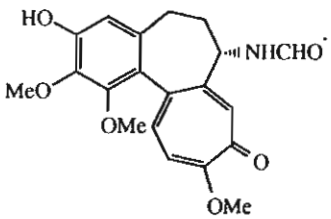
The active principle of *Gloriosa superba* is colchicine which has long been used for the treatment of arthritis. From the dried tuber of *Gloriosa superba*, four tropolone alkaloids were isolated and identified as colchicine, lumicolchicine, 3-demethyl-*N*-deformyl-*N*-deacetylcolchicine and 3-demethylcolchicine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



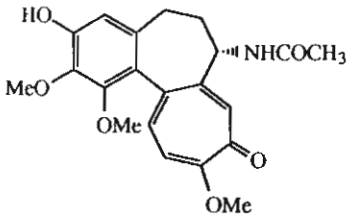
Colchicine



Lumicolchicine



3-Demethyl-*N*-formyl-*N*-deacetylcolchicine



3-Demethylcolchicine

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 ED_{50} of the different "Gloriosa" extracts are shown in the table.

Colchicine exhibits very low ED_{50} value suggesting the potent cytotoxicity of the compound. The chloroform, methanol and petroleum ether extracts of *Gloriosa superba* also show very low ED_{50} value which could result from the presence of colchicine in these extracts.

Compounds	ED_{50} values (μg/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-*N*-formyl-*N*-deacetyl-colchicine	0.0252

It is interesting to note that the ED_{50} value of 3-demethyl-*N*-formyl-*N*-deacetylcolchicine is very close in value to the ED_{50} 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, COL-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 μg/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, prostrate 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 hightly 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 viveririni*(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 ED_{50} values (μg/ml) against KB and HuCCA-1 cell lines

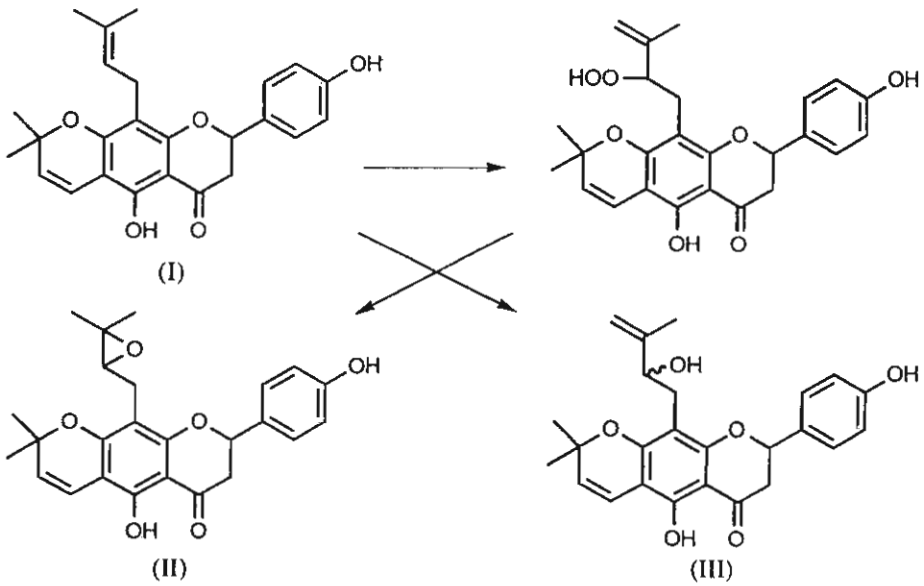
Gloriossa superba	KB	HuCCA-1
Methanol extract	0.5	2.5
3-Demethyl-*N*-formylcolchicine	0.03125	0.0625
3-Demethyl-*N*-formyl-*N*-deacetylcolchicine	0.03125	0.0625

HuCCA-1 = Human cholangiocarcinoma cell line

The ED_{50} values for the cholangiocarcinoma cell line for the methanol extract was found to be 2.5 micrograms per ml, while both the ED_{50} 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 ED_{50} value of about 0.5 microgram per ml of colchicine. These values were approximately two times higher than the ED_{50} 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, dereticulatin 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.

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The Syntheses of Lamellarins and Isoindolobenzazepine Alkaloids

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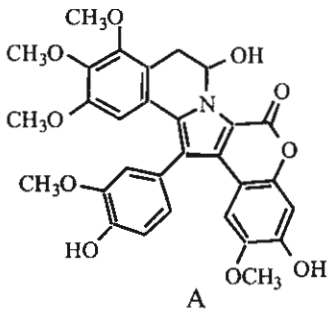
Abstract: Two efficient synthetic routes for the syntheses of lamellarin alkaloid and isoindolobenzazepine alkaloid are described.

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



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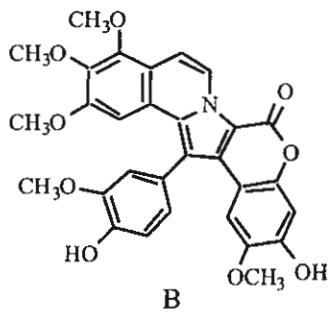

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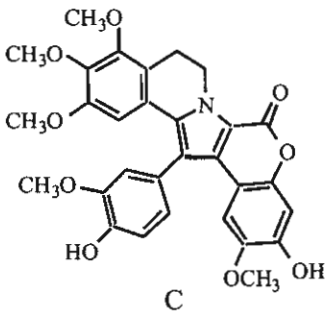

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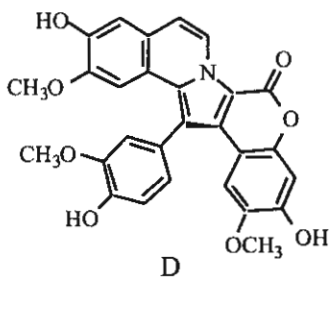
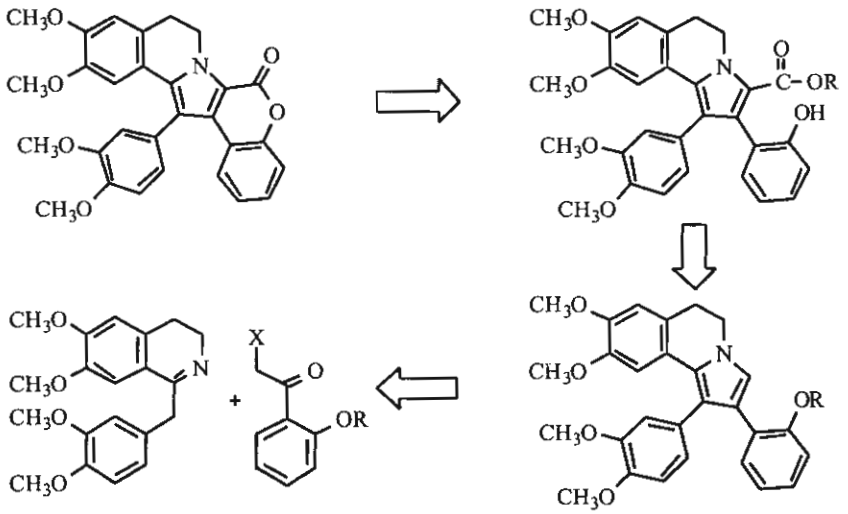


D

*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.

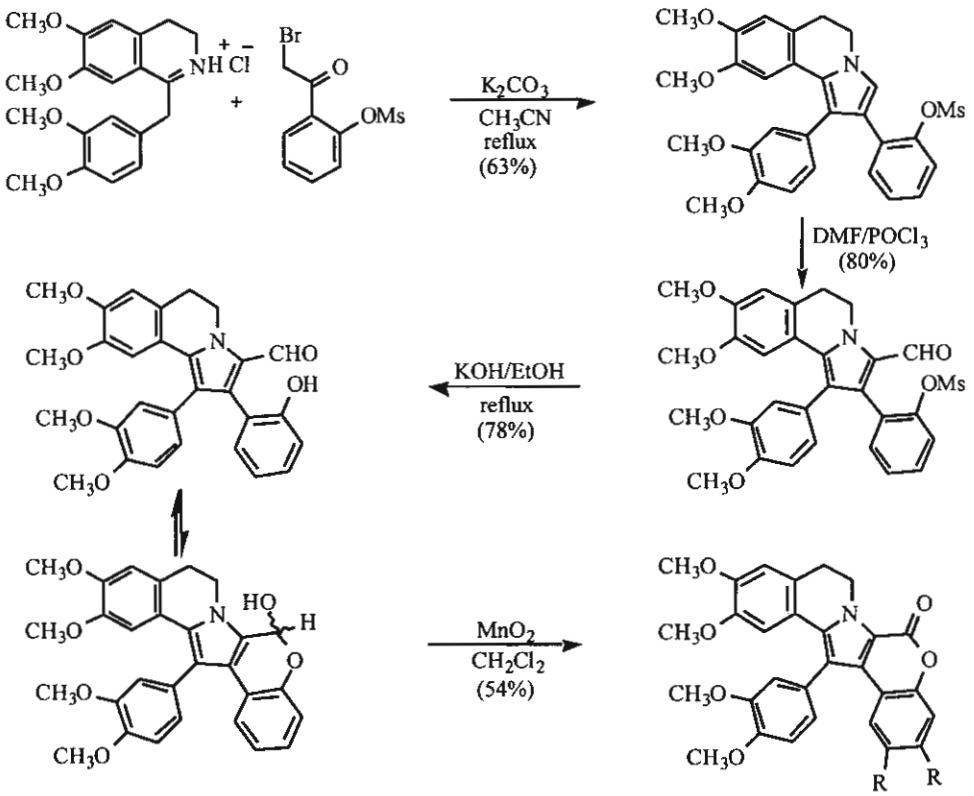
The first four lamellalins were isolated by Faulkner et al. in 1985 and named Lamellarins A, B, C, D. The structure of Lamellarin A was determined by X-ray crystallographic analysis and the structures of the remaining compounds were derived from spectroscopic data (1). More than twenty lamellarins have 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.

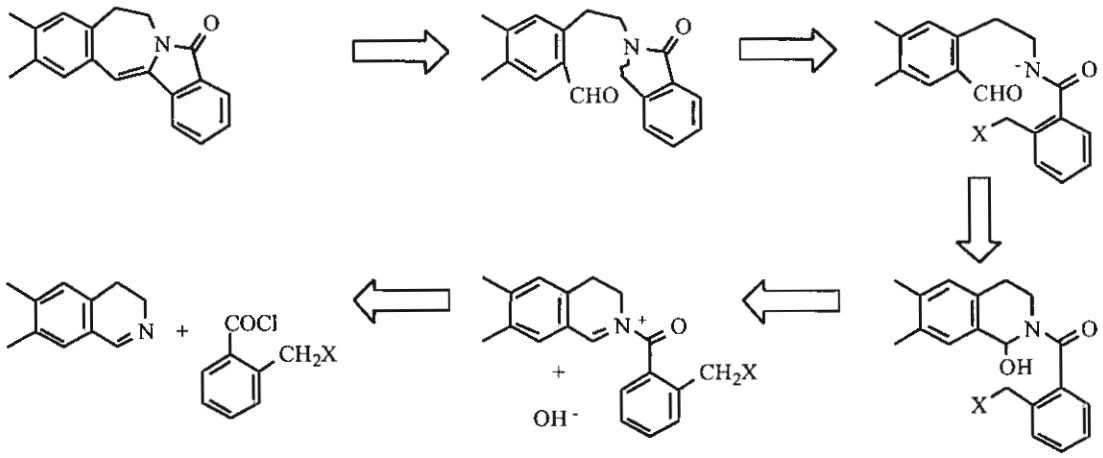
Indeed, the synthesis of the lamellarin skeleton can be accomplished via the above retrosynthetic analysis and this is shown in the scheme I.



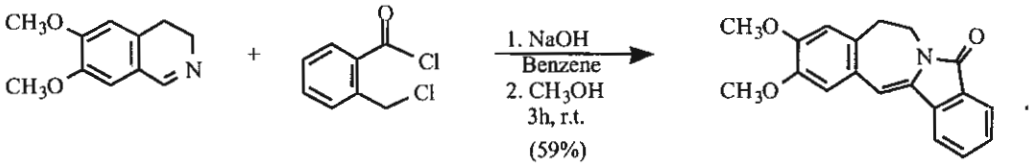
Scheme I

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

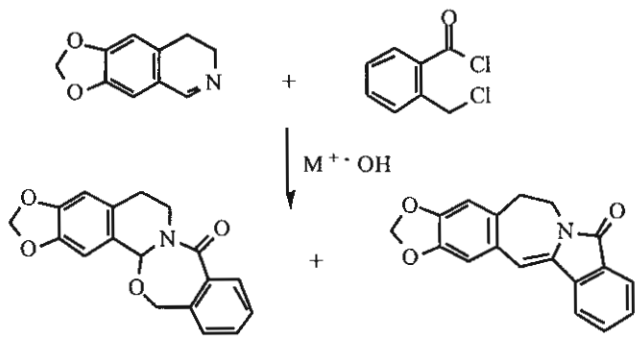
Our research group has also been interested in the synthesis of various benzylisoquinoline-derived alkaloids, including the isoindolobenzazepines (7). Our synthetic approach was based on the retrosynthetic analysis of the isoindolobenzazepine skeleton as shown.



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.



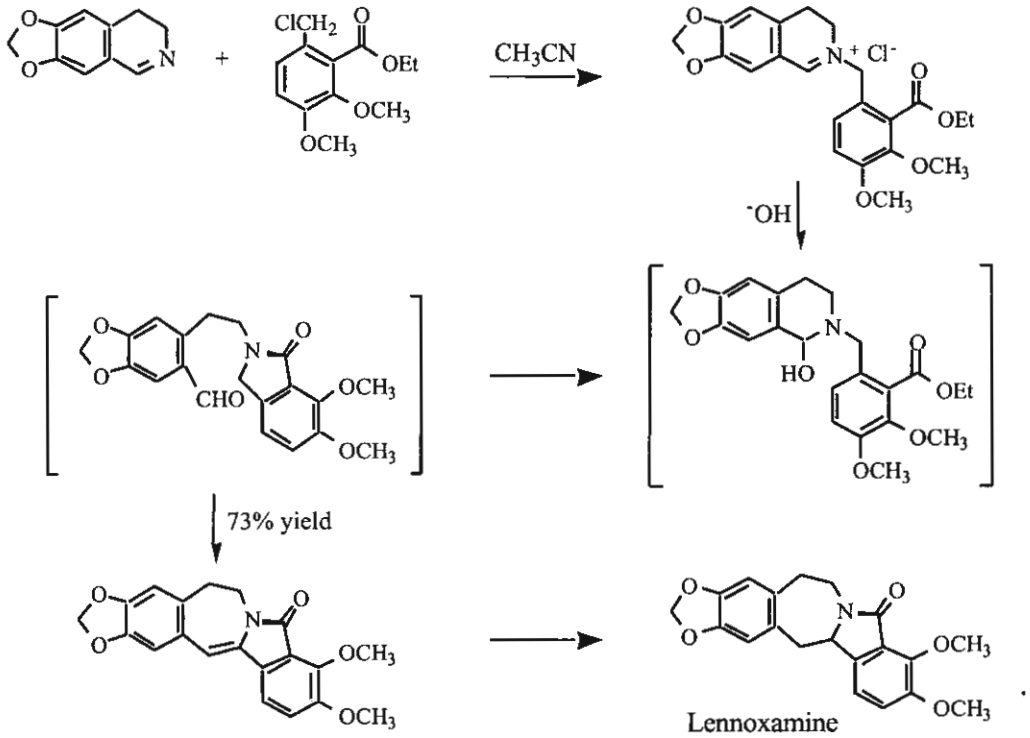
We have extended our study to the reaction of 2-chloromethylbenzoyl chloride with 3,4-dihydroisoquinoline and 3,4-dihydro-6,7-methylenedioxyisoquinoline in the presence of both sodium hydroxide and potassium hydroxide and these are shown.



	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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TETRAHEDRON LETTERS

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

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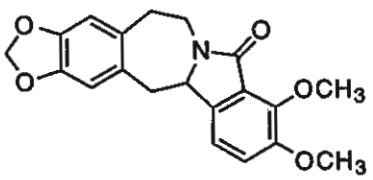
Abstract

A novel, highly efficient synthesis of lennoxamine, a representative of isoindolobenzazepine alkaloid, is described.

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 alkaloids[4] in general and chilenine[5,6] and lennoxamine[6,7] in particular, have captured the interest of many groups of synthetic chemists.



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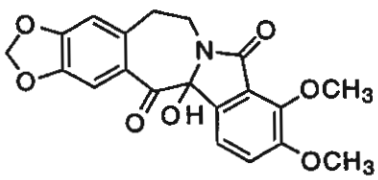

2

We have previously reported[4b] the synthesis of the isoindolobenzazepine (aporhoeadane) 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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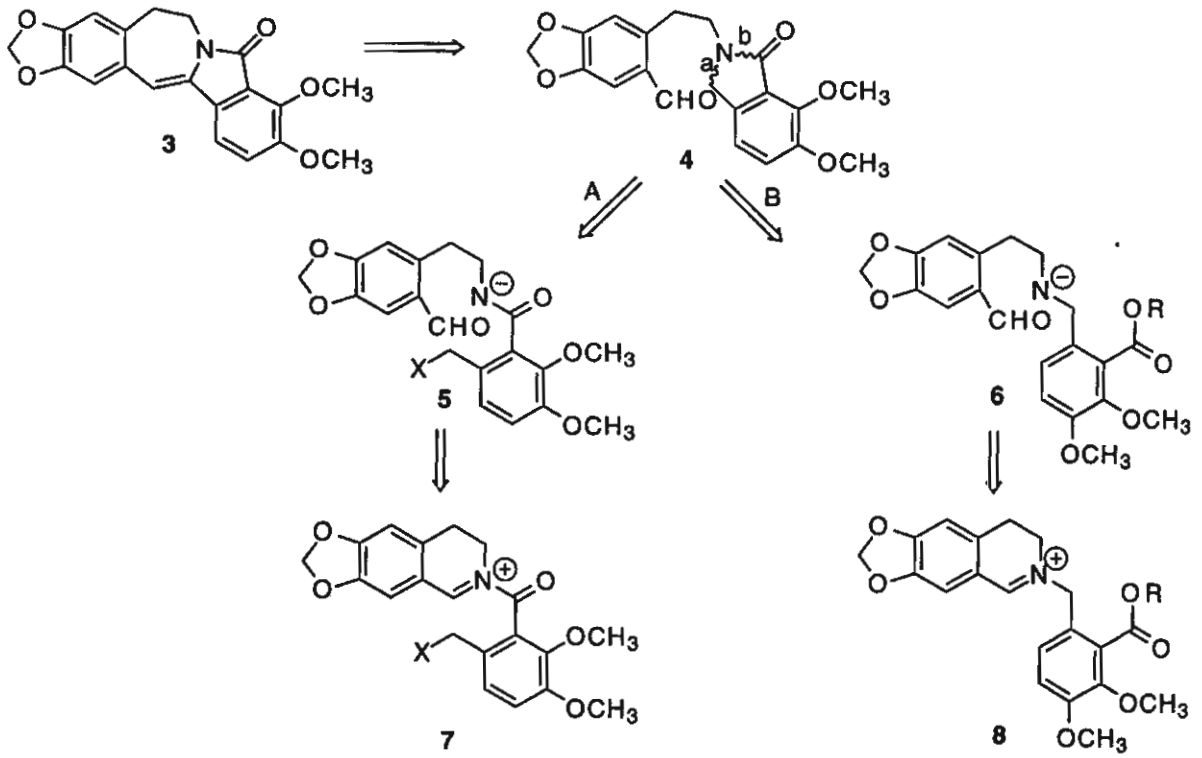
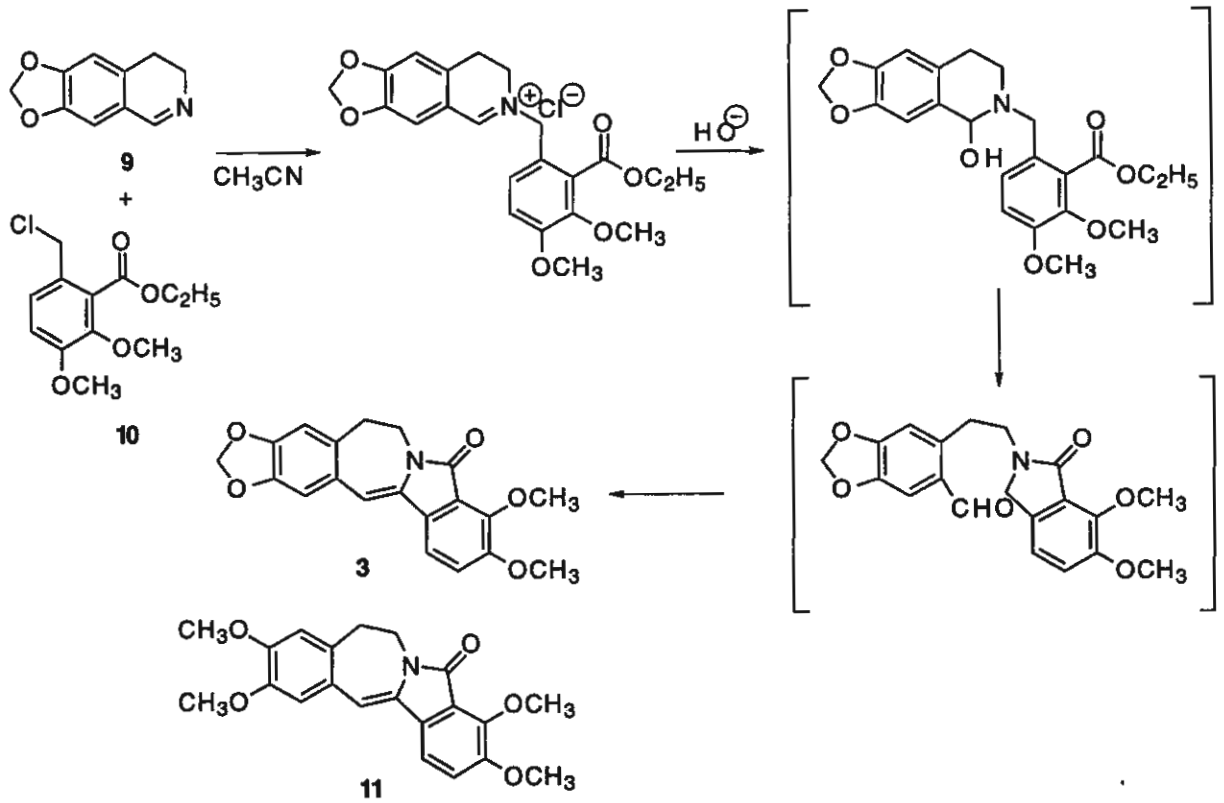
required the synthesis of not readily accessible halomethylbenzoyl chlorides. We have also found that when 6,7-methylenedioxy-3,4-dihydroisoquinoline was used as one of the components in the reaction with chloromethylbenzoyl chloride, the required lactam was obtained in disappointing yield. We have now 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 benzyliminium 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-dimethoxybenzoate[8] **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 analogue[9] in 76 and 80% yields, respectively.

Acknowledgements

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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 $C_{20}H_{17}NO_5$: 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 $C_{21}H_{21}NO_5$: 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; Lit[1], 225°C; Lit[7]a, 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 $C_{20}H_{19}NO_5$: 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, 147.14, 147.46, 152.53, 165.11. EIMS 370(20), 369(85), 353(44), 351(29), 177(100). Anal. calcd for $C_{21}H_{23}NO_5$: C, 68.29; H, 6.23; N, 3.79. Found: C, 68.38; H, 6.32; N, 3.77.

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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, 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 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.

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

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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-

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 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, 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 ED_{50} for the cholangiocarcinoma cell line with the methanol extract of *Gloriosa superba* was found to be 2.5 μg/ml, while the ED_{50} of 3-demethyl-*N*-formyl-*N*-deacetylcolchicine was found to be 0.0625 micrograms per ml, in contrast with the ED_{50} of about 0.02 μg/ml for colchicine. These values were approximately two-times higher than the ED_{50} 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 C_{16} 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 cholchicine with various dienophiles (Brecht et al., 1997). Reaction of singlet oxygen with colchicine produced the cycloaddition product of colchicine peroxide (Scheme 5).

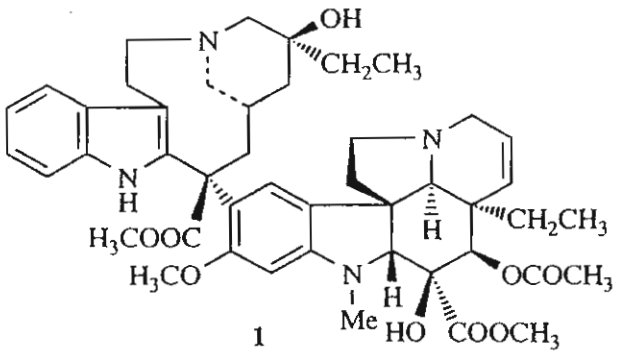
N-Phenyl-1,2,4-triazolinedione (PTAD), the highly reactive nitrogen dienophile, reacted with colchicine. When the two reactants were heated in toluene at 110 °C for half an hour, only the endo adduct was formed. Under relatively drastic conditions, colchicine gave the maleimide adduct. When colchicine 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 a 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. ED_{50} values against KB and HuCCA-1 cell lines.

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

HuCCA-1 = Human cholangiocarcinoma cell line



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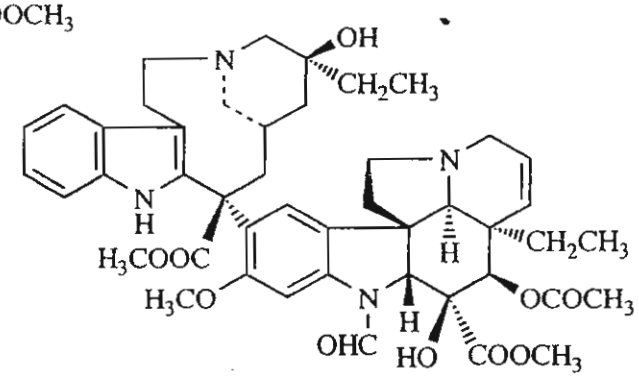

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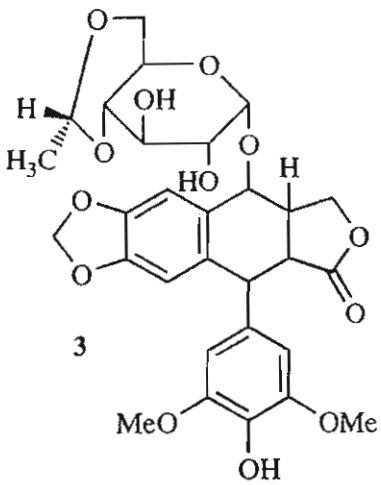

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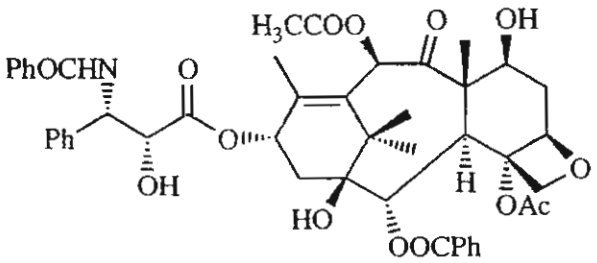

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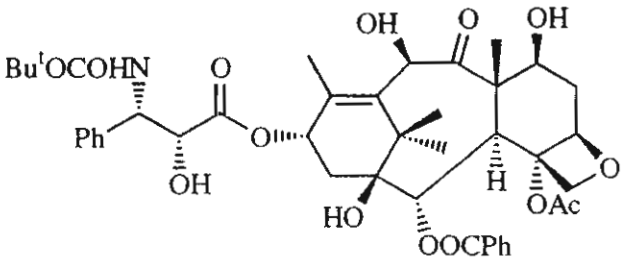

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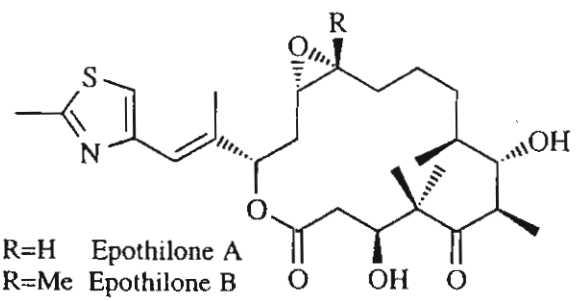
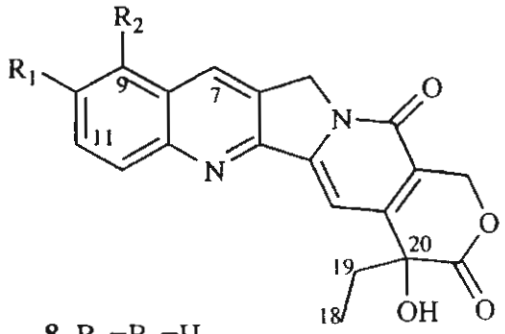
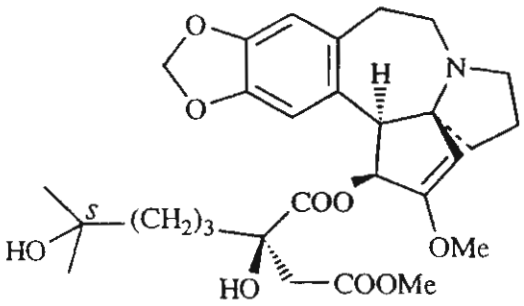


6 R=H Epothilone A
7 R=Me Epothilone B

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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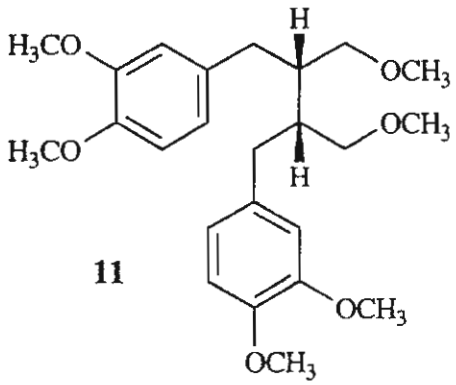

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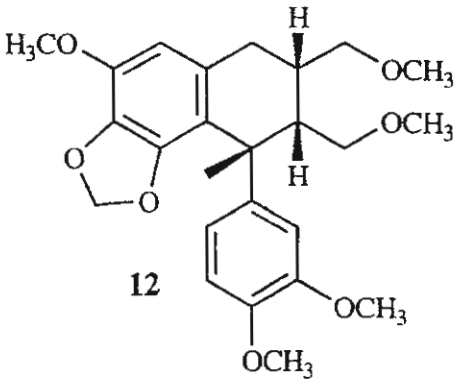

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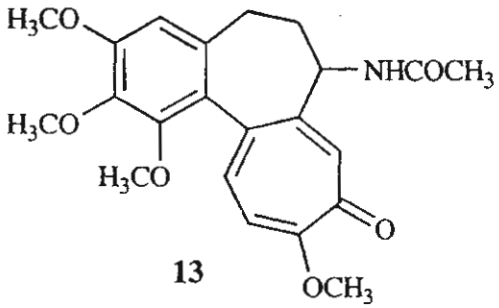

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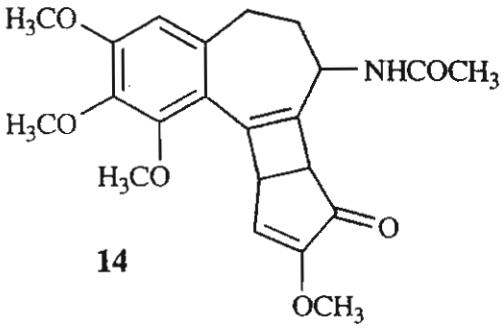

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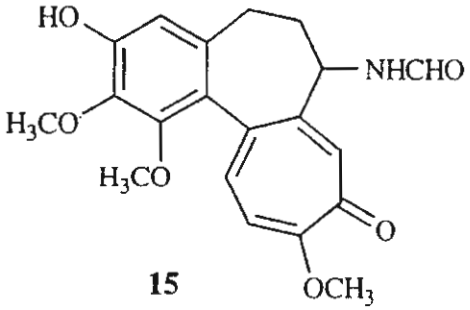

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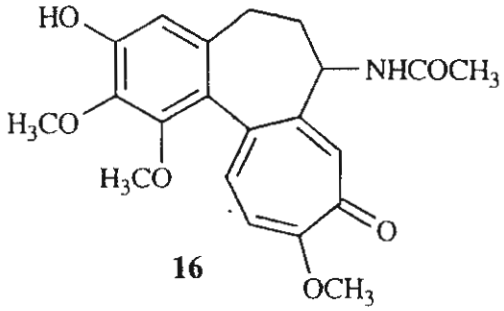

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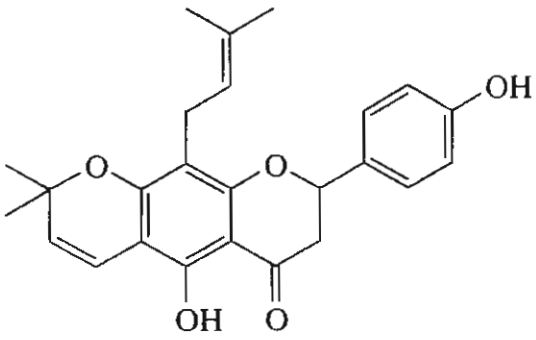
Fig. 2. Structures of compounds 8–16.



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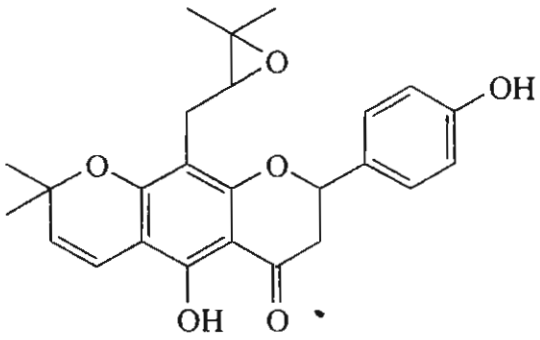

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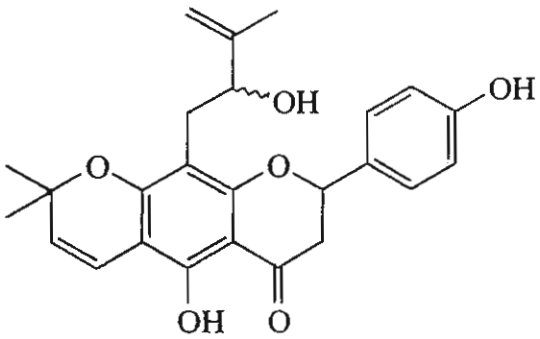

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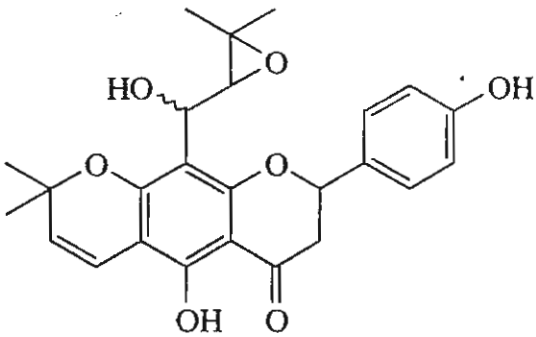

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Fig. 3. Structures of compounds **17–20**.

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.

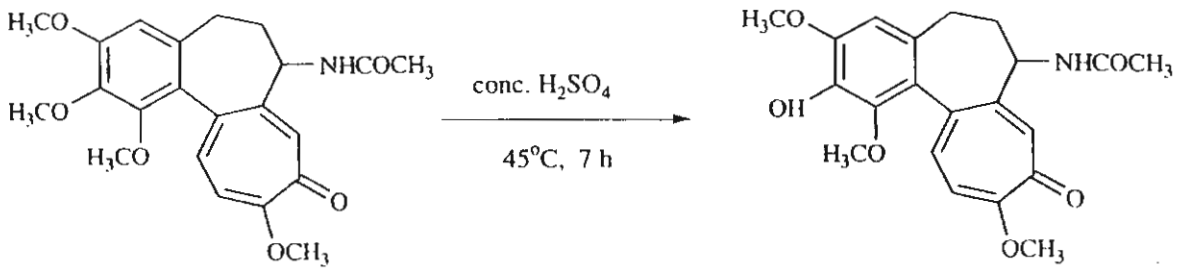
We have recently investigated another plant, *Derris reticulata*, locally known as Cha-aim thai (Mahidol et al., 1997b). Cha-aim thai is a medicinal plant of Thailand used for the relief of thirst and as an expectorant. Our studies led to the isolation of three new pyranoflavanone compounds. Lupinifolin (**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.

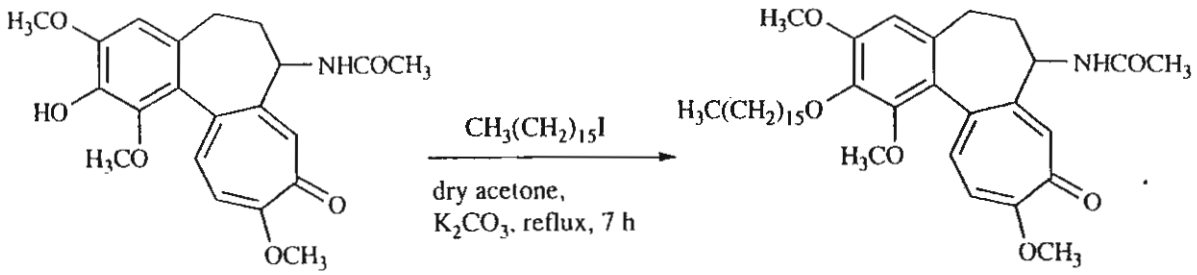
The *in vitro* bioassay evaluation of lupinifolin, epoxylupinifolin and dereticulatin triacetate was also carried out. The results showed that each of them inhibited the P-388 cell line at 0.4–0.5 μg/ml, but were inactive against the KB cell line.

In conclusion, it is our conviction that research on



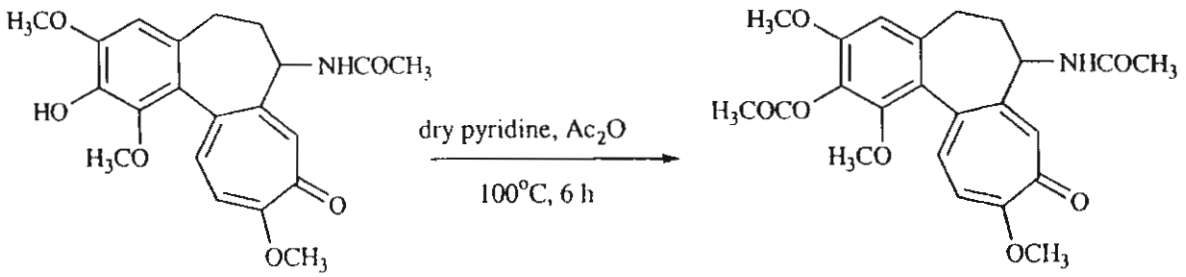
2-Desmethylcolchicine

Scheme 1



2-C_{16}-ether

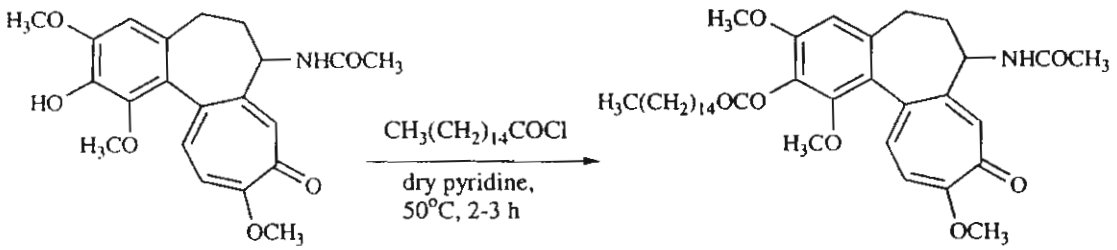
Scheme 2



Colchicine acetate

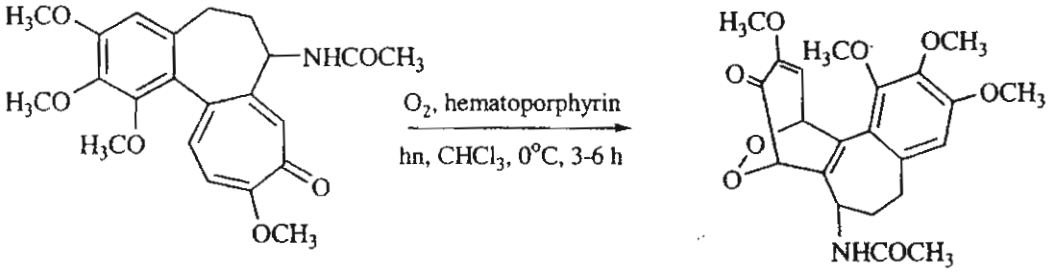
Scheme 3

natural products is most worthwhile despite some signs that the interest in natural product research is waning with the explosive growth of combinatorial chemistry.



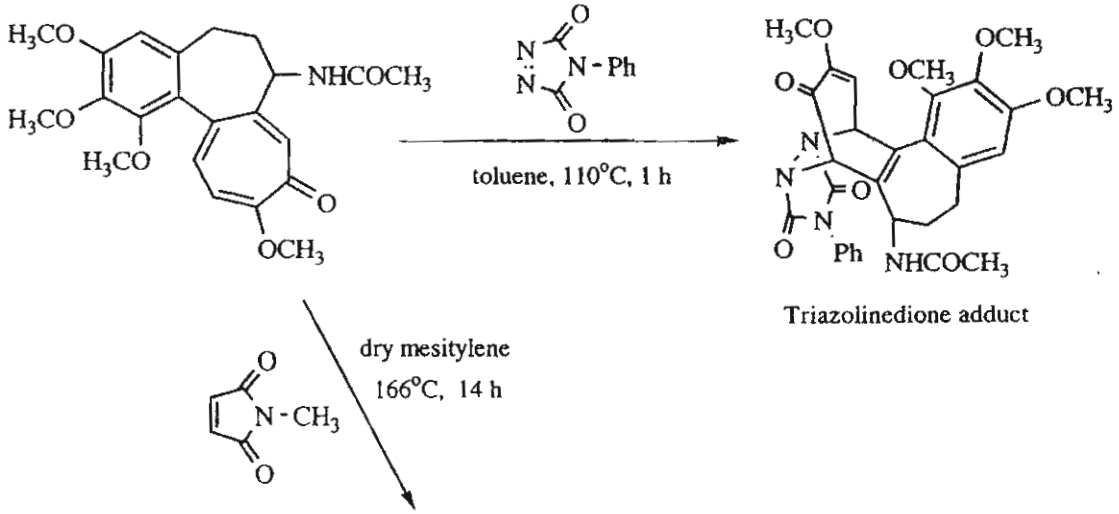
Colchicine palmitate

Scheme 4

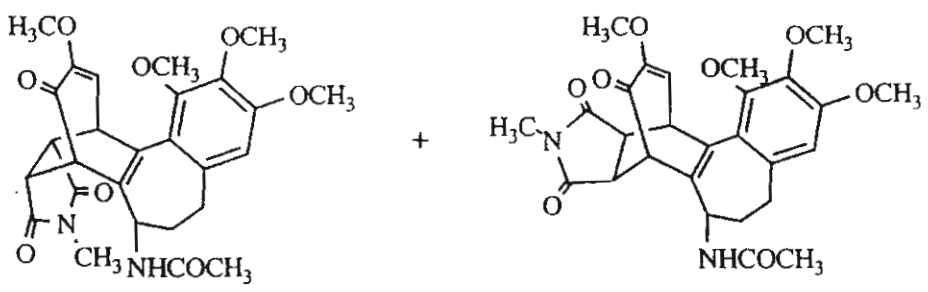


Colchicine peroxide

Scheme 5

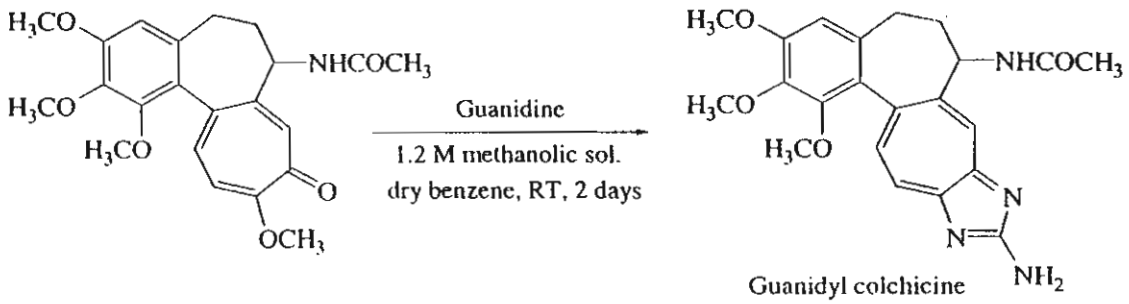


Triazolinedione adduct

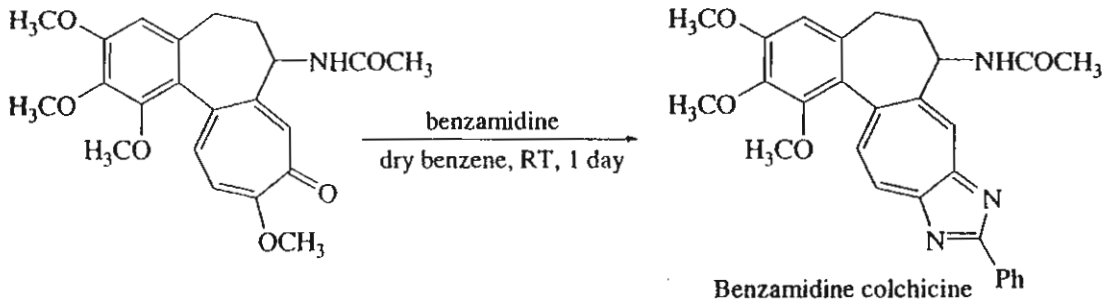


Endo-Maleimide adduct Exo-Maleimide adduct

Scheme 6



Scheme 7



Scheme 8

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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 ($CHCl_3$ or KBr pellets) spectra were measured on a Perkin-Elmer system 2000 FT-IR 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 ^{13}C) spectrometer, using TMS as internal standard. Precoated Si gel 60 PF_{254} plates (Merck, layer thickness 0.2 mm) were used for analytical TLC while preparative TLC was performed on Si gel PF_{254} 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. Nijsiri Ruangrungsi. 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 CH_2Cl_2 at room temperature. After filtration, the extract was evaporated *in vacuo* to dryness (88 g). The CH_2Cl_2 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 F_6 (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

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

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(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). ^{1}H NMR ($CDCl_3$) δ: 5.69 (*m*, H-2), 2.95 (*dd*, *J* = 16.7, 13.2 Hz, H-3α), 2.82, 2.78 (*dd*, *J* = 16.7, 3.2 Hz, H-3β), 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). ^{1}H 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-3α), 2.89, 2.86 (*dd*, *J* = 17.2, 3.2 Hz, H-3β), 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*, O*H*-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**. ^{1}H 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'). ^{1}H 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 ^{1}H 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 C_8 and C_{18}, Hichrom C_{18}) with several solvent systems. However, ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) exhibited clearly two sets of signals with partial overlapping. The ^{1}H 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 ^{1}H 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 ^{1}H 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. ^{1}H NMR spectroscopic assignments of compound **1**.

Proton	Compound **1** (CD_3OD + acetone-d_6)	Compound **1** (pyridine-d_5)
2	5.43, 5.41 (*dd*, *J* = 13.2, 3.0)	5.48, 5.41 (*dd*, 13.0, 2.9)
3α	3.18, 3.15 (*dd*, 17.2, 13.2)	3.24, 3.237 (*dd*, 17.1, 13.0)
3β	2.74, 2.72 (*dd*, 17.2, 3.0)	2.91, 2.89 (*dd*, 17.1, 2.9)
2', 6'	7.34, 7.33 (*d*, 8.6)	7.51, 7.48 (*d*, 8.6)
3', 5'	6.80 (*d*, 8.6)	7.18 (*d*, 8.6)
3"	5.61 (*d*, 10.1)	5.58 (*d*, 10.0)
4"	6.56 (*d*, 10.1)	6.85 (*d*, 10.0)
5", 6"	1.44, 1.434, 1.427, 1.42 (*s*)	1.46, 1.43, 1.41, 1.40 (*s*)
1"'	4.75, 4.74 (*d*, 7.3)	5.47, 5.46 (*d*, 7.2)
2"'	3.50, 3.49 (*d*, 7.3)	4.16, 4.15 (*d*, 7.2)
4"', 5"'	1.14, 1.12, 1.09, 1.08 (*s*)	1.36, 13.6,1.34, 1.32 (*s*)*
OH-5	–	13.06 (*s*)
OH-4'	–	11.81, 11.80 (*s*)

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*
5	158.47
6	103.32*
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

*, # Interchangeable assignments.

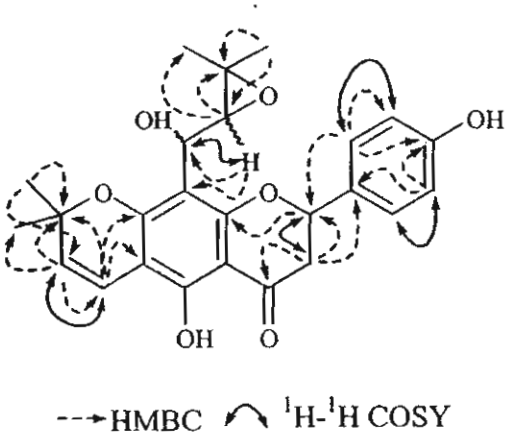
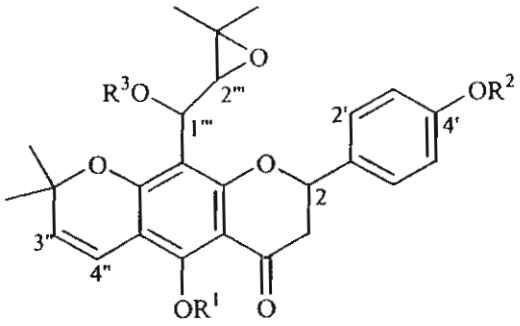


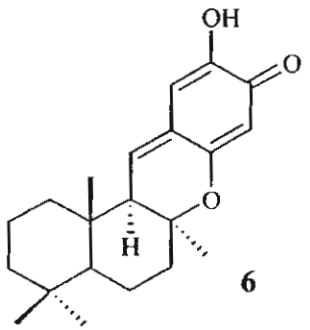
Fig. 1. Summary of important connectivities observed in compound **1** by HMBC and ^{1}H-^{1}H COSY.

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 characteristic 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.



1 $R^1 = R^2 = R^3 = H$
2 $R^1 = R^2 = R^3 = COCH_3$
3 $R^1 = H$, $R^2 = COCH_3$, $R^3 = CH_3$



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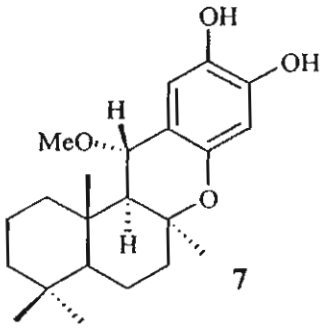

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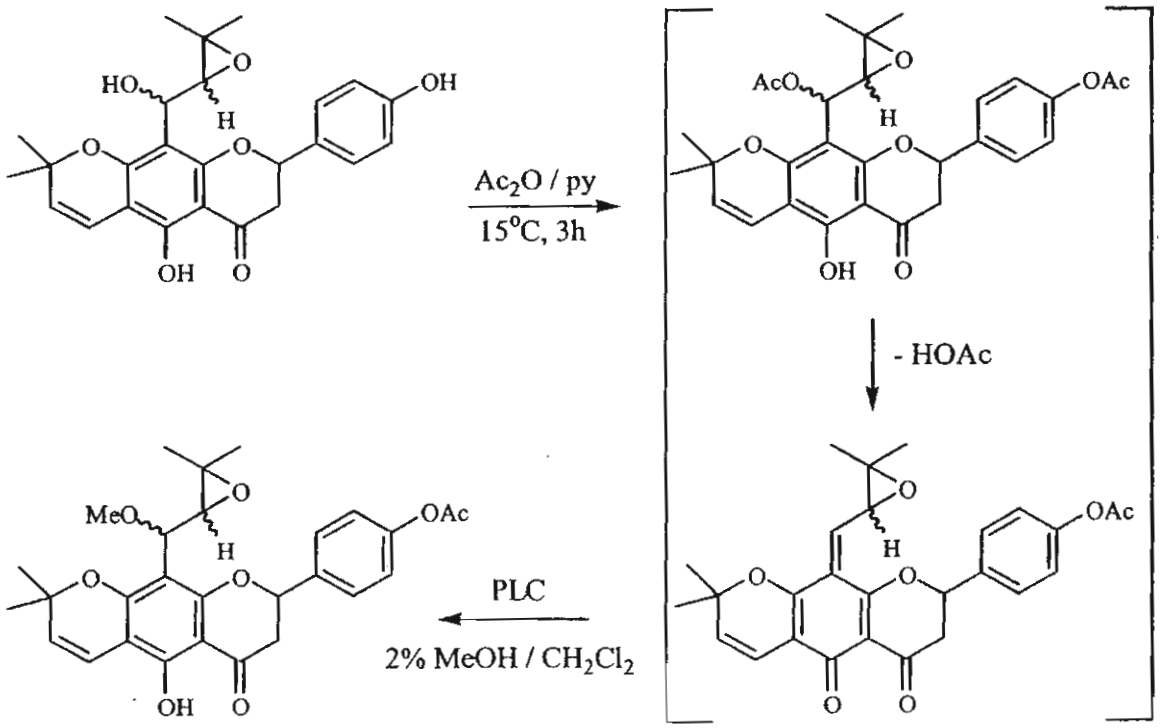
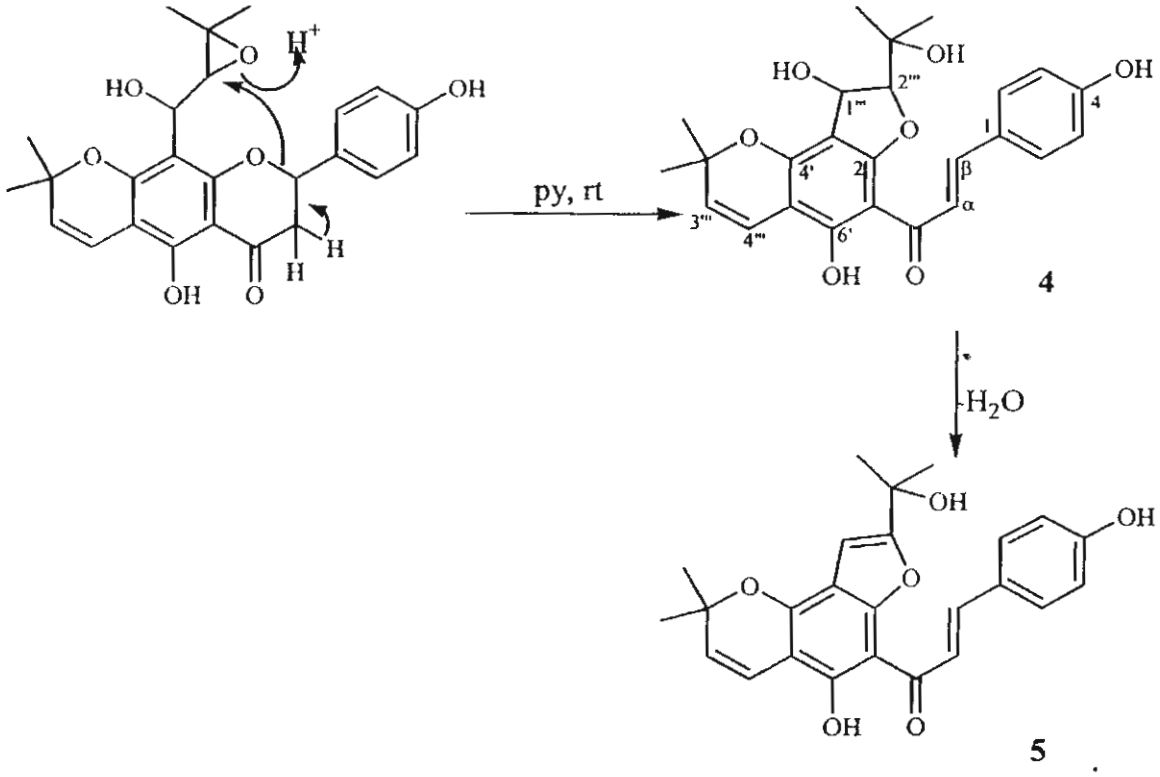


Scheme 1



Scheme 2

ACKNOWLEDGMENT

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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), xanthones (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). ^{1}H-, ^{13}C- 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 UVIDEC-650 double beam spectrophotometers. Optical rotations were measured using JASCO DIP-370 digital polarimeter in $CHCl_3$. Column chromatography was carried out by using silica gel 60 (70–230 mesh ASTM, ≤ 230 mesh, ASTM) and silica gel 60 PF_{254} (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:Et_3N (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-H_2O (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-H_2O (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 CH_2Cl_2-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}; ^{13}C NMR (100 MHz, $CDCl_3$), Table 2; ^1H NMR (400 MHz, $CDCl_3$) 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"), 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}; ^{13}C NMR (100 MHz, $CDCl_3$), Table 2; ^1H NMR (400 MHz, $CDCl_3$) 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", 4"), 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}; ^{13}C NMR (100 MHz, $CDCl_3$), Table 2; ^1H NMR (400 MHz, $CDCl_3$) 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); $[\alpha]_D^{27}$ −8.21° (*c* 0.195, $CHCl_3$).

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 ^{13}C NMR data ($CDCl_3$), 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); $[\alpha]_D^{29}$ −2.45° (*c* 0.245, $CHCl_3$).

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 ^{13}C NMR ($CDCl_3$), 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}; ^{13}C NMR (75 MHz, $CDCl_3$): see Table 2; ^1H NMR (300 MHz, $CDCl_3$) 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); $[\alpha]_D^{29}$ −7.32° (*c* 0.41, $CHCl_3$).

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}; ^{13}C NMR (100 MHz, $CDCl_3$), Table 2; ^1H NMR (400 MHz, $CDCl_3$) 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 ^{1}H NMR (300 MHz) data for compound 5[a].

Position	^{13}C[b]	^{1}H (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'''

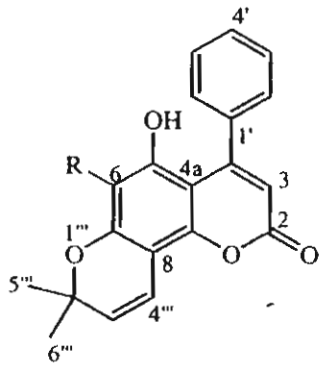
[a]Data were recorded in $CDCl_3$ at 300 MHz for ^{1}H and 75 MHz for ^{13}C NMR.
[b]Multiplicity deduced from DEPT spectroscopy.

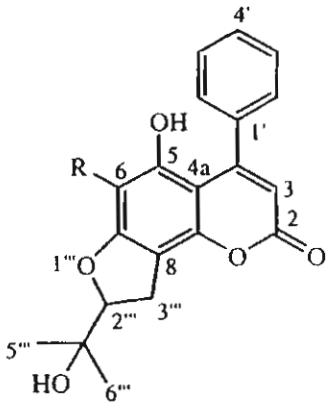
Table 2. ^{13}C NMR data for compounds **1**, **2**, **3**, **6**, **7** in $CDCl_3$ (δ_C).

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

[a]The ^{13}C NMR spectra were recorded at 100 MHz in $CDCl_3$. [b]75 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





1 : R = $COCH_2CH(CH_3)_2$

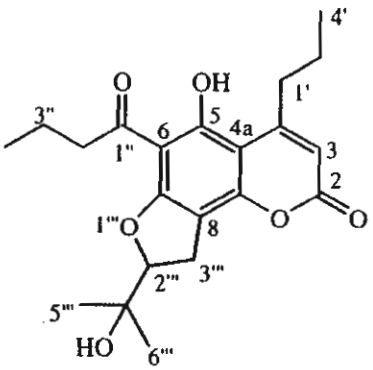
2 : R = $COCH(CH_3)_2$

3 : R = $COCH(CH_3)CH_2CH_3$

4 : R = $COCH_2CH_2CH_3$

5 : R = $COCH(CH_3)CH_2CH_3$

6 : R = $COCH_2CH(CH_3)_2$



7

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° (*c* 0.42, $CHCl_3$). *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 ^{13}C NMR spectra with the aid of ^1H-^1H COSY, ^1H-^{13}C COSY, ^1H-^{13}C 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 ^{13}C NMR (Table 1) showed two sets of resonances of nearly equal intensity and virtually identical chemical

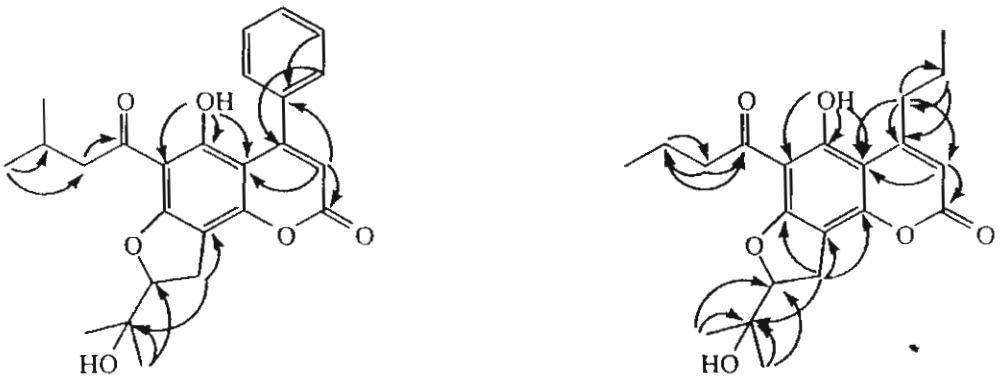


Fig. 2. COLOC correlations of compounds 6 and 7.

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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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 xanthones (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. ^{1}H 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 PF_{254} (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_3)	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_3)	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_3)	28.69	H-5''', 1.52	*s*	–	C-2''', C-3'''
6'''(CH_3)	29.69	H-6''', 1.52	*s*	–	C-2''', C-3'''

*Determined from DEPT spectra

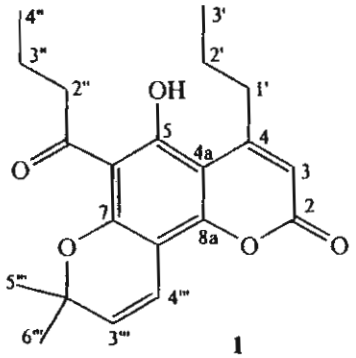
Mammea B/AC cyclo D (**1**) was recrystallized from dichloromethane-hexane to give yellow needles, m.p. 105–106 °C. IR $\nu_{max}CHCl_3$: 3028, 2928, 2855, 1731, 1643, 1615, 1583, 1465, 1426, 1384, 1267, 1189, 1152, 1115 cm^{-1}. UV (MeOH) λ_{max} nm (log ε): 227 (4.245), 285 (4.446), 335 (3.732), 375 (3.586). High resolution FABMS (positive mode) obs. 357.1697 calcd. for $C_{21}H_{24}O_5$+H 357.1701. EIMS *m/z* (rel. int.): 356 ($M^{+\cdot}$, 32), 341 ([M-CH_3]$^+$, 100), 269 ([341-$OCCH_2CH_2CH_3$]$^+$, 2), 227 ([269-$CH_2CH_2CH_3$]$^+$).

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} $CHCl_3$: 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 ($M^{+\cdot}$, 30), 375 ([M-CH_3]$^+$, 100), 357 (20), 347 (14). ^{1}H NMR (400 MHz, $CDCl_3$) 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'''). ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{21}H_{24}O_5$ from ($M^{+\cdot}$ + 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 ($M^{+\cdot}$) and the base peak for (M^+ - CH_3) at *m/z* 341 (100%). Mammea **1** has been detected by GC-MS in *Mammea americana*, and tentatively assigned the structure without ^{1}H and ^{13}C 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 ^{1}H NMR spectrum of compound **1** showed the presence of a 2,2-dimethylchromene ring system [δ



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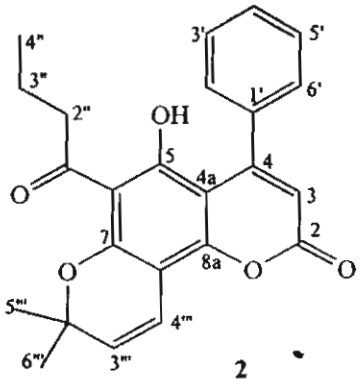

2

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).

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THE FIRST SYNTHESIS OF WRIGHTIADIONE

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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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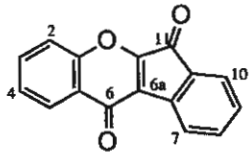
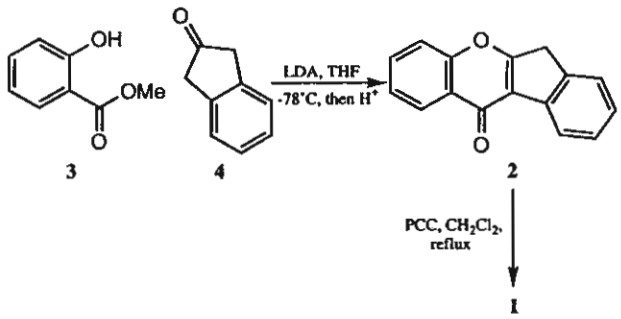


1

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]



3 4 2 1

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_2O, 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_2Cl_2, reflux[11,12]	20	68
9	NHPI*,CH_3CN, reflux[13]	20	25

*NHPI=N-Hydroxyphthalimide

C-11 was not observed, while in the ^{13}C 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 ^{13}C 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 vacum 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 = 0), 1615 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 $C_{16}H_{10}O_2$: 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 string 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=0), 1641 (C=0), 1614, 1605, 1483, 1459, 1371 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 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 ^{13}C NMR (100 MHz, $CDCl_3$) ppm120.7(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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Labdane and pimarane diterpenes from *Croton joufra*

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Abstract

From the chloroform extract of the leaves of *Croton joufra* two novel diterpenes 2α,3α-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 establised by spectroscopic methods. One of the compounds showed weak lethality to brine shrimp.

Keywords: Croton joufra; Euphorbiaceae; Labdane; 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 antidysentery 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 & Permphiphat, 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 μg/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 CH_3-C=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 ^{1}H-^{1}H COSY, HETCOR, COLOC, led to the complete assignments of the ^{1}H and ^{13}C shift values, as shown in Table 1. The important long-range-^{1}H-^{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 $[M+H]^+$ ion at *m/z* 361.23804 corresponding to the molecular formula $C_{22}H_{32}O_4$+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 ^{1}H 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 ^{1}H-^{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 $CH\text{-}CH_2\text{-}CO$ 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 ^{3}J 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 ^{3}J 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-CH_2OCOCH_3 and H-2a/C-19-CH_2OCOCH_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 3β-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 μg/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 μg/ml, respectively.

3. Experimental

3.1 General

Mps uncorrected; ^{1}H 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, CH_2Cl_2-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, $CHCl_3$-MeOH 10:0 to 4:1 and n-hexane-$CHCl_3$ 3:7 to 1:4) to yield **1** (103.6 mg).

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

Pale yellow powder, mp 72-74°; $[\alpha]_D^{25}$ -18.24° ($CHCl_3$, c 0.34); HR-EIMS *m/z*: 304.24150, $[M]^+$, $C_{20}H_{32}O_2$ 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 ^{13}C NMR spectral data are shown in Table 1.

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

Colorless gum; $[\alpha]_D^{25}$ -127.62° ($CHCl_3$, c 0.105); HR-FABMS (glycerol) *m/z*: 361.23804, $[M+H]^+$, $C_{22}H_{32}O_4$+H requires 361.23788; IR (film, cm^{-1}): 3460, 3090, 2931, 2863, 1731, 1648, 1373, 1241, 1094, 1036, 908, 756; ^1H and ^{13}C NMR spectral data are shown in Table 1.

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Table 1 ^{1}H 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 (*m*)	45.0 (*t*)	1.92 (*m*)	34.0 (*t*)
1'	2.11 (*dd*, 4.3, 12.4)			
2	3.70 (*ddd*, 4.2, 9.8, 11.3)	69.1 (*d*)	1.36 (*m*)	27.2 (*t*)
2'			1.85 (*m*)	
3	3.02 (*d*, 9.6)	83.4 (*d*)	3.35 (*dd*, 4.8,11.5)	78.0 (*d*)
4		39.4 (*s*)		41.8 (*s*)
5	1.19 (*br d*, 12.3)	54.5 (*d*)	1.73 (*dd*, 3.9, 14.0)	49.4 (*d*)
6	1.38 (*dddd*, 4.1, 12.8, 12.8, 12.9)	23.6 (*t*)	2.53 (*dd*, 14.0, 17.6)	35.6 (*t*)
6'	1.69 (*m*)		2.63(*dd*, 3.9, 17.6)	
7	1.99 (*ddd*, 4.7, 12.8, 12.9)	37.7 (*t*)		199.5 (*s*)
7'	2.39 (*m*)			
8		147.2 (*s*)		129.0 (*s*)
9	1.73 (*m*)	56.9 (*d*)		164.3 (*s*)
10		40.2 (*s*)		39.3 (*s*)
11	2.19 (*dd*, 7.0, 10.9)	22.3 (*t*)	2.00 (*m*)[a]	23.1 (*t*)
11'	2.37 (*m*)		2.18 (*m*)	
12	5.26 (*t*, 6.4)	130.9 (*d*)	1.28 (*m*)	33.5 (*t*)
12'			1.62 (*m*)	
13		131.9 (*s*)		34.4 (*s*)
14	6.73 (*dd*, 10.8, 17.3)	133.7 (*d*)	2.00 (*m*)[a]	33.2 (*t*)
14'			2.38 (*dd*, 1.5, 17.8)	
15	5.08 (*d*, 10.8)	113.5 (*t*)	5.66 (*dd*, 10.8, 17.5)	144.9 (*d*)
15'	5.17 (*d*, 17.3)			
16	1.76 (*s*)	19.7 (*q*)	4.83 (*dd*, 1.2, 17.5)	111.7 (*t*)
16'			4.93 (*dd*, 1.2, 10.8)	
17	4.50 (*br s*)	108.7 (*t*)	1.02 (*s*)	28.2 (*q*)
17'	4.86 (*br s*)			
18	1.02 (*s*)	28.8 (*q*)	1.15 (*s*)	21.9 (*q*)
19	0.80 (*s*)	16.6 (*q*)	4.23 (*d*, 11.8)	65.0 (*t*)
19'			4.41 (*d*, 11.8)	
20	0.78 (*s*)	15.5 (*q*)	1.11 (*s*)	17.5 (*q*)
CO				171.1 (*s*)
CH_3			2.08 (*s*)	21.0 (*q*)

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

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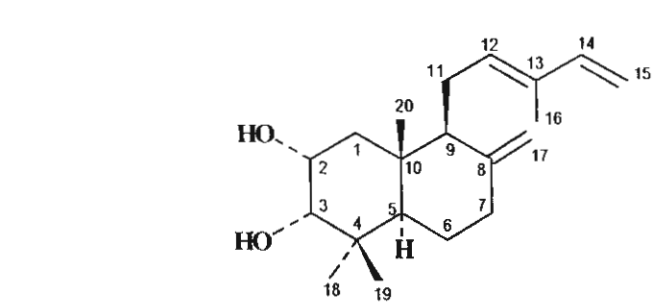
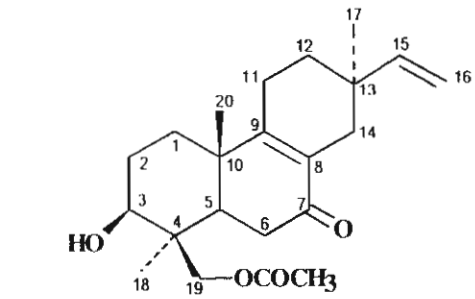


1



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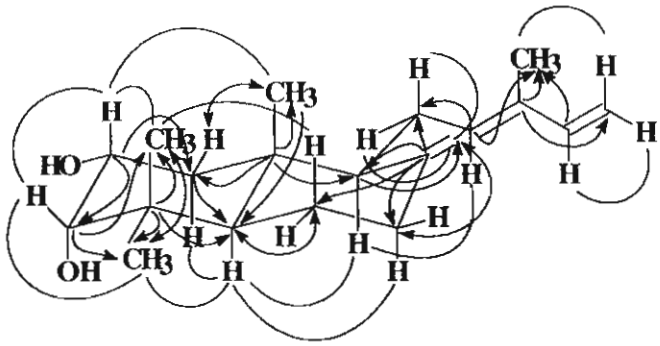

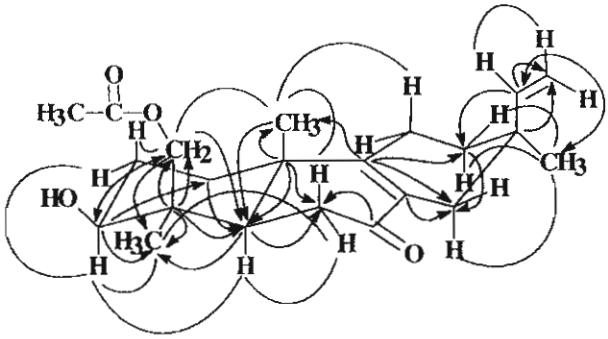

Fig. 1 Structures, key long-range ^{1}H-^{13}C (C H) and NOESY (H H) correlations of **1** and **2**

A SYNTHESIS OF BIPOWINE AND BIPOWINONE

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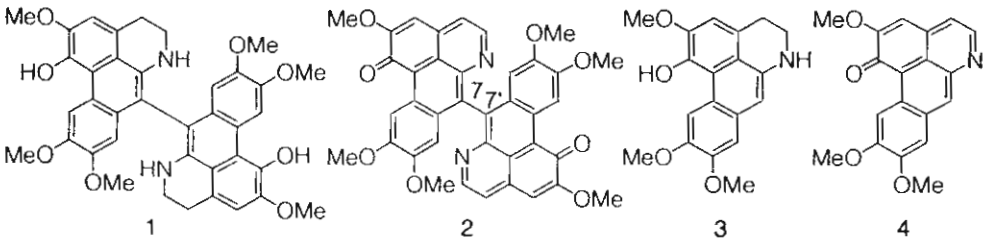
Abstract- Various oxidizing agents were investigated for the synthesis of bipowine (**1**) and bipowinone (**2**), two symmetrical dimeric aporphine alkaloids, from the oxidative coupling of dehydroaporphine (**3**).

Dimeric aporphine alkaloids[1-5] 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 obtained[9] 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 compounds[11-13] have recently been utilized in many organic functional group transformations. The relatively low toxicity of hypervalent iodine compounds[11i] 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_2O (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 Ag_2O. The results are summarized in Table II. Reaction of **3** with Ag_2O[14] 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 ^{13}C 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 procedure[9] 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); ^{1}H NMR (300 MHz, acetone d_6): δ (ppm) 3.13-3.17 (m, 2H, C-4, CH_2-CH_2-NH), 3.38-3.42 (m, 2H, C-5, CH_2-CH_2-NH), 3.88, 3.89, 4.01 (3s, 9H, 3xOCH_3), 6.63 (s, 1H, C-8-Ar*H*), 7.01 (s, 1H, C-7-=C*H*), 7.11 (t, 1H, J = 0.9 Hz, C-3-Ar*H*), 9.27 (s, 1H, C-11-Ar*H*); 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; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.09-3.34 (m, 4H, CH_2-CH_2NH), 3.49, 4.05, 4.07 (3s, 9H, 3xOCH_3), 6.65 (s, 1H, C-8-Ar*H*), 6.86 (s, 1H, O*H*), 7.03 (s, 1H, C-3-Ar*H*), 9.33 (s, 1H, C-11-Ar*H*), ^{13}C NMR (100 MHz, $CDCl_3$-CD_3OD) : 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:

$Hg(OAc)_2$ (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; ^{1}H NMR (400 MHz, $CDCl_3$) : δ 3.36, 4.05, 4.20 (3s, 9H, 3xOC*H*$_3$-9, 2, 10) 6.44 (s, 1H, C-8-Ar*H*), 6.89 (s, 1H, C-3-Ar*H*), 7.45, 8.66 (AB, J_{AB} = 4.4 Hz, 2H, C*H*=C*H*N), 9.91 (s, 1H, C-11-Ar*H*), ^{13}C NMR ($CDCl_3$-CD_3OD) : 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 (OCH_3-2, 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 (Al_2O_3; 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 (Al_2O_3; 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; ^{1}H NMR (400 MHz, $CDCl_3$) : δ 4.03, 4.09, 4.15 (3s, 9H, 3xOC*H*$_3$), 6.78 (s, 1H, C-3-Ar*H*), 6.89 (s, 1H, C-8-Ar*H*), 7.45, 8.66 (AB, J_{ab}= 4.3Hz, 2H,C*H* =C*H* N), 8.70 (s, 1H, C-7-Ar*H*), 9.51 (s, 1H, C-11-Ar*H*), ^{13}C NMR ($CDCl_3$-CD_3OD), 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. (OC*H*$_3$-2,3,10); MS: m/z 321(M^+,73.80), 290(100.00).

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An efficient synthesis of argemonine, a pavine alkaloid†

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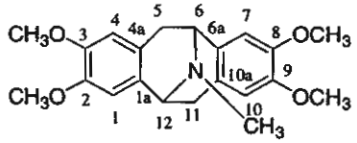
Abstract—A method for the synthesis of 1,2-dihydroisoquinoline derivatives is described and the conversion of the 1,2-dihydroisoquinoline intermediate to a pavine alkaloid via palladium-induced intramolecular hydroarylation reaction and radical cyclization is presented.

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 1[2] 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.

The key steps involve a palladium-induced intramolecular hydroarylation[5] and a radical cyclization[6] 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 synthesis[8] 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′-bromopapaverine[9] **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

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.

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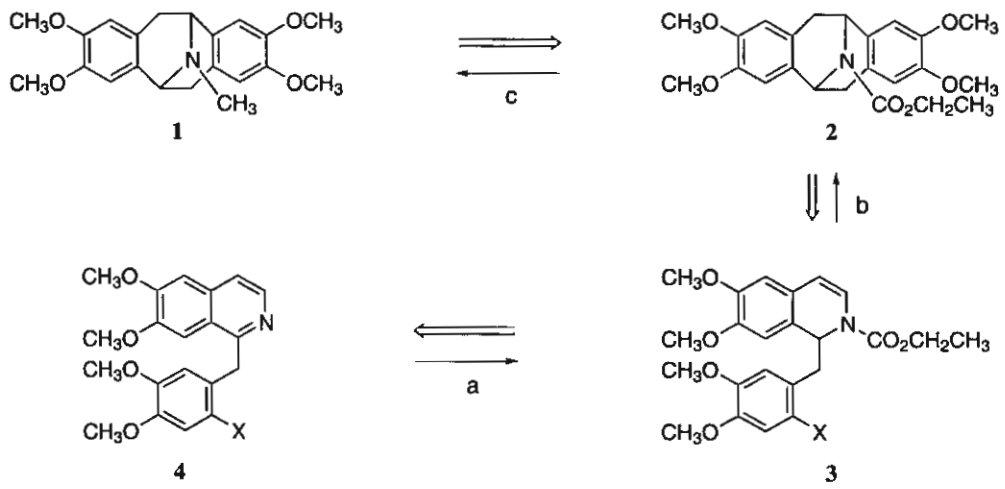
PII: S0040-4039(00)02233-4



Scheme 1. *Reagents and conditions*: (a) i. Bu_3SnH/CH_2Cl_2, ii. $EtOCOCl/CH_2Cl_2$, −78°C→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 $Pd(PPh)_4$ in DMF at 80–90°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 (±)-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**	$Pd(PPh_3)_4/DMF/HCO_2Na$/reflux 24 h	44	34
2	Compound **3b**	Bu_3SnH/AIBN, benzene, reflux 10 h	30	5
3	Compound **3c**	$Pd(PPh_3)_4/DMF/HCO_2Na$/reflux 24 h	56	15
4	Compound **3c**	Bu_3SnH/AIBN, benzene reflux 10 h	42	10

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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, $CDCl_3$) 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, Jab$=7.1$ Hz), 5.94, 6.96 (AB q, 2H, Jab$=7.1$ 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). ^{13}C 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 $C_{23}H_{26}NO_6I$: 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, $CDCl_3$) 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). ^{13}C 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 $C_{26}H_{22}N_2O_3$: C, 66.40; H, 6.59; N, 3.48. Found: C, 66.24; H, 6.46; N, 3.73.

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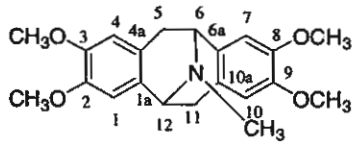
Abstract—A method for the synthesis of 1,2-dihydroisoquinoline derivatives is described and the conversion of the 1,2-dihydroisoquinoline intermediate to a pavine alkaloid via palladium-induced intramolecular hydroarylation reaction and radical cyclization is presented.

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 1[2] 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.

The key steps involve a palladium-induced intramolecular hydroarylation[5] and a radical cyclization[6] 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 synthesis[8] 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′-bromopapaverine[9] **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

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

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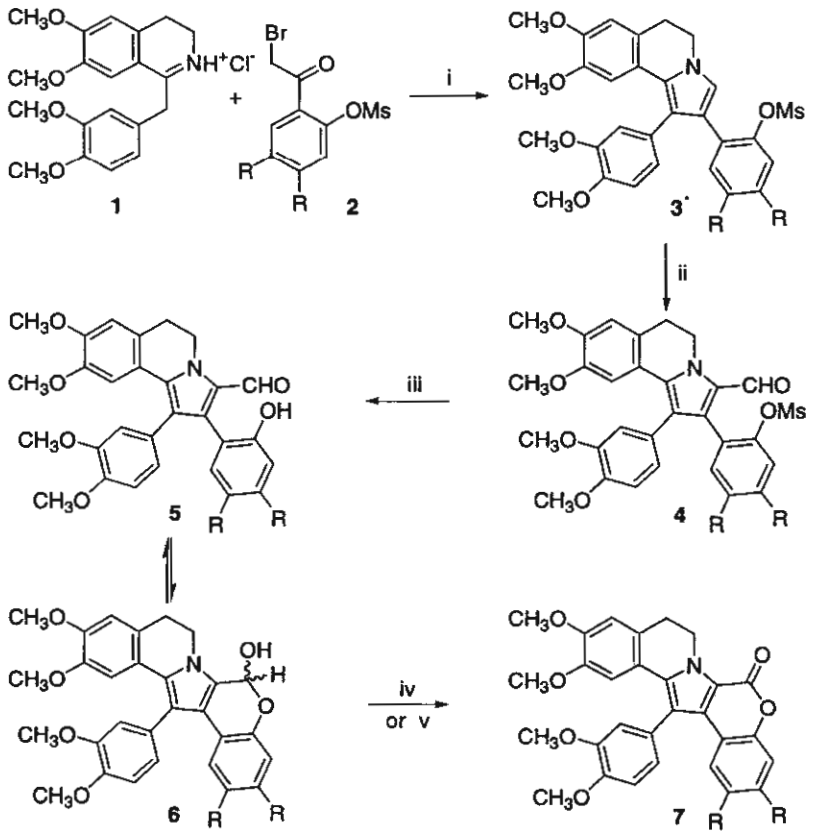
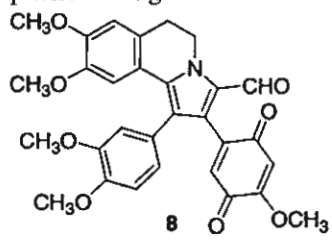


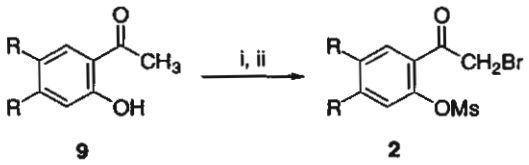
Scheme 1. *Reagents and conditions*: (i) K_2CO_3, CH_3CN, reflux (**3a**, 63%; **3b**, 63%); (ii) DMF, $POCl_3$, rt (**4a**, 80%; **4b**, 82%); (iii) KOH, EtOH, reflux (**5a**, 77%; **5b**, 81%); (iv) MnO_2, CH_2Cl_2, rt (**7a**, 54%; **7b**, 20% and **8**, 37%); (v) $Pd(OAc)_2$, PPh_3, K_2CO_3, DMF,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

have been developed for the synthesis of lamellarins[7] 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.





9 2

Series a R = H
Series b R = OMe

Scheme 2. *Reagents and conditions*: Series a. (i) $MeSO_2Cl$, Et_3N, CH_2Cl_2 (97%); (ii) $BnN^+Me_3Br_3^-$, CH_2Cl_2 (**2a**, 81%): Series b. (i) $MeSO_2Cl$, Et_3N, CH_2Cl_2 (97%); (ii) $BnN^+Me_3Br_3^-$, 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.

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