รายงานวิจัยฉบับสมบูรณ์

สารออกฤทธิ์ต้านการเจริญเติบโตเนื้อเยื่อมะเร็ง เชื้อมาเลเรีย เชื้อวัณโรค และเชื้อจุลินทรีย์จากพืชสมุนไพรไทย

สมยศ สุทธิไวยกิจ และ คณะ

กรกฎาคม 2553

สัญญาเลขที่ DBG 5180018

รายงานวิจัยฉบับสมบูรณ์

สารออกฤทธิ์ต้านการเจริญเติบโตเนื้อเยื่อมะเร็ง เชื้อมาเลเรีย เชื้อวัณโรค และเชื้อจุลินทรีย์จากพืชสมุนไพรไทย

Cytotoxic, Antimalarial, Antituberculous and Antimicrobial Compounds from Thai Medicinal Plants

ผู้วิจัยหลัก
สมยศ สุทธิไวยกิจ

ผู้ร่วมวิจัย และนักวิจัย
มยุรี ชวนกำเนิดการ
ปาริชาต นารีบุญ
วันทนา มงคลวิสุทธิ์
จุฑามณี อยู่ขวัญ
ธิติมา หลินหะตระกูล
ชลธิชา สีกา

ภาควิชาเคมี คณะวิทยาศาสตร์
มหาวิทยาลัยรามคำแหง

ทุนวิจัยพื้นฐานแบบมุ่งเป้า
สมุนไพร ยารักษาโรค และ สารเสริมสุขภาพ
สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย
(ความเห็นในรายงานนี้เป็นของผู้วิจัย
สกว.ไม่จำเป็นต้องเห็นด้วยเสมอไป)

สารบัญ

กิตติกรรมประกาศ

ผู้วิจัยขอขอขอบคุณสำนักงานกองทุนสนับสนุนการวิจัย (สกว.) ที่ให้ทุนวิจัยพื้นฐานแบบมุ่งเป้า “สมุนไพร ยารักษาโรค และ สารเสริมสุขภาพ” ทำให้งานวิจัยนี้บรรลุผลตามความมุ่งหมาย

ขอขอบคุณ รศ.ดร. นิจศิริ เรืองรังษี ภาควิชาเภสัชเวท คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัยที่ช่วยหาเอกลักษณ์ทางพฤกษศาสตร์พืชที่ทำวิจัย

ขอขอบคุณภาควิชาเคมี มหาวิทยาลัยมหิดล วิชาเคมี มหาวิทยาลัยเชียงใหม่ และสถาบันวิจัยจุฬาภรณ์ ที่ตรวจวัดน้ำหนักโมเลกุลสารบางชนิดด้วยเครื่องแมสสเปกโตรมิเตอร์ให้ นอกจากนี้คณะผู้วิจัยขอขอบคุณห้องปฏิบัติการตรวจสอบฤทธิ์ทางชีวภาพ ศูนย์พันธุวิศวกรรมและเทคโนโลยีแห่งชาติ (สวทช.) ที่ทำการทดสอบฤทธิ์ทางชีวภาพสารองค์ประกอบที่แยกได้

งานวิจัยชิ้นนี้จะไม่สำเร็จลุล่วงได้หากไม่ได้รับความร่วมมืออันดีจาก อาจารย์ และเจ้าหน้าที่ของภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยรามคำแหง

สมยศ สุทธิไวยกิจ และคณะผู้วิจัย

Executive Summary

สัญญาเลขที่ DBG 5180018

โครงการวิจัยชื่อ สารออกฤทธิ์ต้านการเจริญเติบโตเนื้อเยื่อมะเร็ง เชื้อมาเลเรีย เชื้อวัณโรคและเชื้อจุลินทรีย์จากพืชสมุนไพรไทย (Cytotoxic, Antimalarial, Antituberculous and Antimicrobial Compounds from Thai Medicinal Plants)

คณะผู้วิจัย: สมยศ สุทธิไวยกิจ มยุรี ชวนกำเนิดการ ปาริชาต นารีบุญ วันทนา มงคลวิสุทธิ์ จุฑามณี อยู่ขวัญ ธิติมา หลินหะตระกูล และ ชลธิชา สีกา
Email address: somyote_s@yahoo.com; s_somyote@ru.ac.th

วัตถุประสงค์ (Objectives):

การวิจัยโครงการนี้จะใช้วิธีทดสอบฤทธิ์ทางชีวภาพ เช่น antimalarial, anti-TB, cytotoxcity assays, antimicrobial assay และ antioxidant assay ใช้ stable DPPH radical เป็นเครื่องมือเพื่อการคัดเลือกพืช และส่วนสกัดของพืช

1. เพื่อแยกสารองค์ประกอบโดยเฉพาะที่ออกฤทธิ์ทางชีวภาพที่น่าสนใจให้บริสุทธิ์ เพื่อนำไปหาโครงสร้าง
2. เพื่อหาสารออกฤทธิ์ชนิดใหม่ จากแหล่งใหม่ หรือแยกสารออกฤทธิ์ที่เคยค้นพบแล้วจากแหล่งใหม่ และจะไม่ละเลยการหาสารที่มีโครงสร้างใหม่ที่อาจไม่แสดงฤทธิ์
3. เพื่อนำสารบริสุทธิ์ที่แยกได้ และสารอนุพันธ์ที่อาจจำเป็นต้องเตรียมไปทดสอบฤทธิ์ทางชีวภาพ
4. เพื่อสังเคราะห์สารที่มีโครงสร้างที่แตกต่างในบางตำแหน่งของโมเลกุลเพื่อนำไปศึกษาฤทธิ์ทางชีวภาพ
5. เพื่อเพิ่มทักษะและสร้างความชำนาญให้กับนักศึกษาระดับบัณฑิตศึกษา และระดับปริญญาตรีที่ร่วมโครงการ ให้เป็นนักวิจัยที่ดีต่อไปในอนาคต
6. เพื่อสร้างองค์ความรู้ใหม่ และผลิตผลงานวิจัยที่ดี เทียบเท่าระดับสากล

ระเบียบวิธีวิจัย (Methodology):

โครงการวิจัยนี้จะเลือกสรรพืชที่ให้ผลบวกกับการทดสอบฤทธิ์ทางชีวภาพในการศึกษา การดำเนินการสำหรับโครงการวิจัย สามารถลำดับได้ดังต่อไปนี้

นำส่วนของพืชมาสกัดด้วยตัวทำละลาย ได้แก่ สกัดด้วยเฮกเซน ไดคลอโรมีเทน และเมทานอล ซึ่งจะทำให้ได้ส่วนสกัดเฮกเซน ส่วนสกัดไดคลอโรมีเทน และส่วนสกัดเมทานอล ตามลำดับ นำส่วนสกัดต่าง ๆ ไปทำการทดสอบฤทธิ์ทางชีวภาพ ซึ่งจะใช้ 2-3 วิธีควบคู่กัน เมื่อพบว่าส่วนสกัดใดให้ผลบวกกับการทดสอบข้างต้น จะทำการแยกสารองค์ประกอบให้บริสุทธิ์ โดยวิธีทางโครมาโตกราฟี นำสารบริสุทธิ์ที่แยกได้ไปหาสูตรโครงสร้างด้วยวิธีทางสเปกโทรสโกปี เมื่อทราบสูตรโครงสร้างแล้ว จะนำสารบริสุทธิ์ที่ได้ในปริมาณมากพอ ไปทดสอบฤทธิ์ทางชีววิทยาที่เกี่ยวข้อง

ผลลัพธ์ วิจารณ์ และสรุปผลการทดลอง (Results, discussion and conclusion): จากการศึกษาพืชต่างๆที่เลือกสรรได้จากการทดสอบการออกฤทธิ์ พบ ต้นหญ้าค้อนกลอง (*Eriosema chinense*) ต้นรัก (*Calotropis gigantea*) และ ต้นปัตตาเวีย (*Jatropha integerrima*)ให้ฤทธิ์น่าสนใจที่สุด และ สามารถแยกสารออกฤทธิ์ และสารใหม่ได้หลายชนิด สารหลายชนิดมีฤทธิ์ทางชีวภาพระดับดีถึงปานกลาง โดยผลงานได้รับการตีพิมพ์แล้วรวม 3 เรื่อง สำหรับพืชชนิดที่ 4 ต้นมะกรูด (*Citrus hystrix*) กำลังอยู่ระหว่างดำเนินการ ผลที่ได้ยังไม่ได้รายงานไว้ในรายงานนี้ และคาดว่าจะทำให้เสร็จสมบูรณ์ได้ราวปลายปีพ.ศ. 2553

จากเงินทุนวิจัยนี้นอกจากจะสามารถผลิตผลงานวิจัยที่สามารถตีพิมพ์ได้แล้ว เงินทุนวิจัยนี้มีส่วนสนับสนุนการศึกษาแก่นักศึกษาทั้งในระดับบัณฑิตศึกษา และปริญญาตรีที่ร่วมในโครงการ ได้ประมาณปีละ 3-4 คน สามารถสร้างนักวิจัยรุ่นเยาว์ปริญญาตรีได้ปีละอย่างน้อย 3-4 คน

งานวิจัยพืชชนิดที่หนึ่ง สารองค์ประกอบ และฤทธิ์ทางชีวภาพของต้นหญ้าค้อนกลอง (Constituents and Bioactivity of *Eriosema chinense*)

บทคัดย่อภาษาไทย

จากส่วนสกัดรากชั้นเฮกเซน และไดคลอโรมีเทนสามารถแยกสารใหม่ได้ 8 ชนิด โดยตั้งชื่อตามชื่อภาษาไทยของพืช ได้แก่ khonklonginols A-H รวมถึงสารที่เคยแยกได้แล้วจากแหล่งอื่นอีก 6 ชนิด เป็นสารฟลาโวนอนด์ 5 ชนิด คือ lupinifolinol, dehydrolupinifolinol, flemichin D, eriosemaone A, lupinifolin และ สารประเภทลิกแนน 1 ชนิดได้แก่ yangambin สารเหล่านี้บางชนิดแสดงฤทธิ์ยับยั้งการเจริญเติบโตเนื้อเยื่อมะเร็ง เช่น มะเร็งเต้านม (Breast Cancer) มะเร็งปอด (human small cell lung cancer, NCI-H187) และมะเร็งในช่องปาก (human oral epidermal carcinoma, KB) และ เชื้อวัณโรคในระดับดีมากถึงปานกลาง

Abstract

Eight new prenylated flavonoids, khonklonginols A-H, together with six known compounds including five flavonoids, lupinifolinol, dehydrolupinifolinol, flemichin D, eriosemaone A, lupinifolin, and one lignan, yangambin, have been isolated from hexane

and dichloromethane extracts of the roots of *Eriosema chinense*. The structures of khonklonginols A-H were elucidated by spectroscopic methods. The compounds were evaluated for cytotoxic activity against the small-cell lung (NCI-H187) and oral epidermal carcinoma (KB) human cell lines as well as for antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra)

งานวิจัยพืชชนิดที่สอง สารองค์ประกอบ และฤทธิ์ทางชีวภาพของต้นรัก (Constituents and Bioactivity of *Calotropis gigantea*)

บทคัดย่อภาษาไทย

จากส่วนสกัดใบชั้นไดคลอโรมีเทน และบิวทานอล สามารถแยกสารใหม่ได้ 3 ชนิด ที่เป็นสารกลุ่มคาร์ดิโนไลด์ (Cardenolides) ที่มีหมู่ไฮดรอกซิที่ตำแหน่ง 15 และ16 ได้แก่ 2α,15β-dihydroxy-19-oxo-uzarigenin, 15β-hydroxycalactinic acid และ 16α-hydroxycalactinic acid methyl ester นอกจากนี้ยังสามารถแยกสารคาร์ดิโนไลด์ที่เคยมีการค้นพบแล้วอีก 9 ชนิด ได้แก่ 16α-hydroxy- calotropagenin, coroglaucigenin, 16α-hydroxycalotropin, calactinic acid, calotoxin, 6′-O-(*E*-4-hydroxycinnamoyl)desglucouzarin, 12β-hydroxycoroglaucigenin, frugoside, calotropagenin รวมทั้งกรดไขมัน 1 ชนิด ได้แก่ 9,12,13-trihydroxyoctadeca-10(*E*),15(*Z*)-dienoic acid และ *R*-(–)-mevalonolactone สารคาร์ดิโนไลด์หลายชนิดให้ผลยับยั้งในระดับดีมากถึงปานกลางต่อการเจริญเติบโตของเซลล์เนื้อเยื่อมะเร็งเต้านม (Breast Cancer) มะเร็งปอด (human small cell lung cancer, NCI-H187) และมะเร็งในช่องปาก (human oral epidermal carcinoma, KB) โดยการออกฤทธิ์ของสารกลุ่มคาร์ดิโนไลด์แตกต่างกันไปตามชนิดของโครงสร้างสเตอริโอเคมี และ ชนิดของน้ำตาลที่เกาะ

Abstract

Two 15β-hydroxycardenolides, 2α,15β-dihydroxy-19-oxo-uzarigenin, 15β-hydroxycalactinic acid and a 16α-hydroxycalactinic acid methyl ester along with eleven known compounds including 16α-hydroxycalotropagenin, coroglaucigenin, 16α-hydroxycalotropin, calactinic acid, calotoxin, 6′-O-(*E*-4-hydroxycinnamoyl) desglucouzarin, 12β-hydroxycoroglaucigenin, frugoside, calotropagenin, 9,12,13-trihydroxyoctadeca-10(*E*),15(*Z*)-dienoic acid and *R*-(–)-mevalonolactone were isolated from the polar fraction of the CH_2Cl_2 extract, and n-BuOH extract of the leaves of this plant. The isolated compounds were evaluated for their inhibitory activities against a panel of cell lines.

งานวิจัยพืชชนิดที่สาม สารองค์ประกอบ และฤทธิ์ทางชีวภาพของต้นปัตตาเวีย (Constituents and Bioactivity of *Jatropha integerrima*)

บทคัดย่อภาษาไทย

จากส่วนสกัดรากชั้นไดคลอโรมีเทนของต้นปัตตาเวีย (*Jatropha integerrima*) สามารถแยกสารใหม่ได้ 5 ชนิด เป็นสารประเภทไดเทอร์พีน (diterpenes) ได้ 3 ชนิด ได้แก่ 2α-hydroxyjatropholone, 2β-hydroxyjatropholone และ 1,5-dioxo-2,3-dihydroxyrhamnofola-4(10),6,11(18),15-tetraene สารประเภทเซสคิวเทอร์พีน(sesquiterpene) 1 ชนิด ได้แก่ 2-keto-5-hydroxyguai-3,11-diene และสารคอนจูเกตระหว่างเซสคิวเทอร์พีนกับคิวมาริน(sesquiterpene-coumarin conjugate) ที่มาโครงสร้างแปลกใหม่ ได้แก่ jatrophadioxan นอกจากนี้ยังได้สารที่เคยค้นพบจากแหล่งอื่นแล้วอีก9 ชนิด โครงรูปของ 2α-hydroxyjatropholone ได้รับการพิสูจน์โดยใช้เทคนิค X-ray crystallography สารเหล่านี้บางชนิดให้ผลออกฤทธิ์ยับยั้งการเจริญเติบโตของเชื้อมาลาเรีย เชื้อวัณโรค และ ยับยั้งการเจริญเติบโตของเซลล์เยื่อมะเร็ง

Abstract

Five new compounds including three diterpenes, 2α-hydroxyjatropholone, 2β-hydroxyjatropholone, and 1,5-dioxo-2,3-dihydroxyrhamnofola-4(10),6,11(18),15-tetraene, one sesquiterpene, 2-keto-5-hydroxyguai-3,11-diene, and a unique sesquiterpene-coumarin conjugate, jatrophadioxan, in addition to nine known compounds, have been isolated from the roots of *Jatropha integerrima*. Structural identification was established from spectroscopic data. Relative configuration of 2α-hydroxyjatropholone was confirmed by X-ray crystallography. Six diterpenes were evaluated for their antiplasmodial, antituberculous and cytotoxic activities.

ข้อเสนอแนะ (Suggestions): จากการศึกษาพืช 3 ชนิดสามารถแยกสารใหม่ และสารที่เคยมีรายงานการค้นพบแล้วหลายชนิด ในจำนวนสารหลายชนิดได้ทำการศึกษาการออกฤทธิ์ทางชีวภาพไปบ้างแล้ว และพบว่าสารหลายชนิดให้ผลการทดสอบในหลอดทดลองอยู่ในเกณฑ์ดีมากถึงปานกลาง แต่การศึกษาเพื่อนำไปพัฒนาเป็นยาอาจจะยังต้องพิจารณาเกี่ยวกับการนำส่งตัวยาให้มุ่งสู่เป้าหมายในสัตว์ทดลองเพิ่มเติมต่อไป ขณะนี้ผู้วิจัยได้พยายามเก็บรวบรวมข้อมูลบางประเภท เช่น ข้อมูลแมสสเปคโทรสโคปี และ ยูวีสเปคโทรโฟโตเมตรีเพิ่มเติม เพราะเชื่อว่าจะเป็นประโยชน์หากต้องการติดตามแยกสารเหล่านี้ใหม่ โดยหวังว่าจะสามารถหลีกเลี่ยงการต้องทำซ้ำใหม่ (Dereplication) เพื่อการศึกษาในเชิงลึกเพิ่มเติมต่อไป

(**Keywords**):*Eriosema chinense, Calotropis gigantea, Jatropha integerrima*

Final Report
(August 2008-June 2010)

Cytotoxic, Antimalarial, Antituberculous and Antimicrobial Compounds from Thai Medicinal Plants

Dr. Somyote Sutthivaiyakit

Department of Chemistry, Faculty of Science

Ramkhamhaeng University, Hua Mark

Bangkok 10240, Thailand

Contract no. DBG 5180018

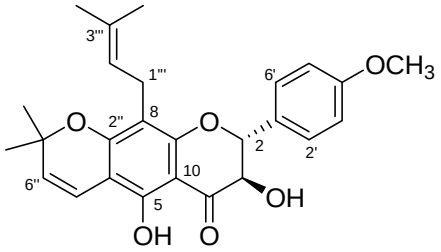
Submitted to
The Thailand Research Fund

Cytotoxic, Antimalarial, Antituberculous and Antimicrobial Compounds from Thai Medicinal Plants

Part I. Constituents and Bioactivity of *Eriosema chinense*

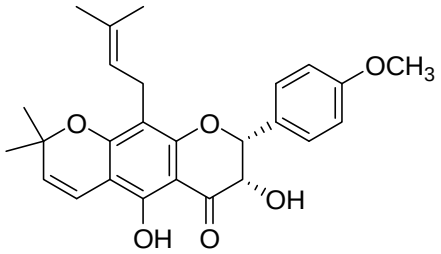
Eriosema chinense is a small plant which was documented as the only one species found in Thailand.[1] There has been no previous report on biological activity and phytochemical investigation of this plant. Our preliminary cytotoxic activity assays indicated the hexane extract of the root to be active showing IC_{50} of ~10 μg/mL using KB and NCI H187 cell lines, it also showed anti-TB activity with MIC value of 50 μg/mL. Chromatographic separation of the hexane and dichloromethane extracts led to the isolation of compounds **1**-**14**.

Compound **1** was isolated as a yellow liquid with molecular formula of $C_{26}H_{28}O_6$ as calculated from HREIMS (found M^+ at *m/z* 436.1873). The FTIR spectrum showed absorption bands for hydroxyl (ν_{max} 3467 cm^{-1}) and conjugated carbonyl (ν_{max} 1626 cm^{-1}) functional groups. The ^1H and ^{13}C NMR spectra showed characteristic sets of signals at δ_H 4.98 (1H, d, J = 12.0 Hz, H-2) and 4.42 (1H, d, J = 11.6 Hz, H-3) and at δ_C 82.9 (CH, C-2) and 72.5 (CH, C-3) of a 3-hydroxy flavanone skeleton.[2] A low field singlet at δ_H 11.41 indicated a C-5 OH group hydrogen-bonded to a carbonyl carbon at C-4. Aromatic proton signals at δ_H 7.46 (2H, d, J = 8.8 Hz, H-2′, H-6′) and 6.96 (2H, d, J = 8.8 Hz, H-3′, H-5′) could be assigned to a 1,4-disubstituted aromatic ring B protons as evident from the HMBC correlations between H-2′ and H-6′ to C-2. The ^3J correlations of H-2′, H-3′ and a singlet at δ_H 3.83 to C-4′ (δ_C 160.3, qC) indicated the attachment of a O*CH₃* group at C-4′. The ^1H NMR signals at δ_H 5.51 (d, J = 10.0 Hz, H-5″), 6.62 (d, J = 10.0 Hz, H-4″), 1.44 (s) and 1.43 (s) showing ^1J correlations with the ^{13}C NMR signals at δ_C 126.2, 115.4, and 28.4 (2×), respectively, were assigned for a dimethylchromene group. ^1H NMR signals at δ_H 3.16 (2H, d, J = 6.8 Hz, H-1‴), 5.11 (dt, J = 6.4, 1.6 Hz, H-2‴) and two singlets at δ_H 1.63 and 1.59, and ^{13}C NMR signals at δ_C 21.4, 122.3, 109.3, 25.7 and 17.8 were assigned for a dimethylallyl group. The key HMBC correlations between H-4″/C-5 (δ_C 156.1) required the placement of a chromene ring at C-6 and C-7, and the correlations between H-1‴ with C-8 (δ_C 131.3, qC) and C-9 (δ_C 159.5, qC) indicated a 3‴, 3‴-dimethylallyl group at C-8. Compound **1** was identified as 3,5-dihydroxy-4′-methoxy-6″,6″-dimethyl-pyrano (2″, 3″: 7,6)-8-(3‴, 3‴-dimethylallyl)-flavanone. This compound was named trivially as khonklonginol A. The absolute configurations at C-2 and C-3 were proposed as 2*R*,3*R* based on the large $J_{2,3} = J_{3,2}$ value of 12.0 Hz indicating H-2 and H-3 to be *trans* and the circular dichroism spectrum which showed a positive $n \rightarrow \pi^*$ Cotton effect at 362 nm.[3] Our data is consistent with those reported for lupinifolinol[4,5] which was also isolated in this study, as well as for jayacanol previously isolated from *Lonchocarpus oaxacensis*.[6] Full assignments of ^1H and ^{13}C chemical shifts are as shown in Tables 1 and 2.



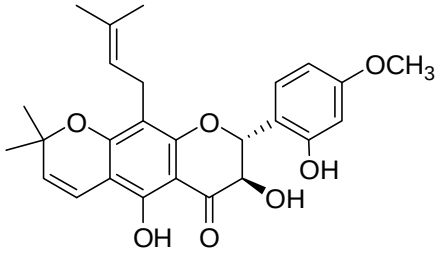
1

Compound **2** was assigned a molecular formula of $C_{26}H_{28}O_6$ from HRMS. The FTIR spectrum also showed absorption bands for hydroxyl (ν_{max} 3436 cm^{-1}) and carbonyl (ν_{max} 1622 cm^{-1}) groups. ^{1}H and ^{13}C NMR spectra showed rather similar pattern of signals as those of compound **1** (Tables 1-2), except for the presence of lesser shielded doublet signals at δ_H 5.64 and 4.71, both mutually coupled with relatively smaller vicinal coupling constant of 5.1 Hz and were assignable to H-2 and H-3 of a 3-hydroxyflavanone skeleton, respectively.[2] The smaller $J_{2,3}$ value indicated compound **2** to possess different stereochemistry at C-3 as of compound **1**, impling H-2 and H-3 to be *cis*. The CD spectrum showed positive Cotton effect at 356 nm.[3] The absolute configurations at C-2 and C-3 of **2,** a 3-epimer of **1**, could be proposed as 2*R*,3*S* and **2** was given a trivial name as khonklonginol B.



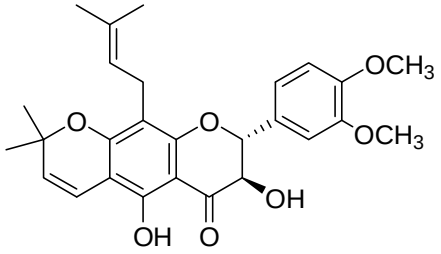
2

Compound **3** was obtained as a pale yellow sticky liquid. The HRMS revealed a molecular formula of $C_{26}H_{28}O_7$. The FTIR spectrum also showed the absorption bands of a hydroxyl (ν_{max} 3391 cm^{-1}) and a carbonyl (ν_{max} 1622 cm^{-1}) groups. ^{1}H and ^{13}C NMR signals are rather similar to those of compound **1** (Tables 1 and 2), the difference was detected at aromatic proton signals. Instead of a 1,4-disubstituted pattern as observed in **1** and **2**, the aromatic ring B was deduced as a trisubstituted ring. Assignment of signals at δ_H 7.42 (d, J = 8.6 H) for H-6′, δ_H 6.58 (brd, J = 8.7 Hz) for H-5′, and 6.54 (d, J = 2.5 Hz) for H-3′ was based on the long range ^{1}H-^{13}C correlations between H-2 (δ_H 5.28)/C-1′ (δ_C 116.3, qC), C-2′ (δ_C 155.3, qC), C-6′ (δ_C 127.9, CH), as well as H-3′/C-1′, C-2′, C-4′ (δ_C 161.2, qC), C-5′ (δ_C 107.3, CH). The ^{3}J correlations of O*CH*$_3$ (δ_H 3.79), H-6′ and H-5′ with C-4′ required placement of the *OCH*$_3$ group at C-4′, thus implying OH at C-2′. Compound **3**, proposed as khonklonginol C, was identified as 3,5,2′-trihydroxy-4′-methoxy-6″,6″-dimethyl-pyrano (2″, 3″: 7,6)-8-(3‴, 3‴-dimethylallyl)-flavanone.



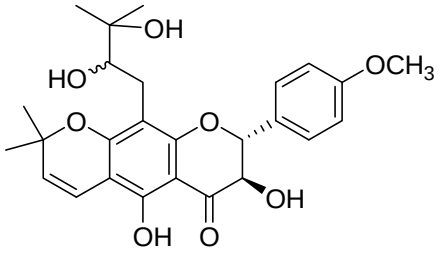
3

Compound **4** was isolated as a yellow liquid and was assigned a molecular formula of $C_{27}H_{30}O_7$ based on the $[M+1]^+$ ion with *m/z* 467.2001 in its HRESIMS spectrum. The FTIR spectrum showed the presence of a hydroxyl (ν_{max} 3430 cm^{-1}) and carbonyl (ν_{max} 1627 cm^{-1}) groups. ^1H NMR spectrum indicated **4** to have similar core skeleton as those of **1** and **3**. The aromatic ring B protons although revealed a trisubstituted pattern as of compound **3** but difference could be detected. The partially overlapped doublet of doublets signal at δ_H 7.08 (J = 8.8, 2.0 Hz), a doublet signal at δ_H 7.07 (J = 2.0 Hz) and a doublet at δ_H 6.91 (J = 8.8 Hz) were assigned for H-6′, H-2′ and H-5′, respectively due to an observation of the ^3J correlations between H-2 (δ_H 4.97)/C-2′ (δ_C 120.2, CH), C-6′ (δ_C 110.2, CH), C-1′ (δ_C 129.0, qC), in addition to correlations between H-2′, H-5′ and H-6′ with C-1′. HMBC correlations also led to assign signals at δ_H 3.90 and 3.91for O*CH*$_3$-3′ and O*CH*$_3$-4′, respectively. Compound **4** was proposed as 3,5-dihydroxy-3′,4′-dimethoxy-6″,6″-dimethyl-pyrano (2″, 3″: 7,6)-8-(3‴, 3‴-dimethylallyl)-flavanone and named as khonklonginol D.



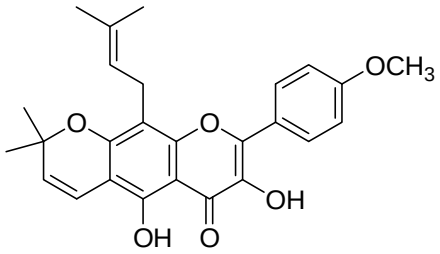
4

Compound **5** was obtained as a yellow sticky liquid and assigned a molecular formula of $C_{26}H_{30}O_8$ from its HRESIMS with $[M+Na]^+$ ion showing *m/z* 493.1382. The FTIR spectrum showed the presence of a hydroxyl (ν_{max} 3401 cm^{-1}) and carbonyl (ν_{max} 1633 cm^{-1}) groups. ^1H and ^{13}C NMR spectra of **5** were rather similar to those of **1** (Tables 1 and 2) except for the absence of signals for a dimethylallyl group. Two sets of partially overlapped signals for oxymethine proton at δ_H 3.45 and 3.42, as well as of benzylic protons at δ_H 2.76, 2.75, 2.53 and 2.51 indicated the presence of two forms of vicinal diol at C-2‴ and C-3‴ as reported for 2‴,3‴-dihydroxylupinifolin.[7] Compound **5** was proposed as 3,5-dihydroxy-4′-methoxy-6″,6″-dimethyl-pyrano (2″, 3″: 7,6)-8-(3‴, 3‴-dimethyl-2‴,3‴-dihydroxypropyl)-flavanone (khonklonginol E).



5

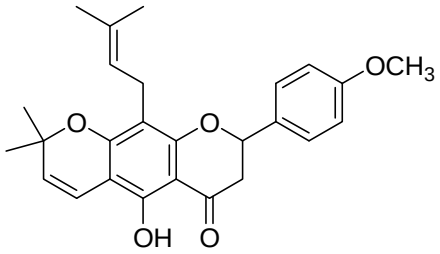
Compound **6** was obtained as pale yellow solid with molecular formula of $C_{26}H_{26}O_6$. IR spectrum showed absorption bands of a hydroxyl (ν_{max} 3316 cm^{-1}) and carbonyl (ν_{max} 1620 cm^{-1}) groups. ^1H NMR spectrum also exhibited signals of a chromene, dimethylallyl and a chelated OH groups (Table 3). Two pairs of doublet signals at δ_H 8.15 and 7.01 (both corresponded to 2H, J = 8.8 Hz) of the 1,4-disubstituted aromatic ring was also observed. The *OCH₃* group resonated at δ_H 3.87 was assigned at C-4′ as indicated from the HMBC correlations between H-2′, H-6′ and O*CH₃*/C-4′. The molecular weight of 2 amu lower than that of compound **1**, with the absence of doublet signals for H-2 and H-3 protons at approximately δ_H 4.98 and 4.42, as observed in **1**-**4**, and the presence of two quaternary carbon signals at δ_C 145.4 and 135.5, in addition to the HMBC correlations of a hydroxyl proton signal at δ_H 6.63/ C-2 (δ_C 145.4) and C-4, impling the presence of a double bond at C-2 and a hydroxyl group at C-3. Compound **6** could thus be established as a flavonol and elucidated as 3,5-dihydroxy-4′-methoxy-6″,6″-dimethyl-pyrano (2″, 3″: 7,6)-8-(3‴, 3‴-dimethylallyl)-flavone and named as khonklonginol F.



6

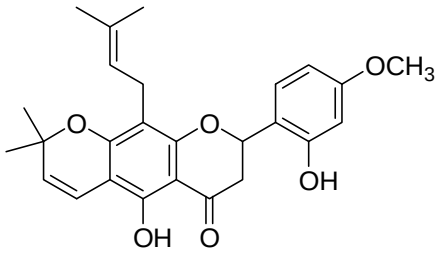
Compound **7** was obtained as a pale yellow amorphous solid, and its mass spectrum exhibited $(M+1)^+$ ion at *m/z* 421.2093 corresponding to a molecular formula of $C_{26}H_{28}O_5$. Infrared spectrum showed absorption bands of a hydroxyl (ν_{max} 2918 cm^{-1}) and carbonyl (ν_{max} 1628 cm^{-1}) groups. ^1H NMR spectrum (Table 3) also showed the presence of a chelated hydroxyl proton, a 1,4-disubstituted aromatic ring, a dimethyl chromene and a dimethylallyl group. The location of each functional group was confirmed by the use of 2D NMR spectroscopic techniques suggesting that these groups are present at similar positions as in **1**-**2**. Two missing doublets at ~ δ_H 4.98 and 4.42 were replaced by resonances for an ABX system at δ_H 5.33 (1H, dd, J = 12.8, 2.7 Hz), 3.03 (1H, dd, J = 12.8, 17.1 Hz) and 2.78 (1H, dd, J = 17.1, 2.7 Hz) of a flavanone. The configuration at C-

2 was assigned to be *S* based on vicinal coupling constant of 12.8 Hz in comparison to those of previously reported flavanones.[8] Compound **7**, khonklonginol G, was thus identified as 5-hydroxy-4′-methoxy-6″,6″-dimethyl-pyrano (2″, 3″: 7,6)-8-(3‴, 3‴-dimethylallyl)-flavanone



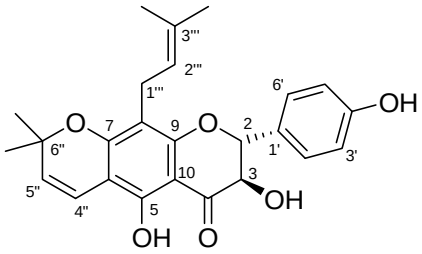
7

Compound **8** was obtained as yellow sticky liquid. The molecular formula of $C_{26}H_{28}O_6$ was based on its HRESIMS spectrum which showed $(M+1)^+$ ion at *m/z* 437.2079. IR spectrum indicated a hydroxyl (ν_{max} 3363 cm^{-1}) and carbonyl (ν_{max} 1624 cm^{-1}) groups. ^1H and ^{13}C NMR data of compound **8** were very similar to those of **7** (Table 3), except for aromatic proton signals at δ_H 6.46 (d, J = 3.5 Hz), 6.49 (dd, J =8.5, 2.2 Hz), and 7.16 (d, J = 8.4 Hz) implying a trisubstituted aromatic ring. The key HMBC correlations between H-2/carbon signals at δ_C 116.6 (qC, C-1′), 155.4 (qC, C-2′) and 127.9 (CH, C-6′), and between H-6′ (δ_H 7.16)/C-2′, C-4′ (δ_C 161.2, qC) as well as O*CH*$_3$/C-4′ required the placement of the OCH_3 and OH groups at C-4′ and C-2′, respectively. Compound **8** could be concluded as 5,2′-dihydroxy-4′-methoxy-6″,6″-dimethyl-pyrano (2″, 3″: 7,6)-8-(3‴, 3‴-dimethylallyl)-flavanone, and proposed khonklonginol H as a trivial name.



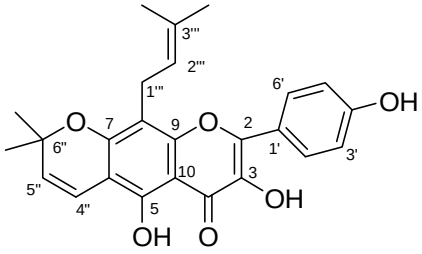
8

Compound **9** was obtained as amorphous solid. ^{1}H and ^{13}C NMR data of compound **9** were very similar to those of **1** (Table 1), except with the absence of OMe signal. Compound **9** could be concluded as a known compound, lupinifolinol.[4,5] The use of 2D NMR technique led to full assignment of ^{1}H and ^{13}C NMR chemical shifts (Table 4).



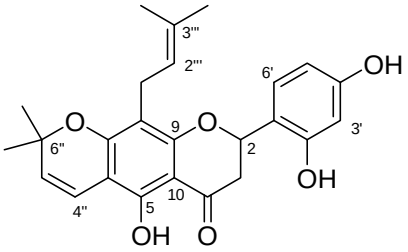
9

Compound **10** was obtained as a liquid with molecular formula $C_{25}H_{24}O_6$ as analysed from its HRESIMS (found 421.1655, calc for $C_{25}H_{25}O_6$ 421.1655). ^{1}H and ^{13}C NMR chemical shifts (Table 5) were in good agreement as those of compound **6** (Tables 3 and 4), except for the absence of OMe signal. Compound **10** could thus be concluded as dehydrolupinifolinol, which was previously reported.[9]



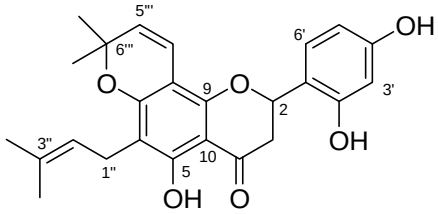
10

Compound **11** was isolated as a solid with molecular formula $C_{25}H_{26}O_6$ based on the HRESIMS (calc for $C_{25}H_{27}O_6$ 423.1802, found 423.1803). The ^{1}H and ^{13}C NMR data were consistent to those of compound **8** but with the absence of a OMe group signal. Compound **11** was therefore proposed as 5,2′,4′-trihydroxy-6″,6″-dimethyl-pyrano (2″, 3″: 7,6)-8-(3‴, 3‴-dimethylallyl)-flavanone. Full assignment of ^{1}H and ^{13}C resonances (Table 6) was based on 2-D NMR experiments. Compound **11** is identical to flemichinD.[10]



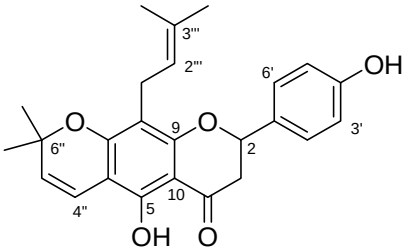
11

Compound **12** was obtained as sticky liquid, with same molecular formula as of **11**. ^{1}H NMR spectrum showed similar pattern of signals as those found in **11**. The key HMBC correlations between H-1″/ C-6, 7, 2″, 3″ and H-4‴/C-7, 9, 2‴ led to conclude **12** as eriosemaone.[10] Full assignment of ^{1}H and ^{13}C resonances of **12** is as showed in Table 7.



12

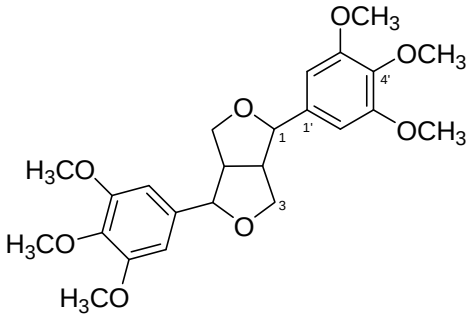
Compound **13** was obtained as sticky liquid. Its ^{1}H and ^{13}C NMR data are in close resemblance to those of compound **7** but with the absence of the OMe signal. Compound **13** was thus identified as lupinifolin.[4,11] The ^{1}H and ^{13}C NMR chemical shifts are as shown in Table 8.



13

Compound **14** was isolated as a solid. ^{1}H-^{1}H-COSY spectrum showed cross-peak between H-1/H-2 and H-2/H-3. HMBC spectrum showed key long-range ^{1}H-^{13}C correlations between H-1/C-2, C-3, C-2′ and C-6′, and between H-2′, H-6′/C-1, C-1′, C-3′, C4′, C-5′ indicating a furanofuran nucleus in **14**. Compound **14** was deduced to be

yangambin,[12] which was previously reported. ^{1}H and ^{13}C NMR data is as shown in Table 9.



14

Table 1 ^{1}H NMR Spectroscopic Data of Compounds **1-5** ($CDCl_3$)[a]

Position	δ_H **1**	δ_H **2**	δ_H **3**	δ_H **4**	δ_H **5**
2	4.98 (d,12.0)	5.64 (d, 5.1)	5.28 (d,12.0)	4.97 (d,11.9)	5.01 (d, 11.9), 4.50 (d,11.9)
3	4.42 (d,12.0)	4.71 (d, 5.1)	4.50 (d,12.0)	4.49 (d,11.9)	4.50 (d,11.9), 4.49 (d, 11.9)
2′	7.46 (d, 8.8)	7.29 (brd, 8.9)	-	7.07 (d, 2.0)	7.43 (d, 8.7)
3′	6.96 (d, 8.8)	6.82 (d, 8.7)	6.54 (d, 2.5)	-	6.96 (d, 8.8), 6.94 (d, 8.8)
5′	6.96 (d, 8.8)	6.82 (d, 8.7)	6.58 (d, 8.7)	6.91 (d, 8.8)	6.96 (d, 8.8), 6.94 (d, 8.8)
6′	7.46 (d, 8.8)	7.29 (d, 8.9)	7.42 (brd, 8.6)	7.08 (dd, 8.8, 2.0)	7.43 (d, 8.7)
4″	6.62 (d, 10.0)	6.58 (d, 9.9)	6.62 (d,10.1)	6.62 (d,10.0)	6.64 (d,10.0), 6.63 (d, 10.0)
5″	5.51 (d, 10.0)	5.48 (d, 9.8)	5.53 (d,10.0)	5.51 (d,10.0)	5.52 (d,10.2)
6″-CH_3	1.44 (s)	1.43 (s)	1.44 (2× s)	1.44 (s)	1.47 (s), 1.45 (s)
	1.43 (s)	1.41 (s)		1.43 (s)	1.44 (s), 1.43 (s)
1‴	3.16 (d, 6.8)	3.21 (d, 7.1)	3.21 (brt, 8.0)	3.16 (brd, 6.9)	3.16 (brd, 6.9)
2‴	5.11 (dt, 6.4, 1.6)	5.10 (t, 7.3)	5.10 (brt, 7.4)	5.13 (brt, 6.8)	5.13 (brt, 6.1)
3‴-CH_3	1.63 (s)	1.69 (s)	1.66 (s) 1.65 (s)	1.62 (s)	1.17 (s), 1.16 (s)
	1.59 (s)	1.62 (s)		1.59 (s)	1.14 (s), 1.135 (s)
OCH_3-4′	3.83 (s)	3.76 (s)	3.79 (s)	3.90 (s)[b]	3.82 (s)
OCH_3-3′		-		3.91 (s)[b]	
O*H*-5	11.41 (s)	11.39 (s)	11.29 (s)[c]	11.41 (s)	11.40 (s)

[a]Coupling constants (*J*) are presented in parentheses in Hz.
[b]Assignment may be reversed. [c]O*H*-2′ in **3** was detected as singlet at δ_H 6.82.

Table 2 ^{13}C NMR Spectroscopic Data of Compounds **1-5** ($CDCl_3$)

Position	δ_C **1**	δ_C **2**	δ_C **3**	δ_C **4**	δ_C **5**
2	82.9 CH	80.0 CH	79.0 CH	83.1 CH	83.1 CH 83.0 CH
3	72.6 CH	71.5 CH	73.1 CH	72.6 CH	72.4 CH
4	196.4 qC	194.6 qC	195.3 qC	196.1 qC	196.4 qC 196.3 qC
5	156.1 qC	156.1 qC	156.1 qC	156.0 qC	156.5 qC
6	103.2 qC	102.9 qC	103.5 qC[a]	103.2 qC	103.4 qC 103.3 qC
7	160.7 qC	160.6 qC	160.9 qC	160.7 qC	160.48 qC 160.45 qC
8	109.3 qC	109.1 qC	109.6 qC	109.3 qC	106.5 qC
9	159.5 qC	158.2 qC	159.0 qC	159.3 qC	159.8 qC
10	100.4 qC	100.9 qC	100.2 qC	100.3 qC	100.5 qC 100.4 qC
1′	128.8 qC[a]	126.8 qC	116.3 qC	129.0 qC	128.1 qC
2′	128.8 CH[a]	128.7 CH	155.3 qC	110.1 CH	128.6 CH
3′	114.0 CH	113.8 CH	103.5 CH[a]	149.1 qC	114.2, 114.1 CH
4′	160.3 qC	159.6 qC	161.2 qC	149.7 qC	160.3 qC
5′	114.0 CH	113.8 CH	107.3 CH	111.0 CH	114.2, 114.1 CH
6′	128.8 CH[a]	128.7 CH	127.9 CH	120.2 CH	128.6 CH
4″	115.4 CH	115.4 CH	115.3 CH	115.4 CH	115.4 CH
5″	126.2 CH	126.1 CH	126.5 CH	126.3 CH	126.2 CH
6″	78.5 qC	78.4 qC	78.7 qC	78.5 qC	79.2 qC
6″-CH_3	28.4 (2×) CH_3	28.4, 28.5 CH_3	28.4 (2×) CH_3	28.3 (2×) CH_3	28.6 (2×), 28.5, 28.4 CH_3
1‴	21.4 CH_2	21.3 CH_2	21.3 CH_2	21.3 CH_2	25.2, 25.1 CH_2
2‴	122.3 CH	122.2 CH	122.0 CH	122.2 CH	79.1, 78.9 CH
3‴	131.3 qC	131.3 qC	131.7 qC	131.3 qC	72.8 qC
3‴-CH_3	25.7, 17.8 CH_3	25.5, 17.9 CH_3	25.8, 17.9 CH_3	25.7, 17.8 CH_3	25.97, 25.91, 23.38, 23.37 CH_3
OCH_3-4′	55.3 CH_3	55.2 CH_3	55.3 CH_3	55.94 CH_3[b]	55.3 CH_3
OCH_3-3′	-	-	-	55.91 CH_3[b]	-

[a]Overlapped signals

[b]Assignment may be reversed.

Table 3 ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds **6-8** ($CDCl_3$)[a]

Position	δ_H **6**	δ_C **6**	δ_H **7**	δ_C **7**	δ_H **8**	δ_C **8**
2	-	145.4 qC	5.33 (dd, 12.8, 2.7	78.6 CH	5.54 (dd, 13.0, 3.0)	77.7 CH
3	-	135.5 qC	3.03 (dd, 17.1, 12.8) 2.78 (dd, 17.1, 2.7)	43.3 CH_2	3.09 (dd, 17.4, 13.1) 2.86 (dd, 17.3, 3.1)	41.9 CH_2
4	-	175.5 qC	-	196.4 qC	-	196.4 qC
5	-	153.0 qC	-	156.6 qC	-	156.8 qC
6	-	104.9 qC	-	102.7 qC	-	103.3 qC
7	-	156.9 qC	-	159.3 qC	-	159.8 qC
8	-	107.7 qC	-	108.6 qC	-	108.8 qC
9	-	153.6 qC	-	159.8 qC	-	158.6 qC
10	-	103.5 qC	-	102.8 qC	-	102.7 qC
1′	-	123.7 qC	-	130.9 qC	-	116.6 qC
2′	8.15 (d, 8.8)	129.3 CH	7.35 (d, 8.4)	127.5 CH	-	155.4 qC
3′	7.01 (d, 8.8)	114.1 CH	6.92 (d, 8.5)	114.1 CH	6.46(d, 2.2)	102.9 CH
4′	-	161.0 qC	-	159.8 qC	-	161.2 qC
5′	7.01 (d, 8.8)	114.1 CH	6.92 (d, 8.5)	114.1 CH	6.49 (dd, 8.5, 2.2)	106.4 CH
6′	8.15 (d, 8.8)	129.3 CH	7.35 (d, 8.4)	127.5 CH	7.16 (d, 8.4)	127.9 CH
4″	6.72 (d, 9.9)	115.7 CH	6.61 (d, 10.0)	115.7 CH	6.62 (d, 9.9)	115.6 CH
5″	5.62 (d, 10.1)	128.1 CH	5.48 (d, 10.0)	125.9 CH	5.50 (d, 9.9)	126.3 CH
6″	-	77.8 qC	-	78.1 qC	-	78.3 qC
6″-CH_3	1.45 (s)	28.3 CH_3	1.41 (s), 1.43 (s)	28.4, 28.3 CH_3	1.42 (s), 1.43 (s)	28.3, 28.4 CH_3
1‴	3.49 (d, 7.0)	21.5 CH_2	3.19 (t, 7.2)	21.5 CH_2	3.20 (t, 7.0)	21.4 CH_2
2‴	5.21 (dt, 7.0, 6.1)	122.2 CH	5.15 (t, 7.2)	122.6 CH	5.09 (t, 7.2)	122.3 CH
3‴	-	131.8 qC	-	131.0 qC	-	131.8 qC
3‴-CH_3	1.67 (s), 1.82 (s)	18.0, 25.7 CH_3	1.63 (s, 2 ×)	17.8, 25.8 CH_3	1.65 (s, 2 ×)	17.8, 25.7 CH_3
OH-5	11.93 (s)		12.24 (s)		12.23 (s)	
OCH_3-4′	3.87 (s)	55.3 CH_3	3.82 (s)	55.3 CH_3	3.77 (s)	41.9 CH_3
OH-	6.63 (br s, *OH*-3)				6.26 (br s, *OH*-2′)	

[a]Coupling constants (*J*) are presented in parentheses in Hz.

Table 4 ^{1}H and ^{13}C NMR Spectroscopic Data of **9** ($CDCl_3$).

Position	δ_H	δ_C	HMBC
2	4.96 (d, 11.9)	82.8 CH	C-3, 4, 2′, 6′
3	4.48 (d, 11.7)	72.5 CH	C-2, 4, 1′
4	-	196.2 qC	
5	-	156.0 qC	
6	-	103.2 qC	
7	-	160.7 qC	
8	-	131.3 qC	
9	-	159.4 qC	
10	-	100.3 qC	
1′	-	128.8 qC	
2′	7.40 (d, 8.4)	129.0 CH	C-2, 3′, 4′, 5′
3′	6.86 (d, 8.8)	115.6 CH	C-2′, 4′, 6′
4′	-	156.3 qC	
5′	6.86 (d, 8.8)	115.6 CH	C-2′, 4′, 6′
6′	7.40 (d, 8.4)	129.0 CH	C-2, 3′, 4′, 5′
4″	6.62 (d, 10.4)	115.6 CH	C-5, 6, 7, 6″, 6″-CH_3
5″	5.51 (d, 9.7)	126.3 CH	C-6, 6″, 6″-CH_3
6″	-	78.5 qC	
6″-CH_3	1.43 (s),1.43 (s)	2×, 28.4 CH_3	C-6″, 5″, 4″, 6″-CH_3
1‴	3.15 (brd, 6.9)	21.3 CH_2	C-7, 8, 9, 2‴, 3‴
2‴	5.10 (dt, 7.4, 1.4)	122.2 CH	C-1‴, 3‴, 3‴-CH_3
3‴	-	109.3 qC	
3‴-CH_3	1.58 (s), 1.62 (s)	17.8 CH_3, 25.8 CH_3	C-8, 2‴, 3‴, 3‴-CH_3
OH-5	11.39 (s)		C-5, 6, 10

Table 5 ^{1}H and ^{13}C NMR Spectroscopic Data of **10** ($CDCl_3$+MeOH-d4 3 drops)

Position	δ_H	δ_C	HMBC
2	-	145.4 qC	
3	-	135.4 qC	
4	-	175.5 qC	
5	-	153.0 qC	
6	-	104.9 qC	
7	-	157.0 qC	
8	-	107.7 qC	
9	-	153.6 qC	
10	-	103.5 qC	
1′	-	123.6 qC	
2′	8.05 (d,8.9)	129.6 CH	C-2, 3′, 6′
3′	6.90 (d,8.9)	115.6 CH	C-1′, 4′, 5′
4′	-	157.6 qC	
5′	6.90 (d,8.9)	115.6 CH	C-1′, 3′, 4′
6′	8.05 (d,8.9)	129.6 CH	C-2, 2′, 5′
4″	6.68 (d,10.0)	115.7 CH	C-5, 6, 7, 6″
5″	5.59 (d, 10.0)	128.2 CH	C-6, 6″, 6″-CH_3
6″	-	77.8 qC	
6″-CH_3	2× 1.41 (s)	2x 28.3 CH_3	6″-CH_3, 6″, 5″
1‴	3.45 (brd,7.1)	21.5 CH_2	C-7, 8, 9, 2‴, 3‴
2‴	5.17 (ddt,7.1,5.8, 1.3)	122.3 CH	3‴-CH_3
3‴	-	131.8 qC	
3‴-CH_3	1.63 (s), 1.77 (s)	18.1 CH_3, 25.7 CH_3	C-8, 2‴, 3‴, 3‴-CH_3
OH-5[a]			

[a]Not observed due to the addition of MeOH-d4 to the sample

Table 6 ^{1}H and ^{13}C NMR Spectroscopic Data of **11** ($CDCl_3$).

Position	δ_H	δ_C	HMBC (H to C)
2	5.52 (dd, 13.0, 2.7)[a]	77.3 CH	C-1′
3	3.10 (dd, 17.3, 13.1)	41.9 CH_2	C-2, 4
	2.83 (dd, 17.3, 2.9)		
4	-	196.6 qC	
5		156.8 qC	
6		103.3qC	
7		158.8 qC	
8		108.8 qC	
9		159.8 qC	
10		102.7 qC	
1′		116.9 qC	
2′		157.2qC	
3′	6.40 (br s)[b]	104.2 CH	C-1′, 2′, 4′
4′	-	155.3 qC	
5′	6.41 (d, 8.7)[b]	107.8 CH	C-1′, 3′, 4′
6′	7.09 (d, 8.7)	128.0 CH	C-2, 2′
4″	6.61 (d, 10.0)	115.6 CH	C-6, 7, 6″
5″	5.49 (d, 10.0)[a]	126.2 CH	C-6″, 6″-CH_3
6″	-	78.3 qC	
6″-CH_3	1.42 (s), 1.43 (s)	28.3 CH_3, 28.4 CH_3	C-3″, 6″, 6″-CH_3
1‴	3.20 (brt, 6.6)	21.5 t CH_2	C-7, 8, 9, 2‴, 3‴
2‴	5.08 (t, 7.2)	122.2 CH	3‴-CH_3
3‴	-	131.8 qC	
3‴-CH_3	2x 1.64 (br s)	17.8 CH_3, 25.7 CH_3	C-2‴, 3‴, 3‴-CH_3
OH-5	12.22 (s)		C-4, 5, 6

[a,b]Overlapped signals

Table 7 ^{1}H and ^{13}C NMR Spectroscopic Data of **12** ($CDCl_3$).

Position	δ_H	δ_C	HMBC (H to C)
2	5.54 (br d, 10.1)	77.4 CH [a]	C-1′
3	3.07 (dd, 17.2, 12.9)	42.0 CH_2	C-2, 4
	2.83 (brd, 14.6)		
4	-	196.4 qC	
5		161.3 qC	
6		110.3 qC	
7		159.8 qC	
8		101.8 qC	
9		154.4 qC	
10		102.5 qC	
1′		116.9 qC	
2′		157.2 qC [b]	
3′	6.39 (br s)	103.9 CH	C-1′
4′	-	157.2 qC [b]	
5′	6.40 (d, 8.3)	107.9 CH	C-1′, 3′
6′	7.12 (d, 8.3)	128.0 CH	C-2, 2′, 4′, 5′
1″	3.23 (d, 7.3)	20.9 CH_2	C-6, 7, 2″, 3″
2″	5.18 (t, 7.3)	122.3 CH	3″-CH_3
3″	-	131.4 qC	
3″-CH_3	1.65 (s), 1.76 (s)	17.9 CH_3, 25.7 CH_3	C-2″, 3″, 5″3″-CH_3
5‴	5.46 (d, 10.1)	126.7 CH	C-8, 6‴, 6‴-CH_3
4‴	6.50 (d, 10.0)	115.6 CH	C-7, 9, 6‴
6‴	-	78.1 qC	
6‴-CH_3	1.40 (s), 1.43 (s)	28.2 CH_3, 28.5 CH_3	C-6‴, 5‴, 6‴-CH_3
OH-5	12.31 (s)		C-5, 6, 10

[a] Obscured by $CDCl_3$ signal. [b]Overlapped signals.

Table 8 ^{1}H and ^{13}C NMR Spectroscopic Data of **13** ($CDCl_3$)

Position	δ_H	δ_C	HMBC (H to C)
2	5.31 (dd, 12.8, 3.1)	78.6 CH	C-4, 1′, 6′
3	3.02 (dd, 17.1, 12.8) 2.78 (dd, 17.1, 3.1)	43.2 CH_2	C-2, 4, 10
4	-	196.4 qC	
5		159.9 qC	
6		102.8 qC	
7		156.6 qC	
8		108.6 qC	
9		159.4 qC	
10		102.6 qC	
1′		131.0 qC	
2′	7.30 (d, 8.5)	127.8 CH	C-2, 4′, 6′
3′	6.86 (d, 8.4)	115.6 CH	C-1′, 4′
4′	-	155.9 qC	
5′	6.86 (d, 8.4)	115.6 CH	C-1′, 4′
6′	7.30 (d, 8.5)	127.8 CH	C-2, 2′, 4′
4″	6.61 (d, 10.1)	115.8 CH	C-5, 6, 7, 6″-CH_3, 6″
5″	5.48 (t, 10.0)	125.9 CH	6″, 6″-CH_3
6″	-	78.1 qC	
6″-CH_3	1.42 (s), 1.41 (s)	28.6 CH_3, 28.4 CH_3	C-4″, 5″,6″-CH_3
1‴	3.18 (brd, 7.4)	21.5 CH_2	C-8, 9, 2‴, 3‴
2‴	5.12 (brt, 7.4)	122.5 CH	C-1‴, 3‴-CH_3
3‴	-	131.0 qC	
3‴-CH_3	2x 1.62 (s)	25.7 CH_3, 18.0 CH_3	C-8, 2‴, 3‴, 3‴-CH_3
OH-5	12.23 (s)	-	C-5, 6, 7, 10

*Overlapped signals

Table 9 ^{1}H and ^{13}C NMR Spectroscopic Data of **14** ($CDCl_3$)

Position	δ_H	δ_C	HMBC
1	4.71 (brd, 4.3)	86.1 CH	C-2,3, 1, 2′, 6′
2	3.08 (m)	54.4 CH	
3	4.27 (dd-like, 9.1, 6.8) 3.87 (m)	71.8 CH_2	C-1, 2
1′		132.1 qC	
2′, 6′	6.56 (s)	102.8 CH	C-1, 1′, 3′, 4′, 5′
3′, 5′	-	147.1 qC	
4′	-	134.3 qC	
OMe-3′	3.88 (s)	56.4 CH_3	C-3′
OMe-4′	3.88 (s)	56.4 CH_3	
OMe-5′	3.88 (s)	56.4 CH_3	C-5′

Cytotoxicity[13,14] against breast cancer (BC), human small cell lung (NCI-H187) and human oral epidermal carcinoma (KB) cell lines and antimycobacterial activity of the isolates were evaluated, results are as indicated in Table 10. Among three 3-hydroxyl flavanones (**1**, **2**, **9**), cytotoxic activity was found to be comparable, implying stereochemistry at C-3 and changes of substituent at C-4′ from OH to OMe and vice versa, to play no clear role in the modulation of activity. Compound **11** with a linear pyrano ring was found to be more active than **12**, a regioisomer of **11** with an angular pyrano ring. Results between **8**, **11** and **13** implied the presence of hydroxyl group at C-2′ and C-4′ in the flavanone nucleus to be vital for cytotoxic activity, and results between **9** and **10**, indicated flavanone to be slightly more active than the corresponding flavonol. Several compounds also showed antituberculous activity against *Mycobacterium tuberculosis* H37Ra. Compounds **11-13** were most active and exhibited inhibitory activity with MIC value of 12.5 μg/mL. Compounds **1**, **8** and **9** were less potent with MIC value of 25 μg/mL. Milder inhibitory activity was observed in **2** and **6** showing MIC values of 50 and 100 μg/mL, respectively.

Table 10. Biological Activity of Some Isolates from *E. chinense*

compound	Anti-TB[a]	KB[b]	NCI H187[b]	Vero cell[b]
1	25	3.1	3.0	7.9
2	50	3.8	4.3	6.9
6	100	6.7	2.4	7.0
8	25	5.4	3.3	6.4
9	25	1.73	3.5	nd[c]
10	12.5	5.8	3.9	11.1
11	12.5	3.3	2.1	nd[c]
12	12.5	5.8	6.0	nd[c]
13	12.5	2.4	6.5	nd
hexane extract	50	12.0	9.9	5.8
ellipticine[d]	-	0.37	0.44	-
doxorubicin[d]	-	0.12	0.042	-
isoniazid[d]	0.023-0.046	-	-	-
streptomycin[d]	0.156-0.313	-	-	

[a]MIC in μg/mL. [b]IC_{50} in μg/mL
[c]nd = not determined. [d]positive control substance

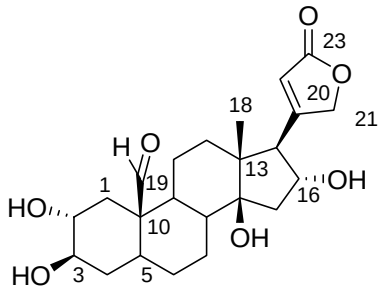
References

(1) Smitinand, T. *Thai Plant Names (Botanical Names-Vernacular Names)*; Funny Publishing: Bangkok, 1980; p 141.
(2) Mabry, T. J.; Marklam, K. R.; Thomas, M. B. *The Systematic Identification of Flavonoids*; Springer-Verlag: New York, 1970; p 268.
(3) (a) Gaffield, W. *Tetrahedron* **1970**, *26*, 4093-4108. (b) Slade, D.; Ferreira, D.; Marais, J. P. J. *Phytochemistry* **2005**, *66*, 2177-2215.
(4) Smalberger, T. M.; Vleggaar, R.; Weber, J. C. *Tetrahedron* **1974**, *30*, 3927-3931.
(5) Singhal, A. K.; Sharma, R. P.; Thyagarajan, G.; Herz, W.; Govindan, S. V. *Phytochemistry* **1980**, *19*, 929-934.
(6) Alavez-Solano, D.; Reyes-Chilpa, R.; Jiménez-Estrada, M.; Gómez-Garibay, F.; Chavez-Uribe, I.; Sousa-Sánchez, M. *Phytochemistry* **2000**, *55*, 953-957.
(7) Mahidol, C; Prawat, H.; Kaweetripob, W.; Ruchirawat, S. *Heterocycles* **2002**, *57*, 1287-1292.
(8) Jang, D. S.; Cuendet, M.; Hawthorne, M. E.; Kardono, L. B. S.; Kawanishi, K.; Fong, H. H. S.; Mehta, R. G.; Pezzuto, J. M.; Kinghorn, A. D. *Phytochemistry* **2002**, *61*, 867-872.
(9) Singhal, A. K.; Sharma, R. P.; Madhusudanan, K. P.; Thyagarajan, G.; Herz, W.; Govindan, S. V. *Phytochemistry* **1981**, *20*, 803-806.
(10) Ma, W. G.; Fuzzati, N.; Li, Q. S.; Yang, C. R.; Stoeckli-Evans, H.; Hostettmann, K. *Phytochemistry* **1995**, *39*, 1049-1061.
(11) Jain, A. C.; Gupta, R. C.; Sarpal, P.D. *Tetrehedron* **1978**, *34*, 3563-3567.
(12) (a) Jeffereries, P. R.; Knox, J. R.; White, D. E. *Aust. J. Chem.* **1961**, *14*, 175-177. (b) Greger, H.; Hofer, O. *Tetrahedron* **1980**, *36*, 3551-3558.
(13) (a) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710-718. (b) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107-1112.
(14) Collins, L. A.; Franzblau, S. G. *Antimicrob. Agents Chemother*. **1997**, *41*, 1004-1009.

Part II. Constituents and Bioactivity of *Calotropis gigantea*

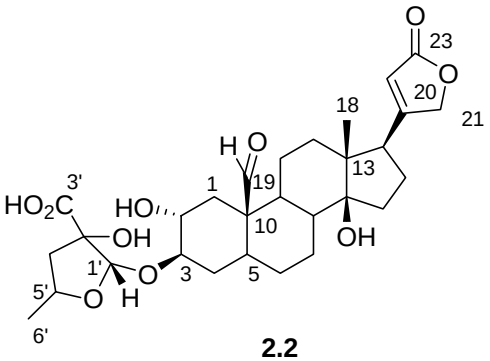
In continuation to our recent study on the bioactive compounds from *Calotropis gigantea*,[2.1] we investigated further the bioactive chemical constituents from the polar fractions of the CH_2Cl_2 extract and butanol extract of the leaves of this plant. Chromatographic separation of the extracts led to the isolation of compounds **2.1-2.14**. Compound **2.3**, which was obtained after chromatographic separation, was found to be unstable and further transformed upon standing at room temperature to give products (**2.3a** and **2.3b**). The isolated compounds were evaluated for their cytotoxic activity against a panel of cell lines.

Compound **2.1** was isolated as a solid, mp = 210-215° C. The HRESIMS spectrum indicated a molecular formula of **1** to be $C_{23}H_{32}O_7$ based on *m/z* 443.2046 $[M+Na]^+$, calcd for $C_{23}H_{32}NaO_7$, 443.2037. The infrared spectrum indicated absorption bands for a hydroxyl (ν_{max} 3401 cm^{-1}) and *α,β*–unsaturated-*γ*- lactone (ν_{max} 1738, 1623 cm^{-1}) functional groups. The ^1H and ^{13}C NMR spectra showed characteristic signals of H-22 (δ_H 6.27), H_2-21 (δ_H 5.22 and 5.0, both with J = 18.2 Hz) and C-20, C-21, C-22 and C-23 at δ_C 174.9, 74.9, 118.2 and 174.9, respectively, of an *α,β*-unsaturated-*γ*-lactone moiety of the cardenolide. The ^{13}C NMR spectrum indicated the presence of twenty three carbon signals assignable to one methyl, eight methylene, nine methine including three oxymethine and one formyl, and five quaternary carbons. The three hydroxyl groups at C-2, C-3 and C-16 were evident from the ^1H-^1H COSY cross-peaks between H-2/H-1 and H-3, and between H-16/H_2-15 and H-17, as well as the long-range HMBC correlations between H-2/C-1, C-3; H-3/C-1, C-2, C-4, and between H-17/C-12, C-13, C-14, C-16, C-21, C-22 and C-23. The ^1H and ^{13}C NMR spectroscopic data of **2.1** (Table 2.1) are similar to those of 16*α*-hydroxycalotropagenin previously obtained from *Asclepias curassavica*.[2.2]



2.1

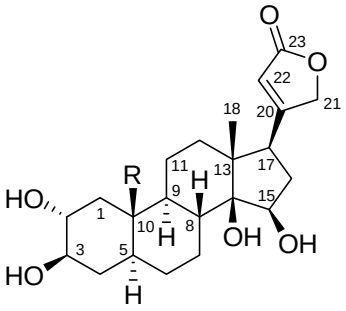
Compound **2.2** was isolated from the butanol extract of the leaves. Compound **2.2** was isolated as colorless solid, mp = 196-198° C with a molecular formula of $C_{29}H_{40}O_{11}$ based on the HRESIMS spectrum. The characteristic IR absorption bands, ^{1}H and ^{13}C NMR shifts of the α,β-unsaturated-γ-lactone moiety were observed as of **2.1**. The dideoxyfuranosyl moiety was detected from the singlet signal of an anomeric proton (H-1′) at δ_H 5.74 and of C-1′ at δ_C 108.7, in addition to doublet signal at δ_H 1.50 (d, J = 6.3 Hz) of H-6′ in ^{1}H NMR spectrum as observed in calactinic methyl ester recently isolated from this plant[2.1] and also from *Asclepias curassavica* [2.3]. The ^{1}H and ^{13}C NMR signals for a methoxy group of the ester function was however missing. Compound **2.2** was thus elucidated as calactinic acid. The ^{1}H and ^{13}C NMR spectroscopic data are shown in Table 2.2**.**



2.2

Compound **2.3** was obtained as a solid, mp. 226-228 °C, and the HR-ESI-MS spectrum indicated molecular formula of $C_{23}H_{32}O_7$. FT-IR spectrum indicated absorption maxima for a hydroxyl (3401 cm^{-1}) and α,β–unsaturated-γ-lactone (1738, 1623 cm^{-1}) functional groups. ^{13}C NMR spectrum exhibited the presence of twenty three carbon signals comprising one methyl, eight methylene, nine methine, including three oxymethine and one formyl, and five quaternary carbons. ^{1}H and ^{13}C NMR spectra showed characteristic signals of an α,β-unsaturated-γ-lactone moiety commonly found in cardenolide (δ_H 6.13 assignable to H-22, δ_H 5.30, 5.03 to H_2-21 and δ_C 175.8, 74.3, 118.3 and 175.2 to C-20, C-21, C-22 and C-23, respectively). The methyl proton signal at δ_H 0.83 (assigned for CH_3-18) showed heteronuclear multiple bond coherence (HMBC) correlations with ^{13}C NMR signals at δ_C 49.4 (C-17) and 81.9 (C-14). The two hydroxyl groups at C-2, and C-3 were evident from HMBC correlations between H-1 (at δ_H 2.97 and 1.34) and C-2, C-3, C-5, C-10 and C-19 (at δ_C 73.3, 76.1, 43.5, 53.4 and 209.5, respectively), in addition to ^{1}H-^{1}H correlation spectroscopy (COSY) cross-peaks between H-2 (δ_H 4.08)/H-1 and H-3 (δ_H 3.90). Vicinal coupling constants, $J_{1a,2}$ of 11.5 Hz, $J_{2,3}$ of 8.9 Hz and $J_{3,4a}$ of 11.3 Hz, were used as evidences for the assignment of orientations of 2-OH and 3-OH groups as α- and β-, respectively. Placement of the third hydroxyl group at C-15 was revealed from ^{1}H-^{1}H COSY cross-peaks between H_2-16 (δ_H 2.75, 1.97)/H-15 (δ_H 4.75) and H-17(δ_H 2.69), as well as HMBC correlations between H-17/C-15(δ_C 72.6), C-16 (δ_C 37.9) and C-21 (δ_C 74.3). The OH-15 was proposed to have β-orientation based on the nuclear overhauser effect spectroscopy (NOESY) spectrum which revealed cross-peaks between H-15/H-7 and H-17. The $J_{15,16}$ value of 8.0 Hz is also consistent to those

values reported in the 15β-hydroxycardenolide analogs.[2.4, 2.5] Compound **2.3** was thus proposed as 2α,15β-dihydroxy-19-oxo-uzarigenin. Full assignment of ^{1}H and ^{13}C NMR data was as shown in Table 2.3. In this study, compound **2.3** was found to be very unstable and further transformed to 19-*nor*-2α, 10,15β- trihydroxyuzarigenin (**2.3a**), and 19-*nor*-10-hydroperoxy-2α,15β-dihydroxyuzarigenin (**2.3b**) upon standing at room temperature for two days with or without solvent in a well capped vial. These types of transformations were previously documented,[2.6, 2.7] but no ^{1}H and ^{13}C NMR spectroscopic evidences of the 10-hydroperoxide derivative were reported. In this study we include full assignment of the ^{1}H and ^{13}C NMR spectroscopic data of these two transformed products. The ^{13}C NMR resonance of C-10 with hydroperoxyl group of **2.3b** was notably found at less shielded position (δ_C 82.9) than that of the corresponding C-10 with a hydroxyl group (δ_C 72.8) of **2.3a**.[2.8]



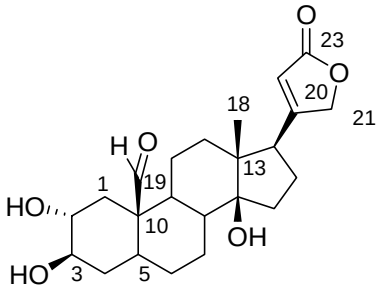
2.3 R = CHO
2.3a R = OH
2.3b R = OOH

Compound **2.4** was a white solid, mp. 222-225°C, its FTIR spectrum revealed absorption bands of a hydroxyl at 3435 and of an α,β-unsaturated-γ-lactone at1743 and1653 cm^{-1}. The HRESIMS suggested a molecular formula of $C_{23}H_{32}O_6$ as indicated from the $[M + Na]^+$ ion at 427.2110 (calcd for $C_{23}H_{32}O_6Na$, 427.2088).

The ^{13}C NMR spectrum exhibited twenty three carbon signals comprising one methyl, nine methylenes, eight methines including two oxymethines and one formyl (δ_C 209. 38), and five quaternary carbons including one carbonyl carbon at δ_C 175.31. The ^{1}H NMR spectrum showed a singlet signal at δ_H 10.20 attributable to an aldehyde proton and signal at δ_H 6.51 (s) assigned for an olefinic proton. The two sets of less shielded doublet signals at δ_H 5.29 and 5.05 (both as d, J = 18.2 Hz) could be assigned to the non-equivalent oxymethylene protons (H_2-21) connecting to an electron withdrawing group, CO-CH=C-*CH_2*-O, as reported in an α,β-unsaturated-γ-lactone moiety of a cardenolide nucleus.[5] The ^{1}H-^{1}H COSY spectrum which indicated cross-peaks between signal at δ_H 6.51 (assigned to H-22) and a doublet of doublet signal at δ_H 2.79 (H-17), in addition to the important long-range ^{1}H-^{13}C correlations from the HMBC spectrum between H-17/ C-20 (δ_C 176.55), C-21 (δ_C 74.23), and C-22 (δ_C 117.94) indicated bonding between C-20 and C-17. The hydroxyl group at C-14 was revealed from the HMBC correlations between H-17/C-14 (δ_H 84.61) and H-18 (δ_H 0.92)/C-14. The presence of two hydroxyl groups at C-2 and C-3 was evident from HMBC correlations between H-1 (δ_H 2.97)/C-2 (δ_C 73.39), C-3 (δ_C 76.14), C-5 (δ_C 43.56), C-10 (δ_C 53.31) and C-19 (δ_C 209.38), in addition to ^{1}H-^{1}H COSY

cross-peaks between H-2 (δ_H 4.05)/H-1 and H-2/H-3 (δ_H 3.94) and a large coupling constant (10.9 Hz) between protons H-2 and H-3 suggested configuration of 2-OH and 3-OH to be α and β, respectively.[2.4]

The use of 2D experiments and related literature data[2.3] led to the assignment of ^{1}H and ^{13}C resonances of **2.4** as shown in Table 2.4. Compound **2.4** was concluded to be calotropagenin, which was previously obtained from the latex and leaves of this plant.[2.15]

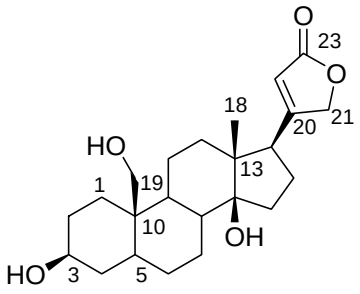


2.4

Compound **2.5** was afforded as a white solid with mp. 246-248 °C. The FTIR spectrum revealed absorption bands at 3400 and 1738 cm^{-1} of a hydroxyl and ester function, respectively. It was assigned the molecular formula of $C_{23}H_{34}O_5$, as deduced from the HRESIMS.

The ^{13}C NMR spectrum showed twenty three carbon signals comprising one methyl, ten methylenes, seven methines and five quaternary carbons. The ^{1}H NMR spectrum showed signals for α,β-unsaturated-γ-lactone moiety at δ_H 4.93 and 4.73 (both as dd, J = 1.4, 18.1 Hz, H_2-21) and δ_H 5.79 (s) for H-22. The important long-range ^{1}H-^{13}C HMBC correlations between H-22/C-17 (δ_C 50.86), C-20 (δ_C 175.79) and C-23 (δ_C 175.43) implied that the C-20 of butanolide ring joined to C-17. The oxymethylene signals at δ_H 3.63, 3.75 and δ_C 59.18 were assigned for –CH_2OH group at C-10 based on HMBC correlations between H-1 (δ_H 0.68, 2.78)/C-3 (δ_C 70.48), C-19 (δ_C 59.18).

The use of 2D NMR experiments and related literature data led to the assignment of ^{1}H and ^{13}C resonances of compound **2.5** as shown in Table 2.5. The structure of compound **2.5** was thus proposed as coroglaucigenin.[2.4]

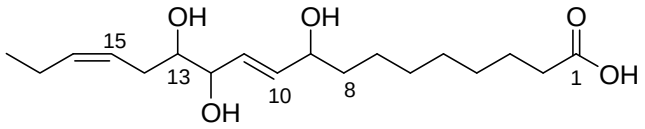


2.5

Compound **2.6** was obtained as white solid, its IR spectrum displayed absorption band for OH and C=O groups at 3351 and 1695 cm^{-1}, respectively. It was assigned the molecular formula of $C_{18}H_{32}O_5$ as deduced from the HRESIMS ($[M + Na]^+$ ion *m/z* 351.2147, calcd for $C_{18}H_{32}NaO_5$, 351.2139).

The ^{13}C NMR spectrum showed methyl carbon signal at δ_C 14.02, several methylene carbon signals from δ_C 20.58 to 36.74, four olefinic carbons at δ_C 124.40, 129.54, 134.31 and 135.62 and a carboxyl carbon at δ_C 176.96. Three oxygenated carbons at δ_C 71.61, 74.34 and 74.27, corresponded to oxymethine protons at δ_H 4.01 (dd, J = 6.1, 12.1 Hz), δ_H 3.89 (t, J = 5.7 Hz) and δ_H 3.41 (dt, J = 5.1, 7.8 Hz), respectively, and confirmed the presence of three hydroxyl groups. Analysis of the COSY, HMQC, and HMBC spectra provided evidences for the presence of CH_3-CH_2-CH=CH-CH_2-CH(OH)-CH(OH)-CH=CH-CH(OH)-CH_2-fragment. Considering the molecular weight of compound **2.6** and the number of CH_2 groups displayed in ^{13}C NMR spectrum, this fragment should be separated from the terminal carboxyl group by a -$(CH_2)_7$- chain. The *E* configuration of the double bond between C-10–C-11 was assigned on the basis of the coupling constant of the olefinic protons (H-10 and H-11), which resonated as doublet of doublets at δ_H 5.69 (dd, J = 5.8, 15.7 Hz) and δ_H 5.60, (dd, J = 5.8, 15.7 Hz), respectively. The vicinal coupling constant of 10.8 Hz between H-15 and H-16 suggested a *Z* configuration for the C-15–C-16 double bond, which was corroborated by the high-field chemical shift δ_C 1.97 of the allylic C-17.

The use of 2D experiments and related literature data led to the assignment of ^1H and ^{13}C chemical shifts of **2.6** (Table 2.6). Compound **2.6** was thus proposed as 9,12,13-trihydroxyoctadeca-10(*E*),15(*Z*)-dienoic acid.[2.8]



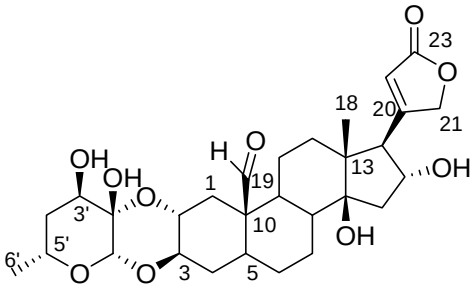
2.6

Compound **2.7** was obtained as a white solid, mp. 184-186 °C. The FTIR spectrum revealed absorption bands a hydroxyl and an ester function similar to those of **2.6**. It was assigned the molecular formula of $C_{29}H_{40}O_{10}$ (HRESIMS).

The ^{13}C NMR spectrum showed the presence of twenty nine carbons including two methyls, eight methylenes, twelve methines and six quaternary carbons, among which one is an olefinic and one a carbonyl carbon. The ^1H and ^{13}C NMR spectra of **2.7** showed signals at δ_H 4.73 and 4.81 (d, J = 18.3 Hz, H_2-21), δ_C 74.29 (C-21) and signals at δ_H 5.86 (s, H-22), δ_C 175.65 (C-22) of an α,β-unsaturated-γ-lactone moiety. The aldehyde group attached to C-10 was observed as singlet at δ_H 9.91(δ_C 207.9). The ^1H-^1H COSY cross-peaks between H-2 (δ_H 3.80)/ H-1 (δ_H 2.38, 1.05) and H-3 (δ_H 3.88), as well as the HMBC correlations between H-1/C-19 (δ_C 207.9), C-2 (δ_C 68.9) and C-3 (δ_C 71.8) indicated the presence of two OH groups at C-2 and C-3. The doublet signal at δ_H 1.20 (H_3-6′) which showed ^1H-^1H COSY correlations to multiplet at δ_H 3.54 (H-5′) as well as correlations between H-5/H-4′ (δ_H 1.73, 1.49) and H-4′/H-3′ (δ_H 3.59) indicated the presence of 4,6-dideoxyhexosulose moiety. The arrangement of H-3′ as α-orientation was based on $J_{3',4'}$ value of 6.8 Hz which is closed to value reported for this proton of related compound.[22] Attachment of C-1′ to

C-3 oxygen atom was observed from the ^{3}J ^{1}H-^{13}C correlation between H-1′(δ_H 4.45) /C-3. The oxymethine signal at δ_H 4.39 was assigned for H-16 due to the ^{1}H-^{1}H COSY cross-peaks between H-16 (δ_H 4.39)/ H-17 (δ_H 2.49), as well as HMBC correlations between H-17/C-14 (δ_H 84.41), C-16 (δ_H 76.12). The orientation of OH-16 as α could be seen from the $J_{16,\ 17}$ of 4.5 Hz which is in good agreement with value reported in 16α-hydroxycalotropin, previously isolated from *Asclepias curassavica*.[2.2]

The use of 2D experiments and related literature data[2.2] led to the assignment of ^{1}H and ^{13}C chemical shifts. Compound **2.7** was thus proposed as 16α-hydroxycalactin. Full assignment of ^{1}H and ^{13}C NMR data was as shown in Table 2.7.

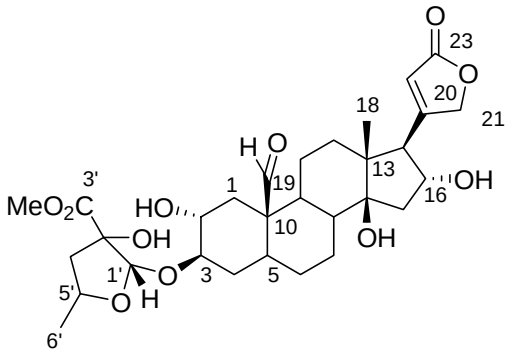


2.7

Compound **2.8** was obtained as white solid, mp. 230-232 °C. The FTIR spectrum revealed the presence of a hydroxyl (3430 cm^{-1}) and C=O (1741 cm^{-1}) group. It showed dark quenching spot under UV 254 light. Its molecular formula of $C_{30}H_{42}O_{11}$ was deduced from the HRESIMS which showed a $[M + Na]^+$ ion with *m/z* 601.2625 (calcd for $C_{30}H_{42}O_{11}Na$, 601.2613).

The ^{13}C NMR spectrum showed the presence of thirty carbons including three methyls, nine methylenes, nine methines, seven quaternary carbons including three carbonyl at δ_C 207.81, 173.38, 171.61, respectively. The ^{1}H and^{13}C NMR spectra signals for α,β-unsaturated-γ-lactone moiety were observed at δ_H 4.72, 4.86 (both as d, J = 18.1 Hz) assignable to H_2-21 and at δ_H 5.92 (s) to H-22. The aldehyde proton was observed as singlet at δ_H 9.91 (s). The ^{1}H-^{1}H COSY cross-peaks between H-2 (δ_H 3.37)/H-3 (δ_H 3.23), H-1 (δ_H 2.56, 0.91) as well as HMBC correlations between H-1/C-2 (δ_C 70.31), C-3 (δ_C 85.12) and C-10 (δ_C 51.90) indicated the presence of two OH groups at C-2 and C-3. The presence of a sugar moiety as furanose was evident from an anomeric group signal at δ_H 4.84 (s, H-1′) and δ_C 108.62 (C-1′) as well as the ^{1}H-^{1}H COSY correlations between H-5′/H-4′(δ_H 2.20, 2.09) and H-6′(δ_H 1.32, d, J = 6.2 Hz) and HMBC correlations between H-1′/C-2′ and C-5′, and between H-4′ to C-1′, C-2′ and C-5′. The attachment of C-1′ of sugar part to C-3 by an acetal linkage was evident from HMBC cross-peak between H-1′/C-3. A methyl ester group (COOMe, δ_H 3.69 , and δ_C 171.61, 52.42) was placed at C-2′ as reported in calactinic acid methyl ester.[2.1, 2.3] The oxymethine proton at δ_H 4.47 which showed cross-peak with H-17 at δ_H 2.51 (d, J = 4.3 Hz) in the ^{1}H-^{1}H COSY spectrum disclosed the presence of 16-hydroxyl group. The $J_{16,17}$ value of 4.3 Hz, which is closed to the values reported in 16α-hydroxycalotropagenin[2.9], and **2.7** indicated an α-oriented *OH*-16 group.

Compound **2.8** could thus be concluded as being 16α-hydroxycalactinic acid methyl ester. Full assignment of ^{1}H and ^{13}C chemical shifts was obtained using 2D spectroscopic data (Table 2.8).

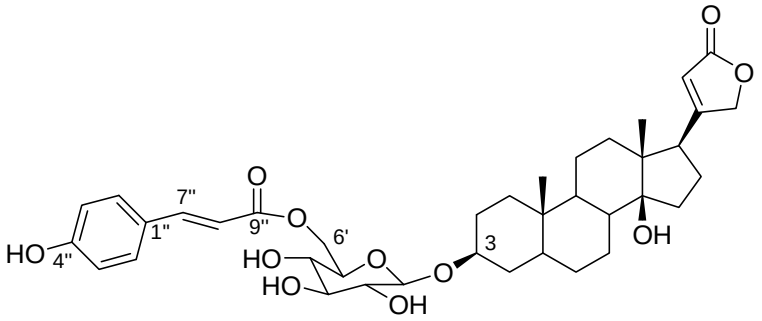


2.8

Compound **2.9** was obtained as a white solid, mp. 138-140 °C. The FTIR spectrum showed hydroxyl group at 3401 cm^{-1}and C=O absorption band at 1739 cm^{-1} thus indicated the presence of α,β-unsaturated-γ-lactone. Compound **2.9** was assigned the molecular formula of $C_{38}H_{50}O_{11}$ (HRESIMS, gave $[M + Na]^+$ ion at *m/z* 705.3252, calcd for $C_{38}H_{50}O_{11}Na$, 705.3237).

The ^{1}H and ^{13}C NMR signals for α,β-unsaturated-γ-lactone moiety was detected at δ_H 4.74 and 4.92 (both as dd, J = 18.3, 1.5 Hz) assignable for H_2-21 and δ_H 5.78 (s) for H-22. The HMBC spectrum showed that H_3-18 (δ_H 0.75) correlated with C-12 (δ_C 39.68, CH_2), C-17 (δ_C 50.77, CH), C-13 (δ_C 48.84, qC) and C-14 (δ_C 85.00, qC). The HMBC spectrum showed correlations of H_3-19 (δ_H 0.66) to C-1 (δ_C 37.04, qC), C-10 (δ_C 35.72, qC), C-5 (δ_C 44.27, CH) and C-9 (δ_C 48.84, CH). The ^{1}H and ^{13}C NMR chemical shifts at δ_H 3.56 and δ_C 78.8 revealed an oxymethine group most probable at position C-3. The presence an *E*-*p*-substituted cinnamoyl group was implied from the ^{1}H NMR doublets at δ_H 7.53 and 6.20 (both 1H d, J = 15.9 Hz) and doublet signals at δ_H 7.32 and 6.74 (both 2H d, J = 8.7 Hz). A glucosyl moiety was suggested by the presence of characteristic ^{1}H NMR signals of an anomeric proton, H-1′, at δ_H 4.29 (d, J = 7.7 Hz), H_2-6′at δ_H 4.40 (dd, J = 6.0, 12.0 Hz), 4.34 (dd, J = 2.0, 12. 0 Hz) and overlapped signals of H-2′-H-5′ between δ_H 3.22-3.44. The large $J_{1',2'}$ coupling constant indicated the β -D-glucopyranosyl ring.[2.16] The HMBC cross-peak between H-1′/C-3 (δ_C 78.67) indicated a C-3-*O*-C-1′ linkage. The long-range HMBC correlation between H_2-6′ and a carbonyl carbon signal at δ_C 167.86 implied connectivity between the carbonyl carbon of cinnamoyl group and O-C-6′ of the glucopyranosyl group.

The use of 2D experiments and related literature data led to the assignment of ^{1}H and ^{13}C chemical shifts as shown in Table 2.9. Compound **2.9** was concluded to be 6′-*O*-(*E*-4-hydroxycinnamoyl) desglucouzarin.[2.9]



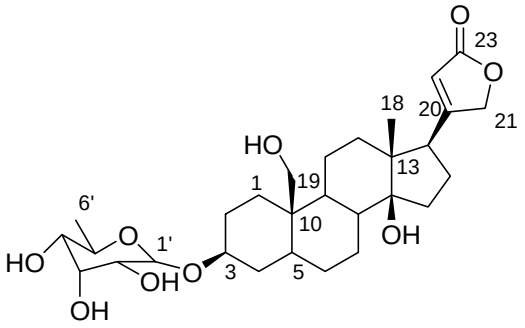
2.9

Compound **2.10** is a white solid, mp. 186-188 °C. The FTIR spectrum revealed absorption bands at 3430 and 1733 cm^{-1} of a hydroxyl and ester function, respectively. The ^1H NMR signals at δ_H 4.93, 4.75 (both as d, J = 18.3 Hz) and δ_H 5.79 (s) indicated the presence of an α,β-unsaturated-γ-lactone. Its molecular formula was assigned $C_{29}H_{44}O_9$ as deduced from the HRESIMS. (*m/z* 559.2878, calcd for $C_{29}H_{44}O_9Na$, 559.3051).

The presence of 6-deoxyallose with a β-linkage was deduced from ^1H and ^{13}C NMR and ^1H-^1H COSY spectra. The ^1H-^1H COSY spectrum showed correlations between H-2′ (δ_H 3.25)/H-1′ (δ_H 4.63) and H-3′ (δ_H 4.02), and between H-4′ (δ_H 3.14)/ H-3′and H-5 ′(δ_H 3.60,). The ^1H and ^{13}C NMR data for the aglycone moiety were almost identical with those reported for compound **2.5** except at C-3 where a glycosylation shift (δ_H 3.60, H-3) was observed.[2.10] All the vicinal protons in the sugar unit were mutually coupled, as indicated by the ^1H-^1H COSY spectrum, and the magnitude of the vicinal coupling constants between protons ($J_{1',2'}$ = 7.9 Hz; $J_{2',3'}$ = 2.9 Hz; $J_{3',4'}$ = 2.9 Hz; $J_{4',5'}$ = 9.5 Hz and $J_{5',6'}$ = 6.2 Hz) showed that the substituents at positions 2′, 4′ and 5′ of the pyranoside are equatorial, while that at 3′ axial. The H-1′ resonated downfield at δ_H 4.63 due to a 1, 3 diaxial deshielding effect by an axial 3′β-hydroxyl group.[2.11, 2.12] The 7.9 Hz trans-diaxial coupling between the anomeric proton (H-1′) and the adjacent H-2′, indicated that 2′-hydroxyl group is equatorial. It could be confirmed that the sugar moiety is a β-D-glycopyranose.[2.11, 2.13]

The HMBC cross-peak between H-1′ and C-3 (δ_C 77.3) indicated C-1′ of the sugar moiety attached to C-3 by a C-*O*-C linkage. The methyl group signals at δ_H 0.82, δ_C 15.8 was assigned for C-18 since H_3-18 showed HMBC correlations with signals of C-14 and C-17. The oxymethylene group signals at δ_H 3.60 and 3.70 (both as d, J = 11.8 Hz), δ_C 59.4 was assigned for C-19 due to the HMBC correlations between H-1(δ_H 2.16)/C-3 and C-19 (δ_C 59.4).

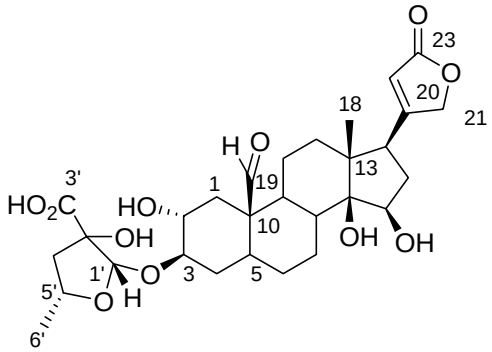
The use of 2D experiments and related literature data led to the assignment of ^1H and ^{13}C resonances of compound **2.10** as shown in Table 2.10. This compound was identified as frugoside.[2.10]



2.10

Compound **2.11** was obtained as white solid, mp. 290-294 °C. The molecular formula of $C_{29}H_{40}O_{11}$ was deduced from the HRESIMS (a $[M + Na]^+$ ion *m/z* 587.2463, calcd for $C_{29}H_{40}O_{11}Na$, 587.2457).

The ^{13}C NMR spectrum showed the presence of twenty nine carbons comprising two methyls, nine methylenes, eleven methines, seven quaternary carbons including two carbonyls at δ_C 175.48, 175.48, respectively. The ^1H NMR shifts of the α,β-unsaturated-γ-lactone moiety were observed at δ_H 5.29 (d, J = 18.4 Hz) and 5.04 (d, J = 18.0 Hz) assigned for H_2-21 and signal at δ_H 6.14 (s) for H-22. The aldehyde proton was observed as singlet at δ_H 10.10 (s). The ^1H-^1H COSY cross-peaks between H-15 (δ_H 4.74)/H-16 (δ_H 1.90, 2.68) and H-16/H-17 (δ_H 2.68) were detected. The 15-oxymethine proton resonated as a triplet at δ_H 4.74 with J value of 7.5 Hz indicating OH-15 as β-oriented (similar to that of **2.3**). The dideoxyfuranosyl moiety was detected from signals of a dioxygenated methine group at δ_H 5.65 (s, H-1′) and δ_C 107.64 (H-1′), in addition to a doublet signal at δ_H 1.53 (d, J = 5.7 Hz) of H-6′, as observed in calactinic methyl ester recently isolated from *Asclepias curassavica* and also from this plant.[2.1, 2.3] Compound **2.11** was thus elucidated as 15β-hydroxycalactinic acid. The ^1H and ^{13}C NMR data of **2.11** are as shown in Table 2.11.

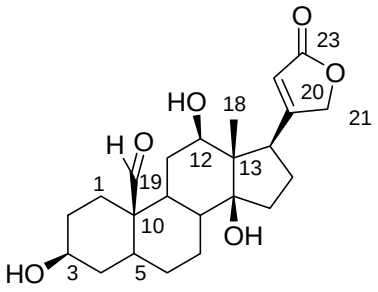


2.11

Compound **2.12** was afforded as a white solid, mp. 254-256 °C. It was assigned the molecular formula of $C_{23}H_{34}O_6$, as deduced from the HRESIMS ($[M + Na]^+$ ion *m/z* 429.2260, calcd for $C_{23}H_{34}O_6Na$, 429.2244).

The ^{13}C NMR spectrum exhibited the presence of twenty three carbon signals comprising one methyl, nine methylenes, eight methines and five quaternary carbons among which one is an olefinic carbon and one is a carbonyl carbon. The ^{1}H NMR spectrum showed signals for an α,β-unsaturated-γ-lactone moiety at δ_H 5.13 and 5.26 (both as d, J = 18.1 Hz) assignable for H_2-21 and at δ_H 6.29 (s) for H-22. The oxymethylene signals at δ_H 4.08, 4.18 (both d, J =11.4 Hz) and δ_C 59.23 indicated the presence of the 19-hydroxy group (as found in compounds **2.5** and **2.10**). This was further supported by the HMBC correlations between H-19/C-1 (δ_C 32.7), C-5 (δ_C 45.5), C-9 (δ_C 47.7), C-10 (δ_C 39.9). A placement of the hydroxyl group at C-12 (δ_C 75.3) was revealed from HMBC correlations between H-17 (δ_H 3.79)/C-12 and C-18 (δ_C 10.6). The orientation of H-12 (δ_H 3.67) as β could be deduced from the J-values of the doublet of double signal at δ_H 3.67 (dd, J = 5.1, 10.5 Hz) as previously reported in the 12β-coroglaucigenin.[2.4]

The use of 2D experiments and related literature data led to the assignment of ^{1}H and ^{13}C resonances of compound **2.12** as shown in Table 16. This compound was thus proposed as 12β-coroglaucigenin.[2.4]

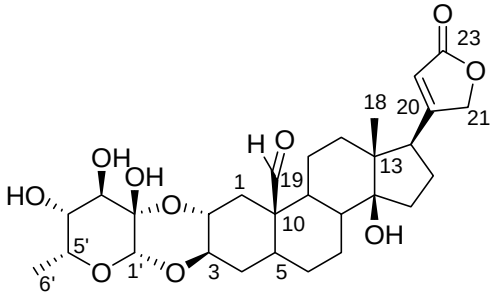


2.12

Compound **2.13** was obtained as a white solid, mp = 264-266°C. The molecular formula of $C_{29}H_{40}O_{10}$ was deduced from the HRESIMS which showed a $[M+Na]^+$ ion at *m/z* 571.2509 (calcd for $C_{29}H_{40}NaO_{10}$, 571.2508).

The ^{13}C NMR spectrum showed the presence of twenty nine carbons including two methyls, nine methylenes, twelve methines and six quaternary carbons among which one is an olefinic and one a carbonyl carbon. The ^{1}H NMR spectrum showed signals for α,β-unsaturated-γ-lactone moiety at δ_H 5.04 (d, J = 18.2 Hz) and 5.29 (d, J = 18.2 Hz) assigned for H_2-21 and 6.14 (s) for H-22, in addition an aldehyde proton at δ_H 10.10 (s). Proton signal at δ_H 2.61, assignated for H-1a, showed HMBC correlations with carbon signals at δ_C 208.71, 69.97 and 72.76, and also exhibited ^{1}H-^{1}H COSY cross-peaks with signals at δ_H 1.17 assigned for (H-1b) and 4.60 for (H-2). Multiplet at δ_H 4.60 and doublet of triplet at δ_H 4.37 (J = 10.7, 4.2 Hz) which showed ^{1}H-^{1}H COSY correlations to each other, were assigned to H-2 and H-3, respectively. The presence of 6-deoxyhexosulose moiety was disclosed from the observation of an anomeric proton (δ_H 5.55 (s), H-1′) and doublet signal at δ_H 1.73. The ^{1}H-^{1}H COSY cross-peaks between H_3-6′ (δ_H 1.73)/H-5′ (δ_H 4.05), H-5′/ H-4′ (δ_H 4.28) and H-4′/ H-3′ (δ_H 4.52) were also observed. The key HMBC correlations between H-1′/C-3 (δ_C 72.60) and H-2/C-2′(δ_C 94.10) indicated attachment of C-1′ to O-C-3 and C-2′ to O-C-2.

The use of 2D NMR experiments and related literature data led to the assignments of ^{1}H and ^{13}C chemical shifts (Table 2.13). Compound **2.13** was concluded to be calotoxin, which was previously obtained from latex and leaves of this plant.[2.11, 2.17]

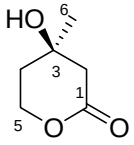


2.13

Compound **2.14** was afforded as yellow sticky oil. The FTIR spectrum showed absorption bands of a hydroxyl (3417 cm^{-1}) and a carbonyl (1732 cm^{-1}) group.

The ^{13}C NMR spectrum showed the presence of six carbons including one methyl, three methylene, and two quaternary carbons. The ^{1}H-^{1}H COSY spectrum showed cross-peaks between δ_H 1.88 (H-4)/H-5 (δ_H 4.31, δ_H 4.57) and H-4/H-2 (δ_H 2.49, δ 2.63). The HMBC spectrum indicated important correlations between H-5/C-4 (δ_C 35.9), C-3 (δ_C 68.1), C-1 (δ_C 170.6), as well as between H-2/C-6 (δ_C 29.8) and C-4 (δ_C 35.9).

Compound **2.14** was concluded to be mevalonalactone. The use of 2D experiments and related literature data[2.14] led to the assignment of ^{1}H and ^{13}C resonances of compound **2.14** (Table 2.14). The specific rotation (α_D) value of −6.67 (*c* 0.36, $CHCl_3$) is consistent to the value reported for *R*-(–)- isomer.[2.14]



2.14

Table 2.1 ^{1}H and ^{13}C NMR Spectroscopic Data of **2.1** (in C_5D_5N)

Position	δ_H (J in Hz)	δ_C mult	HMBC
1	2.95 dd (12.8, 4.5), 1.24 m	40.5 CH_2	C-2, 3, 5, 6, 9, 10. 19
2	4.03 ddd (11.5, 9.7, 4.6)	73.3 CH	C-1, 3
3	3.94 ddd (10.8, 9.2, 4.6)	76.1 CH	C-1, 2, 4
4	2.02 brd (13.1), 1.67 ddd (14.3, 13.3, 11.7)	38.1 CH_2	C-2, 3, 5, 6, 10
5	1.45a	43.4 CH	C-7
6	1.49, 1.21	28.2 CH_2	C-7, 10
7	2.51, 1.93	28.4 CH_2	C-5, 8, 9
8	1.89	42.8 CH	C-6, 7, 11
9	1.45a	48.9 CH	C-7, 10, 11, 19
10	-	53.2 qC	
11	1.77, 1.38	22.8 CH_2	C-8, 13
12	1.93, 1.63	40.5 CH_2	C-11, 14, 17, 18
13	-	49.5 qC	
14	-	84.7 qC	
15	2.45 dd (13.1, 8.5), 2.56 dd (13.4, 7.8)	42.3 CH_2	C-13, 14, 16, 17
16	5.09 obs dd (8.1, 4.4)	76.9 CH	C-13, 15, 17
17	3.05 d (4.0)	62.2 CH	C-12, 13, 14, 16, 21, 22, 23
18	0.96 s	16.4 CH_3	C-12, 13, 14, 17
19	10.19 s	209.2 CH	C-1, 5
20	-	174.9 qC	
21	5.22 d (18.1) , 5.07 d (18.3)	74.7 CH_2	C-17, 22, 23
22	6.27 s	118.2 CH	C-13, 17, 21, 23
23	-	174.9 qC	

Table 2.2 ^{1}H and ^{13}C NMR Spectroscopic Data of **2.2** (in C_5D_5N).

Position	δ_H (J in Hz)	δ_C mult	HMBC
1	1.22[a], 2.91 dd (4.83, 12.84)	39.7 CH_2	C-2, 3, 6, 9, 10, 19
2	3.91 ddd (4.9, 8.64, 11.21)	71.1 CH	C-1, 3
3	3.89 ddd (10.9, 8.6, 4.7)	84.3 CH	C-2, 4, 5, 1’
4	1.43	34.7 CH_2	C-2, 3, 5, 6
5	1.25	48.7 CH	C-3, 6, 9, 10
6	2.44 dt (10.3, 2.4), 1.32	27.9 CH_2	C-3, 5, 7, 8
7	1.29, 1.63	22.4 CH_2	C-4, 5, 8
8	1.78	42.7 CH	C-1, 5, 6, 9, 14
9	1.22[a]	42.9 CH	C-5, 8, 10, 11, 14
10	-	52.5 qC	
11	1.72, 1.22	28.1 CH_2	C-8, 9
12	1.32, 1.19	39.3 CH_2	C-9, 13, 14, 18
13	-	50.0 qC	
14	-	84.3 qC	
15	1.96, 1.83	32.5 CH_2	C-13, 14, 16, 17, 20
16	2.07, 2.01	27.3 CH_2	C-13, 14, 15, 17, 20
17	2.74 dd (9.0, 4.6)	51.3 CH	C-12, 13, 14, 15, 16, 20, 21, 22
18	0.89 s	16.0 CH_3	C-12, 13, 14, 17
19	10.00 s	208.6 CH	C-1, 9, 10
20	-	176.1 qC	
21	5.25 d (18.2), 5.01 d (18.0)	73.9 CH_2	C-17, 20, 22,23
22	6.15 s	117.9 CH	C-17, 21, 23
23	-	174.5 qC	
1′	5.74	108.7 CH	C-3, 4’, 5’
2′	-	85.8 qC	
3′	-	174.8 qC	
4′	2.87 dd (12.9, 5.8), 2.58 dd (13.0, 9.8)	41.8 CH_2	C-1’, 2’. 3’, 5’, 6’
5′	4.88 dt (9.8, 5.8)	76.9 CH	C-1’, 4’
6′	1.50 d (6.3)	22.9 CH_3	C-4’, 5’

[a]Overlapping signals

Table 2.3. ^{1}H and ^{13}C NMR Spectroscopic Data of **2.3**, **2.3a** and **2.3b** (in C_5D_5N).

Position	δ_H **2.3**	δ_H **2.3a**	δ_H **2.3b**	δ_C **2.3**	δ_C **2.3a**	δ_C **2.3b**
1	2.97 dd (12.8, 4.8), 1.34	2.80 dd (13.1, 4.8), 1.53 dd (12.7, 11.8)	3.43 dd (13.4, 4.6), 1.49	40.6 CH_2	44.2 CH_2	39.3 CH_2
2	4.08 ddd (11.5, 8.9, 4.8)	4.53 ddd (11.5, 9.0, 4.9)	4.56 ddd (11.3, 9.3, 4.7)	73.3 CH	73.3 CH	73.3 CH
3	3.90 ddd (11.3, 8.9, 4.8)	3.97 ddd (11.3, 9.1, 4.8)	3.95 ddd (11.3, 9.1, 4.7)	76.1 CH	76.3 CH	76.1 CH
4	1.95, 1.62 ddd (12.8, 11.3, 11.3)	2.20 dd (12.1, 11.9), 1.90	2.10 dt (12.2, 11.8), 1.88	38.5 CH_2	36.4 CH_2	37.2 CH_2
5	1.91	1.40	1.56	43.5 CH	44.3 CH	44.6 CH
6	2.00, 1.49	1.47	1.68, 1.34	28.6 CH_2	28.7 CH_2	28.4 CH_2
7	2.40, 1.90	2.31, 1.80	2.33, 1.82	27.2 CH_2	26.7 CH_2	27.0 CH_2
8	2.48	2.28	2.41	42.8 CH	41.1 CH	41.6 CH
9	1.43	1.39[a)]	1.45	48.4 CH	49.5 CH	48.8 CH
10	-	-	-	53.4 qC	72.8 qC	82.9 qC
11	1.70, 1.21	1.72	2.48, 1.90[c)]	22.6 CH_2	72.8 CH_2	82.9 CH_2
12	1.63, 1.30	1.39[a)]	1.35	38.2CH_2	21.3 CH_2	23.0 CH_2
13		-	-	49.1 qC	38.7 qC	39.2 qC
14	OH-14 5.30	-	-	81.9 qC	38.7 qC	39.2 qC
15	4.75 t (8.0)	4.77 t (7.7)	4.71 t (7.3)	72.6 CH	49.1 CH	49.3 CH
16	2.75, 1.97	2.67[b)], 1.94	2.67[d)], 1.92[c)]	37.9 CH_2	81.9 CH_2	82.2 CH_2
17	2.69	2.66[b)]	2.66[d)]	49.4 CH	73.2 CH	73.2 CH
18	0.83 s	0.98 s	1.04 s	16.9 CH_3	38.0 CH_3	38.1 CH_3
19	10.20 s	-	-	209.5 CH	-	-
20	-	-	-	175.8 qC	48.3 qC	175.8 qC
21	5.30 dd (18.3, 1.7) , 5.03 dd (18.3, 1.7)	5.33 d (18.6), 5.01 dd (18.2, 1.5)	5.30[h)], 4.96 d (18.2)	74.3 CH_2	17.0 CH_2	74.1 CH_2
22	6.13 s	6.10 s	6.06 s	118.3 CH	118.1 CH	118.1 CH
23	-	-	-	175.2 qC	174.8 qC	174.8 qC

Table 2.4. ^{1}H and ^{13}C NMR Spectroscopic Data of **2.4** (in C_5D_5N).

Position	δ_H (J in Hz)	δ_C mult	HMBC
1	2.97 dd (4.6, 12.9) 1.40 m	40.5 CH_2	C-2, 3, 5, 10, 19
2	4.05 ddd (4.6, 8.9, 10.9)	73.4 CH	C-1, 3
3	3.94 ddd (4.6, 8.9, 10.9)	76.1 CH	C-2, 4
4	2.03 m, 1.64 m	38.2 CH_2	C-2, 3, 5, 6, 10
5	1.47 m	43.6 CH	C-6, 10, 19
6	2.52 m, 1.50 m	28.2 CH_2	C-5, 7, 8
7	1.91 m, 1.31 m	28.5 CH_2	C-6, 8, 9, 14
8	1.87 m	42.9 CH	C-7, 9, 14
9	1.26 m	49.0 CH	C-7, 8, 9, 11
10	-	53.3 qC	-
11	1.70 m, 1.34 m	22.7 CH_2	C-8, 9, 10
12	1.28 m	39.5 CH_2	C-11, 13, 14, 18
13	-	50.2 qC	-
14	-	84.6 qC	-
15	1.96 m, 2.00 m	32.7 CH_2	C-13, 14, 16, 17
16	2.11 m, 2.01 m	27.4 CH_2	C-13, 14, 15, 17
17	2.79 dd (4.1, 9.4)	51.4 CH	C-12, 13, 14, 16, 20, 21, 22
18	0.92 s	16.2 CH_3	C-12, 13, 14, 17
19	10.20 s	209.4 CH	C-1
20	-	176.6 qC	-
21	5.05 d (18.2) 5.29 d (18.2)	74.2 CH_2	C-17, 20, 21, 22, 23
22	6.51 s	117.9 CH	C-17, 20, 21, 23
23	-	175.3 qC	-

Table 2.5 ^{1}H, ^{13}C NMR Spectroscopic Data of **2.5** (in $CDCl_3$-CD_3OD, 30:1)

Position	δ_H (*J* in Hz)	δ_C mult	HMBC
1	0.68 td (3.6, 13.7) 2.78 dt (3.6, 13.5)	31.4 CH_2	C-2, 3, 5, 9, 10, 19
2	1.33 m, 1.79 m	31.2 CH_2	C-1, 3, 4, 9
3	3.53 m	70.5 CH	-
4	1.30 m, 1.56 m	37.8 CH_2	C-2, 3, 5, 6, 10
5	0.88 m	49.9 CH	C-7, 19
6	1.03 m, 1.26 m	28.0 CH_2	C-7, 8, 9, 10
7	1.54 m, 1.48 m	22.7 CH_2	C-5, 8
8	1.59 m	41.9 CH	C-7, 13, 14
9	1.03 m	44.4 CH	C-10, 19
10	-	39.1 qC	-
11	1.11 m, 1.93 m	26.9 CH_2	C-9, 10, 13
12	1.23 m, 1.41 m	40.3 CH	C-8, 9, 13, 14, 18
13	-	49.8 qC	-
14	-	85.3 qC	-
15	1.61 m, 1.99 m	32.6 CH_2	C-13, 14, 16, 17
16	1.74 m, 2.08 m	27.3 CH_2	C-13, 15, 17, 20
17	2.65 dd (5.4, 9.5)	50.9 CH	C-12, 13, 16, 20, 21, 22
18	0.83 s	15.7 CH_3	C-12, 13, 14, 17
19	3.63 d (11.9) 3.75 d (11.9)	59.2 CH_2	C-1, 5, 9, 10
20	-	175.8 qC	-
21	4.73 dd (1.4, 18.1) 4.93 dd (1.4, 18.1)	73.8 CH_2	C-20, 22, 23
22	5.79 s	117.2 CH	C-17, 20, 21, 23
23	-	175.4 qC	-

Table 2.6. ^{1}H, ^{13}C NMR Spectroscopic Data of **2.6** (in $CDCl_3$-CD_3OD, 30:1)

position	δ_H (*J* in Hz)	δ_C mult	HMBC
1	-	177.0 qC	-
2	1.97 t (7.20)[a]	34.0 CH_2	C-1, 3, 4, 5
3	1.51 t (6.92)	24.7 CH_2	C-1, 2, 5
4	1.23 brs[b]	29.0[c] CH_2	C-2, 3, 5, 6
5	1.23 brs[b]	28.8[c] CH_2	C-3, 4, 6, 7
6	1.23 brs[b]	28.7[c] CH_2	C-4, 5, 7, 8
7	1.23 brs[b]	25.0[c] CH_2	C-5, 6, 8
8	1.42 brs[b]	36.7 CH_2	C-7, 9, 10
9	4.01 dd (6.1, 12.1)	71.6 CH	C-7, 8, 10, 11
10	5.69 dd (15.7, 5.8)	135.6 CH	C-8, 9, 11, 12
11	5.60 dd (15.7, 5.8)	129.5 CH	C-8, 9, 10, 12, 13
12	3.89 t (5.7)	74.3[d] CH	C-10, 11, 13, 14
13	3.41 dt (5.1, 7.8)	74.3[d] CH	C-11, 12, 14, 15
14	2.12 quintet (7.5), 2.26[a]	30.7 CH_2	C-12, 13, 15, 16, 17
15	5.32 m	124.4 CH	C-13, 14, 16, 17
16	5.43 m	134.3 CH	C-13, 14, 17, 18
17	1.97 quintet (7.4)	20.6 CH_2	C-14, 15, 16, 18
18	0.88 t (7.5)	14.0 CH_3	C-16, 17

[a-c]Methylene envelope. [d]Overlapping signals

Table 2.7. ^{1}H and ^{13}C NMR Spectroscopic Data of **2.7**

position	δ_H (J in Hz)	δ_C mult	HMBC
1	2.38 dd (4.4, 12.6) 1.05 t (12.2)	35.8 CH_2	C-2, 3, 5, 9, 10, 19
2	3.80 td (4.4, 10.3)	68.9 CH	C-3
3	3.88 ddd (4.2, 10.1, 11.7)	71.8 CH	C-2
4	1.68 m, 1.34 m	33.1 CH_2	C-2, 3, 5, 9
5	1.45 m	43.2 CH	-
6	2.11 m, 1.85 m	27.4 CH_2	C-8
7	1.62 m	27.5 CH_2	C-9
8	1.45 m	42.1 CH	C-14
9	1.29 m	48.4 CH	C-5
10	-	52.7 qC	-
11	1.65 m	22.0 CH_2	C-10, 13
12	1.55[a]	39.9 CH_2	C-9
13	-	48.7 qC	-
14	-	84.4 qC	-
15	1.99 dd (7.8, 13.4) 1.89 dd (8.7, 13.3)	40.7 CH_2	C-13, 14, 16, 17
16	4.39 td (8.1, 4.5)	76.1 CH	-
17	2.49 d (4.5)	60.5 CH	C-12, 13, 14, 16, 20, 22
18	0.69 s	15.6 CH_3	C-12, 13, 14, 17
19	9.91 s	207.9 CH	C-1
20	-	173.2 qC	-
21	4.73 d (18.3) 4.81 d (18.3)	74.3 CH_2	C-20, 22
22	5.86 s	117.7 CH	C-17, 21, 23
23	-	175.7 qC	-
1′	4.45 s	95.6 CH	C-3, 2′
2′	-	91.2 qC	-
3′	3.59[b]	72.8 CH	C-1′, 2′
4′	1.73 dd (6.8, 11.0) 1.49[a]	38.3 CH_2	C-2′,3′
5′	3.54[b]	68.1 CH	C-1′
6′	1.20 d (6.2)	20.8 CH_3	C-4′, 5′

[a-b] Overlapping signals

Table 2.8. ^{1}H and ^{13}C NMR Spectroscopic Data of **2.8**

position	δ_H (J in Hz)	δ_C mult	HMBC
1	2.51 dd (13.2, 5.0), 0.91 t (12.4)	37.8 CH_2	C-2, 3, 5, 9, 19
2	3.38	70.3 CH	
3	3.30	85.1 CH	
4	1.52, 1.14	34.2 CH_2	
5	1.28	42.5 CH	
6	2.10	27.2 CH_2	
7	1.70, 1.19	29.6 CH_2	
8	1.41 dt (12.1, 2.9)	42.0 CH	
9	1.22	48.1 CH	
10	-	51.9 qC	
11	1.78, 1.52	27.5 CH_2	
12	1.57	40.1 CH_2	
13	-	48.7 qC	
14	-	84.3 qC	
15	1.87, 1.50	40.6 CH_2	C-13, 17
16	4.43[a]	76.5 CH	
17	2.508 d (4.26)	60.5 CH	C-12, 13, 15, 16, 20, 21, 22
18	0.71 s	15.5 CH_3	C-12, 13, 17
19	9.88 s	207.8 CH	
20	-	173.4 qC	
21	4.85 d (18.2), 4.72 d (18.2)	74.3 CH_2	C-20, 22
22	5.88 s	117.6 CH	C-17, 20, 21, 23
23	-	173.0 qC	
1′	4.84 s	108.6 CH	C-2′, 3′, 4′, 5′
2′	-	84.2 qC	
3′	-	171.6 qC	
4′	2.23 dd (13.1, 10.1), 2.04 dd (13.3, 5.7)	39.9 CH_2	C-1′, 5′, 6′
5′	4.43[a]	76.2 CH	
6′	1.32 d (6.2)	21.9 CH_3	C-4′, 5′
OMe	3.76 s	52.4 CH_3	C-3′

[a] Overlapping signals

Table 2.9. ^{1}H and ^{13}C NMR Spectroscopic Data of **2.9**

position	δ_H (J in Hz)	δ_C mult	HMBC
1	0.79 m, 1.56 m	37.0 CH_2	C-9, 19
2	1.81 m, 1.86 m	28.5 CH_2	-
3	3.56 m	78.7 CH	C-1′
4	1.54 m, 1.60 m	34.1 CH_2	-
5	0.88 m	44.3 CH	-
6	1.41 m, 1.45 m	29.6 CH_2	-
7	1.30 m	27.3 CH_2	-
8	1.45 m	41.3 CH	-
9	0.71 m	48.8 CH	C-5, 10
10	-	35.7 qC	-
11	1.25 m, 1.33 m	21.0 CH_2	-
12	1.36 m, 1.38 m	39.7 CH_2	-
13	-	48.8 C	-
14	-	85.0 qC	-
15	1.22 m, 1.56 m	32.7 CH_2	C-14, 17
16	1.78 m, 1.91 m	26.9 CH_2	-
17	2.64 dd (5.3, 9.5)	50.8 CH	C-12, 13, 16, 20, 22
18	0.75 s	15.6 CH_3	C-12, 13, 17
19	0.66 s	12.0 CH_3	C-1, 5, 9, 10
20	-	175.5[a] qC	-
21	4.74 dd (18.3, 1.5) 4.92 dd (18.3, 1.5)	73.8 CH_2	C-20, 22
22	5.78 s	117.3 CH	C-20, 21, 23
23	-	175.5[a] qC	-
1′	4.29 d (7.7)	100.9 CH	C-3
2′	3.22 m	73.4 CH	C-1′, 3′
3′	3.35 m	76.4 CH	C-3′,5′
4′	3.30 m	70.4 CH	C-3′,5′
5′	3.44 m	73.9 CH	C-2′,4′
6′	4.34 dd (12.0, 2.0) 4.40 dd (12.0, 6.0)	63.5 CH_2	C-9″
1″	-	156.7 qC	-
2″	7.32 d (8.7)	130.0 CH	C-4″, 6″,7″
3″	6.74 d (8.7)	115.9 CH	C-4″, 5″
4″	-	159.4 qC	-
5″	6.74 d (8.7)	115.9 CH	C-3″, 4″
6″	7.32 d (8.7)	130.0 CH	C-2″, 4″, 7″
7″	7.53 d (15.9)	145.5 CH	C-1″, 6″, 8″, 9″
8″	6.20 d (15.9)	114.1 CH	C-9″
9″	-	167.9 qC	-

Table 2.10. ^{1}H, ^{13}C NMR Spectroscopic Data of **2.10** (in $CDCl_3$-CD_3OD, 30:1)

position	δ_H (*J* in Hz)	δ_C mult	HMBC
1	0.67 dt (3.6, 13.5) 2.16 dt (3.6, 13.5)	31.5 CH_2	C-2, 3, 4, 5, 10, 19
2	1.39 m, 1.83 m	29.4 CH_2	C-1
3	3.60 m	77.3 CH	C-1
4	1.31 m , 1.63 m	34.3 CH_2	C-2, 3, 5
5	1.09 m	44.2 CH	C-10, 19
6	1.24 m, 1.93 m	28.1 CH_2	C-7, 8, 10
7	1.00 m, 1.96 m	27.3 CH_2	C-6, 8, 9
8	1.66 m	41.9 CH	C-9, 10
9	0.86 m	49.9 CH	10, 11, 19
10	-	39.3 qC	-
11	1.14 m, 1.47 m	22.6 CH_2	C-13
12	1.26 m, 1.44 m	40.3 CH_2	C-11, 13, 14, 18
13	-	49.8 qC	-
14	-	85.3 qC	-
15	1.55 m, 2.01 m	32.6 CH_2	C-14, 16, 17
16	1.76 m, 2.06 m	26.9 CH_2	C-15, 20
17	2.68 dd (5.3, 9.2)	50.8 CH	C-12, 13, 15, 16, 20, 21, 22
18	0.82 s	15.8 CH_3	C-12, 14, 17
19	3.60 d (11.8) 3.70 d (11.8)	59.4 CH_2	C-1, 5, 9, 10
20	0.82 s	175.8 qC	-
21	4.93 d (18.3) 4.75 d (18.3)	73.8CH_2	C-20, 22
22	5.79 s	117.2 CH	C-17, 20, 21, 23
23	-	175.4 qC	-
1′	4.63 d (7.9)	97.8 CH	C-3, 2′, 5′
2′	3.25 dd (2.9, 7.9)	70.8 CH	C-1′, 2′, 5′
3′	4.02 t (2.9)	71.1 CH	C-1′, 5′
4′	3.14 dd (2.9, 9.5)	72.7 CH_2	C-4′, 5′, 6′
5′	3.60 dq (6.2, 9.5)	69.7 CH	C-1′, 2′, 3′,6′
6′	1.19 d (6.2)	17.6 CH_3	C-1′, 4′, 5′

Table 2.11. ^{1}H and ^{13}C NMR Spectroscopic Data of **2.11** (in C_5D_5N)

position	δ_H (J in Hz)	δ_C mult	HMBC
1	2.90 m, 1.25 m	39.9 CH_2	C-2, 19
2	3.99 m	71.0 CH	C-3
3	3.89 m	81.9 CH	C-2
4	1.99 m, 1.31 m	33.8 CH_2	-
5	1.88 m	43.2 CH	-
6	1.53 m	28.52 CH_2	-
7	2.42 m, 1.89 m	27.2 CH_2	-
8	2.48 m	42.8 CH	-
9	1.27 m	48.5 CH	C-7
10	-	52.9 qC	-
11	1.67 m, 1.26 m	22.5 CH_2	-
12	1.32 m	38.5 CH_2	-
13	-	49.1 qC	-
14	-	82.0 qC	-
15	4.74 t (7.5)	72.7 CH	-
16	2.68[a] , 1.90 m	38.5 CH_2	C-14, 17
17	2.68[a]	49.4 CH	-
18	0.92 s	17.0 CH_3	C-13, 14, 15
19	10.10 s	209.7 CH	C-1
20	5.29 d (18.4)	176.1 qC	-
	5.04 d (18.0)		
21	6.14 s	74.5 CH_2	C-20, 23
22	2.90, 1.25	118.3 CH	C-17, 20, 21, 23
23	-	175.5[b] qC	-
1′	5.65[c]	107.6 CH	C-3
2′	-	Nd	-
3′	-	175.5 [b] qC	-
4′	1.88	43.0 CH_2	-
5′	1.88	43.0 CH_2	-
6′	1.53 d (5.7)	23.1 CH_3	C-4′, 5′

[a-b] Overlapping signals

Table 2.12 ^{1}H and ^{13}C NMR Spectroscopic Data of **2.12** (in C_5D_5N)

position	δ_H (J in Hz)	δ_C mult	HMBC
1	0.89 td (3.2, 13.1) 2.72 d (13.1)	32.7[a] CH_2	C-2, 3, 5, 10, 19
2	2.07 m, 2.21 m	33.0 CH_2	C-1, 3
3	4.00 m	70.9 CH	C-2, 4, 5
4	1.81 m, 1.93 m	39.6 CH_2	C-3, 5, 10
5	1.22 m	45.5 CH	C-4, 10
6	2.44 m	29.0 CH_2	C-8
7	1.25 brs	28.6 CH_2	C-5
8	2.26 m	42.8 CH	C-14
9	1.14 m	47.7 CH	C-8, 10, 11, 19
10	-	39.9 qC	-
11	2.38 m	32.7[a] CH_2	C-8, 9, 12, 13
12	3.67 dd (10.5, 5.1)	75.3 CH	C-17, 18
13		57.1 qC	
14		85.7 qC	
15	1.98 m	33.6 CH_2	C-13, 14, 16, 17
16	2.03 m, 2.16 m	28.0 CH_2	C-14, 15, 20
17	3.79 t (7.7)	46.8 CH	C-12, 13, 14, 15, 16, 20, 21, 22
18	1.29 s	10.6 CH_3	C-12, 13, 14
19	4.08 d (11.4) 4.18 d (11.4)	59.2 CH_2	C-1, 5, 9, 10
20	-	177.3 qC	-
21	5.13 d (18.12) 5.26 d (18.12)	74.4 CH_2	C-20, 22, 23
22	6.29 s	117.5 CH	C-20, 21, 23
23	-	175.3 qC	-

[a] Overlapping signals

Table 2.13 ^{1}H and ^{13}C NMR Spectroscopic Data of **2.13** (in C_5D_5N)

position			
1	1.17 t (12.4)	36.8 CH_2	C-2, 3, 5, 9, 10, 19
	2.61 dd (12.4, 4.3)		
2	4.60 m	70.0 CH	C-1, 3
3	4.37 dt (10.7, 4.2)	72.6 CH	C-2, 4
4	1.54 m, 1.73[a] m	34.26 CH_2	C-2, 3, 10
5	1.29 m	49.0 CH	-
6	1.39 m	28.2[b] CH_2	C-5, 7
	2.52 d (10.7)		
7	1.32 m, 1.62 m	22.6 CH_2	C-8
8	1.32 m	43.7 CH	C-14
9	1.80 m	42.9 CH	C-10
10	-	53.4 qC	-
11	1.45 m, 1.93 m	28.2[b] CH_2	C-14
12	1.35 m	39.4 CH_2	C-14, 18
13	-	50.1 qC	-
14	-	84.5 qC	-
15	2.00 m, 2.15 m	32.7 CH_2	C-14, 16, 17
16	1.76 m, 2.06 m	27.4 CH_2	C-14
17	2.79 dd (6.7, 9.0)	51.4 CH	C-13, 14, 16, 20
18	0.90 s	16.2 CH_3	C-12, 13, 14, 17
19	10.10 s	208.7 CH	C-1, 9, 10
20	-	176.5 qC	-
21	5.04 d (18.2)	74.2 CH_2	C-20, 21, 23
	5.29 d (18.2)		
22	6.14 s	118.0 CH	C-17, 22
23	-	175.3 qC	-
1′	5.55 s	95.5 CH	C-3, 2′
2′	-	94.1 qH	-
3′	4.52[b] d (3.0)	75.5 CH	C-1′, 2′, 3′,4′, 5, ′6′
4′	4.28 dd (2.6, 8.7)	73.1 CH	C-1′, 2′, 3′,5′, 6′
5′	4.53[b]	70.4 CH	C- 4, 6′
6′	1.73[a] d (6.2)	19.1 CH_3	C-1′, 4′,5′

[a-b] Overlapping signals

Table 2.14 ^{1}H and ^{13}C NMR Spectroscopic Data of **2.14** in $CDCl_3$

position	δ_H (J in Hz)	δ_C mult	HMBC
1	-	170.6 qC	-
2	2.49 d (17.4)	44.7 CH_2	C-1, 3, 4, 6
3	2.64 dd (1.4, 17.4)	68.1 qC	-
4	1.88 m	35.9 CH_2	C-1, 2, 3, 5, 6
5	4.31 ddd (4.8, 9.2, 11.3)	66.0 CH_2	C-1, 3, 4
	4.57 ddd (5.4, 9.2, 11.3)		
6	1.36 s	29.8 CH_3	C-1, 2, 3, 4, 5

Ten compounds (**2.1-2.4, 2.8-2.13)** were evaluated for their cytotoxic activity against oral epidermal carcinoma (KB), breast cancer (MCF7) and human small cell lung cancer (NCI-H187) cell lines. Results are as shown in Table 2.15. Compounds **22.10** (frugoside) and **2.13** (calotoxin) showed most potent inhibitory activity against all cell lines.

Table 2.15 Cytotoxic Activities of the Isolated Compounds.

compound	KB[a]	MCF 7[a]	NCI-H187[a]
2.1 (16*α*-hydroxy calotropagenin)	inactive	inactive	Inactive
2.2 (calactinic acid)	17.15 (31.28)	inactive	Inactive
2.3 (2α,15*β*-dihydroxy-19-oxo-uzarigenin)	inactive[b]	inactive[b]	inactive[b]
2.4 calotropagenin,	2.56 (6.33)	42.87 (106.06)	19.42 (48.04)
2.8 (16*α*-hydroxy calactinic acid methyl ester)	0.94 (1.63)	46.61 (80.60)	5.74 (9.93)
2.9 (6′-*O*-(*E*-4-hydroxy cinnamoyl)desglucouzarin)	11.81 (17.30)	inactive	39.56 (57.97)
2.10 (frugoside)	0.02 (0.03)	1.96 (3.65)	0.11 (0.20)
2.11 (15*β*-hydroxy calactinic) acid.	inactive	inactive	Inactive
2.12 (12*β*-coroglaucigenin)	0.68 (1.67)	34.35 (84.55)	6.24 (15.36)
2.13 (calotoxin)	0.002 (0.003)	3.26 (5.95)	0.002 (0.003)
ellipticine[c]	0.448 (1.82)	-	0.684 (2.78)
doxorubicin[c]	0.249 (0.46)	0.57 (1.05)	0.035 (0.06)

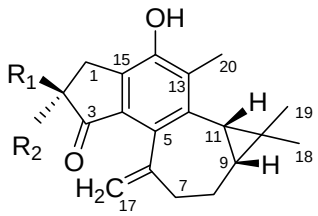
References and Notes

(2.1) Lhinhatrakool, T.; Sutthivaiyakit, S. *J. Nat. Prod.* **2006** *69*, 1249-1251.
(2.2) Abe, F.; Mori, Y.; Yamauchi, T. *Chem Pharm Bull* **1992** *40*, 2917-20.
(2.3) Roy, M. C.; Chang, F.-R.; Huang, H.–C.; Chiang, M. Y. –N.; Wu, Y. -C. *J. Nat. Prod.* **2005** *68*, 1494-1499.
(2.4) Abe, F.; Mori, Y.; Yamauchi, T. *Chem. Pharm. Bull.* **1991**, *39*, 2709-2711.
(2.5) El-Askary, H.; Holzl, J.; Hilal, S.; El-Kashoury, E. *Phytochemistry* **1993**, *34*, 1399-1402.
(2.6) The transformation in **2.3** was likely to occur through nucleophilic attack of an active oxygen species, such as peroxide radical, at the bridge-head formyl group. The addition product after rearrangement (as in Baeyer Villiger oxidation), and further hydrolysis, may give rise to **2.3a** and **2.3b**. See: Hudlický, M., "Oxidations in Organic Chemistry," ACS Monograph 186, American Chemical Society, Washington DC, 1990, pp. 180-181.
(2.7) In previous report, ^{13}C NMR chemical shifts of the quaternary carbons baring OOH groups were also found to resonate at less shielded positions than those with OH groups. See: Lee, C. -K.; Fang, J.-M.; Cheng, Y. -S. *Phytochemistry* **1995**, *39*, 391-394.
(2.8) Oueslati, M. H.; Jannet, H. B.; Mighri, Z.; Chriaa, J.; and Abreu, P. M. *J. Nat. Prod.* **2006**, *69*, 1366-1369 (.
(2.9) Martin, R. A.; Lynch, S. P.; Schmitz, F. J.; Pordesimo, E. O.; Toth, S.; Horton, R. Y. *Phytochemistry* **1991**, *30*, 3935-3939.
(2.10) Elgamal, M. H. A.; Hanna, A. G. ; Morsy, N. A. M.; Duddeck, H.; Simom, A.; Gati, T.; Toth. G. *J. Mol. Struct.* **1999**, *477*, 201-208.
(2.11) Cheung, H. T. A.; Chiu, F. C. K.; Watson, T. R.; Wells, R. J. *J. C. S. Perkin I.* **1986,** 55-59.
(2.12) Grout, D. H. G.; Curtis, R. F.; Hassall, C. H.; Jones, T. L. *Tetrahedron Lett.* **1963**, 63-67.
(2.13) Cheung, H. T. A.; Nelson, C. J.; Watson, T. R. *J. C. S. Perkin I.* **1988**, 1851-1857.
(2.14) Frye, S. V.; Eliel, E. L. *J. Org. Chem.* **1985**, *50*, 3402-3404.
(2.15) Seiber, J. N.; Nelson, C. J. ; Lee, S. M. *Phytochemistry* **1982**, *21*, 2343-2348.
(2.16) McEwan, T.; McInnes, A. G.; Smith, D. G. *Carbohydr. Res.* **1982**, *104*, 161-168.
(2.17) Cheung H. T. A., Nelson C. J., *J. Chem. Soc. Perkin Trans. I*, **1989**, 1563—1570.

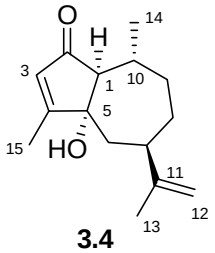
Part III. Constituents and Bioactivity of *Jatropha integerrima*

In continuation to our recent studies of biologically active compounds from *Jatropha integerrima* Jacq. (Euphorbiaceae), [3.1-3.2] we investigated further the constituents of the hexane extract of the roots and isolated five new compounds, including three diterpenes (**3.1**-**3.3**), one sesquiterpene (**3.4**), and a sesquiterpene-coumarin conjugate (**3.5**). Nine known compounds, 1*β*-hydroxy-10*β*H-guaia-4, 11-dien-3-one (3.6),[3.3] 4-hydroxy-10-epirotundone (3.7),[3.4] citlalitrione (3.8),[3.5] stigmast-4-en-6*β*-ol-3-one (3.9),[3.6] jatropholone A (**3.10**),[3.7] jatropholone B (**3.11**),[3.7] caniojane,[3.8] and 1,11-*bisepi*-caniojane[3.8] were also isolated.

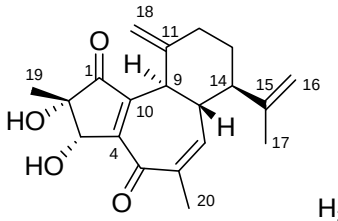
Compound **3.1** was obtained as pale yellow plates with mp 208-210 °C. The HRESIMS spectrum gave an $[M + H]^+$ ion at *m/z* 313.1719 corresponding to the molecular formula $C_{20}H_{24}O_3$. The FTIR spectrum had absorption maxima indicating OH (3225 cm^{-1}), carbonyl (1693 cm^{-1}) and aromatic (1595 cm^{-1}) groups. The presence of an exocyclic methylene group was revealed by methylene proton signals at δ 5.17 and 4.63 (1H each), and ^{13}C NMR signals at δ 115.1 (CH_2) and 135.3 (qC). The ^{13}C NMR signals at δ 150.3 (qC), 146.0 (qC), 138.1 (qC), 134.3 (qC) 132.1 (qC), 129.7 (qC), as well as signals of a methyl group at δ_H 2.25 (s) and δ_C 13.3 (CH_3) indicated a fully substituted aromatic ring with one OH and one methyl substituent groups. Two mutually coupled methine proton signals [δ 1.55 (1H, d, J = 8.1 Hz) and 0.94] both showed HMBC correlations to the carbon resonances of two quaternary methyl groups [δ 28.1 (C-18) and 16.1 (C-19)], and to the aromatic carbon resonance at δ 138.1 (C-12) indicated connectivity between a cyclopropane moiety and an aromatic nucleus as found in jatropholone A (**3.10**) and B (**3.11**)[3.7] also isolated in the present study. The differences were a methyl proton singlet at δ_H 1.40 instead of a doublet at approximately δ_H 1.27, as well as two sets of AB doublets (benzylic methylene protons) at δ_H 3.10 and 2.99 (both with J = 16.3 Hz) instead of two sets of doublet of doublets at ca δ_H 3.25 and 2.50 as reported for **3.10** and **3.11**, indicating the presence of an additional OH group at C-2. Long-range ^1H, ^{13}C correlations were observed between δ_H 3.10 (H-1) and 1.40 (H-16) to δ_C 207.1 (C-3). Thus, compound **3.1** was identified as 2-hydroxyjatropholone. Full assignments of the ^1H and ^{13}C NMR resonances (see Table 3.1) were based on ^1H-^1H COSY, HMQC and HMBC experiments. The relative configuration of **3.1** was established from an X-ray single-crystal analysis (Figure 3.1), indicating an α-oriented 2-OH group.



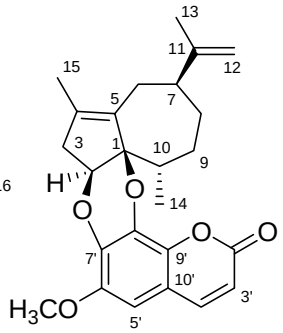
3.1 $R_1 = CH_3$, $R_2 = OH$
3.2 $R_1 = OH$, $R_2 = CH_3$
3.10 $R_1 = CH_3$, $R_2 = H$
3.11 $R_1 = H$, $R_2 = CH_3$



3.4



3.3



3.5

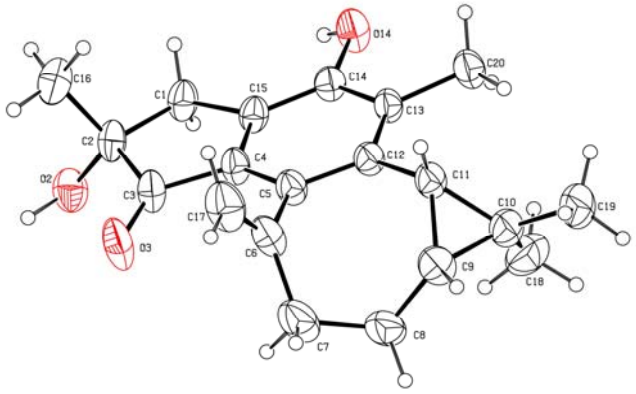


Figure 3.1 ORTEP drawing of **3.1**

Compound **3.2** was a colorless oil having molecular formula $C_{20}H_{24}O_3$ (HRESIMS). The FTIR, ^1H and ^{13}C NMR spectra of **3.2** were similar to those of compound **3.1**. The difference in the ^1H NMR spectrum of **3.2** was the two benzylic methylene protons (H_2-1) found as an obscured triplet at δ_H 3.07 with coupling constant of 17.1 Hz instead of AB doublets (δ_H 3.10 and 2.99) as found in **3.1**. Full assignments of ^1H and ^{13}C NMR resonances are given in Table 3.1. Compound **3.2** was thus 2β-hydroxyjatropholone, the C-2-epimer of **3.1**.

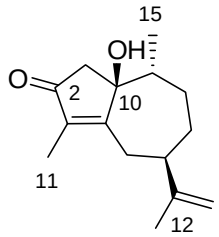
Compound **3.3** was isolated as a colorless solid, mp 144-146 °C, with molecular formula $C_{20}H_{24}O_4$ (HRESIMS). The FTIR spectrum indicated absorption maxima for OH (3434 cm^{-1}), and conjugated carbonyl (1732 and 1651 cm^{-1}) groups. The ^{13}C NMR spectrum showed 20 signals indicating three methyl, four methylene, five methine and eight quaternary carbons including two keto carbons (see Table 3.1). The ^1H-^1H COSY spectrum indicated connectivity from H-7 (δ 5.82) to H-9 (δ 3.19) and from H-8 (δ 2.60) to H-12 (δ 2.40 and 2.24). Two exocyclic double bonds were evident from ^1H NMR signals at δ 4.75 and 4.14, and ^{13}C NMR signals at δ 148.1 (qC) and 108.3 (CH_2), in conjunction with the ^1H NMR chemical shifts at δ 4.84 and 4.80, and the ^{13}C NMR resonances at δ 146.5 (qC) and 113.3 (CH_2). HMBC correlations of H-3/C-1, C-4, C-10, C-19 and of H-7/C-5, C-14, C-20, in combination to the HMBC correlations between H-9/C-4, C-8, C-10 and C-18 led to the establishment of a rhamnofolane skeleton with double bonds at C-4(10), C-6(7), C-15(16) and C-11(18), together with keto groups at C-1 and C-5, and OH groups at C-2 and C-3. Relative configurations at C-8, C-9, C-14 were deduced from coupling constants and were consistent with those reported for curcusones A-D.[9] The 3-OH group had α-orientation due to the presence of homoallylic coupling between H-3 and H-9 (J = 1.6 Hz) as found in 2-epijatrogrossidione.[3.8] The NOESY spectrum showed a cross-peak between H-3/H_3-19 implying that both H-3 and H_3-19 were β–oriented. Compound **3.3** was thus proposed to be 1,5-dioxo-2,3-dihydroxyrhamnofola-4(10),6,11(18), 15-tetraene.

Compound **3.4** was obtained as colorless needles ($C_{15}H_{22}O_2$). The IR spectrum showed absorption maxima for OH (3435 cm^{-1}) and an α,β-unsaturated carbonyl (1688 and 1622 cm^{-1}) groups. The ^{13}C NMR spectrum indicated the presence of three methyl, four methylene, four methine and four quaternary carbons including one carbonyl, two olefinic and an oxygenated quaternary carbons. The ^1H-^1H COSY spectrum indicated cross-peaks between H-3/H-15 and between H-12 and H-13/H-7, and sequential connectivities from H-1 to H-6. A guaiane sesquiterpene skeleton with a keto group at C-2, an OH at C-5, and double bonds at C-3(4) and C-11(12) was indicated based on HMBC correlations of H-3/C-1, C-2, C-5 and C-15, and of H-7/C-5, C-11, C-12 and C-13. Compound **3.4** was proposed to be 2-keto-5-hydroxyguai-3,11-diene. Relative configurations at C-1, C-10 and C-7 were deduced from NOE effects and coupling constants between related protons. The NOESY spectrum showed a cross-peak between H-1 and H_3-14. The $J_{1,10}$ and $J_{6,7}$ values of ca 10 and 12 Hz, respectively, indicated that H-1 and H-10 were both α-oriented, and that the isopropenyl group was β-oriented.[3.10] The ^1H and ^{13}C NMR assignments are as given in Table 3.2.

Compound **3.5** was isolated as a yellow solid. The HRESIMS indicated a molecular formula $C_{25}H_{28}O_5$ and the FTIR spectrum showed absorption maxima consistent with conjugated carbonyl (1732 cm^{-1}) and olefinic (1614 and 983 cm^{-1}) groups. The ^1H NMR spectrum exhibited signals indicating an α,β-unsaturated carbonyl moiety as two sets of doublets at δ 7.54 and 6.22, both with coupling constant of 9.4 Hz, as well as ^{13}C NMR signals at δ 160.9 (qC), 144.9 (qC), 143.7 (CH), 140.6 (qC), 140.0 (qC), 129.1 (qC), 113.2 (CH), 110.6 (qC), and 100.4 (CH) that indicated the presence of a coumarin nucleus (see Table 3.2). NOE interactions between H-3′/H-4′, H-4′/H-5′, and 6′-

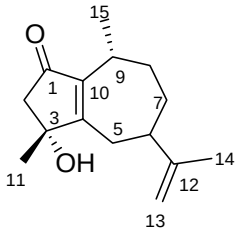
OC*H*$_3$/H-5′ were detected in the NOESY spectrum. The guaiane sesquiterpene skeleton was established from the ^{1}H-^{1}H COSY spectrum which indicated connectivity of an oxymethine proton (H-2, δ 4.96) to H-3, and sequentially from H-6 to H$_3$-14, in combination to the long-range ^{1}H, ^{13}C correlations between H-6/C-1, C-4, C-8 and C-11, as well as between H-14/C-1, C-9, and H-12/C-7, C-11 and C-13 in its HMBC spectrum. The key long range ^{1}H-^{13}C correlations between H-2/C-3, C-10 and C-7′ required an ether linkage between C-2 and C-7′. An NOE effect between H-2/H$_3$-14 indicated the C-10 methyl group and H-2 to be in close proximity. The relative configuration at C-7, although it could not be obtained from the NOESY spectrum, was deduced from the $J_{6,7}$ value of ca 13 Hz which revealed that the dihedral angle between one of the H$_2$-6 protons and H-7 was close to 180°, thus indicating a β-oriented isopropenyl group.[3.10] The presence of an additional ether linkage between C-1 and C-8′ was expected from the molecular formula. Accordingly, the structure of **3.5** was assigned as shown and this compound has been given the name jatrophadioxan.

Compound **3.6** was obtained as colorless oil, which showed dark spot under UV light at 254 nm wavelength. It showed yellowish orange color after treatment with anisaldehyde-sulfuric acid reagent. The molecular formula of $C_{15}H_{22}O_2$ was obtained from the $[M + H]^+$ ion at *m/z* 235.1689. The use of 2D NMR techniques led to identified compound **3.6** as 10β-hydroxy-guaia-3(4),12(13)-dien-2-one (or 1β-hydroxy-10βH-guaia-4, 11-dien-3-one,[3.3]). ^{1}H and ^{13}C NMR chemical shifts are as shown in Table 3.3.



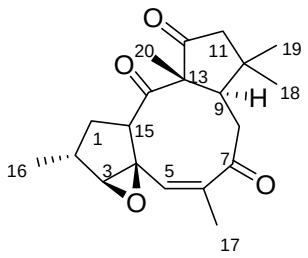
3.6

Compound **3.7** was obtained as colorless oil, which gave dark spot under UV light at 254 nm with $[\alpha]_{589}^{29}$ -56.1582 (*c* 0.8850, $CHCl_3$). It showed yellowish orange spot after staining with anisaldehyde-sulfuric acid reagent. The molecular formula of $C_{15}H_{22}O_2$ was obtained from $[M - H]^+$ ion at *m/z* 233.1545. The 2D NMR data indicated compound **3.7** to be identical to 3-hydroxy-9-epirotundone (or 4-hydroxy-10-epirotundone[3.4]). ^{1}H and ^{13}C NMR chemical shifts are as shown in Table 3.4.



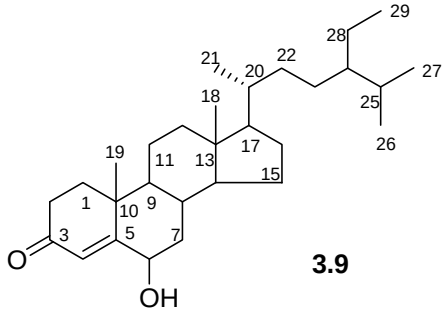
3.7

Compound **3.8** was afforded as colorless needles, mp. 188-190°C. It showed dark spot under UV light at 254 nm wavelength and gave sky blue to greenish gray color after staining with anisaldehyde-sulfuric acid reagent. It showed molecular formula of $C_{20}H_{26}O_4$ as indicated from the $[M + H]^+$ ion at *m/z* 331.1912 with $[\alpha]_{589}^{28}$ -154.89 (*c* 0.09, $CHCl_3$). It was identified as citlalitrione[3.5] based on 2D NMR techniques. Assignment of ^1H and ^{13}C NMR chemical shifts are as indicated in Table 3.5.



3.8

Compound **3.9** was isolated as a colorless solid, mp. 198-200 °C. It gave dark spot under UV light at 254 nm wavelength and showed yellow to red color after staining with anisaldehyde-sulfuric acid reagent. It showed $[M + H]^+$ ion at *m/z* 429.3726, which indicated molecular formula of $C_{29}H_{48}O_2$. The 2D NMR spectra revealed compound **3.9** to be stigmast-4-en-6*β*-ol-3-one.[3.6] Full assignment of ^1H and ^{13}C NMR data is as shown in Table 3.6.



3.9

Table 3.1. ^{1}H and ^{13}C NMR Spectroscopic Data of **3.1**, **3.2** and **3.3** ($CDCl_3$, δ ppm, mult. *J* in Hz)[a]

position	**3.1**		**3.2**		**3.3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	3.10 d (16.3), 2.99 d (16.3)	37.6, CH_2	3.07 t (17.1)	37.2, CH_2	-	209.5, qC
2	-	77.7, qC	-	77.5, qC	-	74.0, qC
3	-	207.1, qC	-	206.1, qC	4.83 t (1.6)	74.7, CH
4	-	134.3, qC	-	134.6, qC	-	157.9, qC
5	-	135.3, qC	-	135.7, qC	-	198.2, qC
6	-	146.0, qC	-	144.4, qC	-	142.0, qC
7	2.71 ddd (15.0, 6.3, 2.3) 2.62 ddd (15.0, 7.0, 5.0)	33.3, CH_2	2.60 obs dd (10.4, 4.7)	33.4, CH_2	5.82 dq (5.2, 1.6)	136.0, CH
8	1.82 m, 0.80 m[a]	21.4, CH_2	1.81 (ddd, 10.0, 7.9, 3.5) 0.87 m[b]	21.4, CH_2	2.60 ddd (12.3, 12.3, 5.2)	43.6, CH
9	0.94 ddd (12.5, 8.1, 5.5)	26.0, CH	0.93 ddd (11.1, 8.6, 3.9)[b]	25.9, CH	3.19 brd (12.3)	45.0, CH
10	-	19.6, qC	-	19.6, qC	-	146.8, qC
11	1.55 d (8.1)	28.3, CH	1.56 d (8.3)	28.3, CH	-	148.1, qC
12	-	138.1, qC	-	137.9, qC	2.40 ddd (12.6, 4.4, 2.7) 2.24 brdt (12.6, 4.5)	36.5, CH_2
13	-	132.1, qC	-	132.4, qC	1.89 m 1.45 dt (12.9, 4.4)	34.3, CH_2
14	-	150.3, qC	-	150.1, qC	2.32 dt (11.9, 4.1)	51.9, CH
15	-	129.7, qC	-	128.7, qC	-	146.5, qC
16	1.40 s	26.1, CH_3	1.37 s	25.7, CH_3	4.84 brs 4.80 brs	113.3, CH_2
17	5.17 t (1.8) 4.63 t (2.1)	115.1, CH_2	5.27 d (1.4) 4.73 brs	116.2, CH_2	1.57 s	18.6, CH_3
18	1.22 s	28.1, CH_3	1.22 s	28.1, CH_3	4.75 brs 4.14 brs	108.3, CH_2
19	0.80 s[a]	16.1, CH_3	0.81 s	16.2, CH_3	1.43 s	24.0, CH_3
20	2.25 s	13.3, CH_3	2.26 s	13.4, CH_3	1.84 t (1.6)	18.7, CH_3

[a,b]Overlapped signals

Table 3.2. ^{1}H and ^{13}C NMR Spectroscopic Data of **3.4** and **3.5** ($CDCl_3$, δppm, mult. *J* in Hz)

position	**3.4**		**3.5**	
	δ_H	δ_C	δ_H	δ_C
1	2.16 d (10.1)	67.2, CH	-	90.8, qC
2		205.8, qC	4.96 t (7.6)	73.7, CH
3	5.80 s	128.7, CH	2.50 br d (7.6)	39.4, CH_2
4	-	177.6, qC	-	136.3, qC
5	-	82.5, qC	-	137.4, qC
6	1.92 brd (14.1) 1.37 dd (14.1, 11.8)	40.7, CH_2	2.45 br d (13.4) 2.18 t (12.7)	29.8, CH_2
7	2.53 dt (11.9, 2.7)	41.1, CH	1.82[a]	49.6, CH
8	1.86 m, 1.37 brt (12.0)	36.3, CH_2	1.82, [a] 1.46[b]	36.3, CH_2
9	1.63 tt (12.1, 2.0), 1.56 obs ddt (12.5, 5.8, 1.7)	35.2, CH_2	1.46,[b] 1.35 dd (12.9, 9.7)	32.5, CH_2
10	1.76 obs ddq (10.3, 6.4, 1.7)	34.3, CH	2.33 dd (9.2, 7.3)	42.3, CH
11	-	150.8, qC	-	150.4, qC
12	4.67 brs , 4.65 brs	109.1, CH_2	4.71 , 4.68	108.9, CH_2
13	1.68 s	20.6, CH_3	1.73 s	20.4, CH_3
14	1.04 d (6.4)	23.8, CH_3	1.04 d (7.2)	16.9, CH_3
15	2.01 s	12.8, CH_3	1.64 s	14.1, CH_3
2′			-	160.9, qC
3′			6.22 d (9.4)	113.2, CH
4′			7.54 d (9.4)	143.7, CH
5′			6.44 s	100.4, CH
6′				144.9, qC
7′				129.1, qC
8′				140.6, qC
9′				140.0, qC
10′				110.6, qC
-OCH_3			3.85 (s)	56.4, CH_3

[a,b]Overlapped signals

Table 3.3. ^{1}H and ^{13}C NMR Spectroscopic Data of Compound **3.6** ($CDCl_3$)

Position	δ_H (ppm, *J* in Hz)	δ_C (ppm)	HMBC
1	2.26 (d, 18.1) 2.42 (d, 18.2)	44.5 CH_2	C-2, 3, 4, 9, 10
2	-	207.3 qC	-
3	-	136.8 qC	-
4	-	174.0 qC	-
5	2.63 (d, 12.0) 2.41 (t, 11.9)	31.3 CH_2	C-3, 4, 6, 7, 10, 12
6	1.93 (m)[a]	49.2 CH	C-5, 7, 8, 12
7	1.83 (m), 1.46 (m)	36.0 CH_2	C-6, 8, 9, 12
8	1.43 (m), 0.96 (m)	31.6 CH_2	C-6, 7, 15
9	1.94 (m)[a]	41.7 CH	C-1, 7, 8, 15
10	-	81.8 qC	-
11	1.68 (s)	7.5 CH_3	C-2, 3, 4
12	-	149.5 qC	-
13	4.75 (br d, 0.66) 4.73 (q, 1.5)	109.6 CH_2	C-6, 12, 14
14	1.75 (s)	20.5 CH_3	C-6, 12, 13
15	1.07 (d, 7.0)	17.8 CH_3	C-8, 9, 10

[a]Overlapping signals.

Table 3.4. ^{1}H and ^{13}C NMR Spectroscopic Data of Compound **3.7** ($CDCl_3$)

Position	δ_H	δ_C	HMBC
1	-	204.7 qC	-
2	2.60 (d, 16.3) 2.46 (d, 16.1)	51.5 CH_2	C-1, 3, 4, 10, 11, 15
3	-	75.7 qC	-
4	-	174.8 qC	-
5	2.48 (m)[a]	28.3 CH_2	C-1, 3, 4, 6, 7, 11, 12
6	2.53 (m)[a]	44.5 CH	C-4, 5, 7, 8, 12, 13, 14
7	1.96 (dddd, 5.5, 12.9, 12.3, 13.4) 1.60 (m)	28.9 CH_2 [b]	C-5, 6^{2J}, 8^{5J}, 9, 12
8	1.82 (dddd, 2.7, 5.7, 12.1, 13.5)	28.9 CH_2[b]	C-6, 7, 9, 10, 15
9	2.57 (m, $w_{1/2}$= 12.6)	28.3 CH	C-1, 4, 7, 8, 10, 15
10	-	145.4 qC	-
11	1.40 (s)	26.5 CH_3	C-2, 3, 4
12	-	149.6 qC	-
13	4.71 (obs t, 0.72) 4.69 (br s)	109.6 CH_2	C-5, 6, 7, 12, 14
14	1.73 (s)	20.1 CH_3	C-6, 12, 13
15	1.13 (d, 7.2)	17.7 CH_3	C-8, 9, 10

[a-b]Overlapping signals.

Table 3.5. ^{1}H and ^{13}C NMR Spectroscopic Data of Compound **3.8** ($CDCl_3$)

Position.	δ_H (ppm, *J* in Hz)	δ_C (ppm)	HMBC
1	1.32 (br d, 13.75), 1.94 (obs. m)[a]	34.9 CH_2	C-2, 3, 4, 14, 15, 16
2	2.33 (br q. 7.68)[b]	33.8 CH	C-3, 4, 15, 16
3	3.27 (s)	72.9 CH	C-2, 5, 15, 16
4	-	67.6 qC	-
5	5.49 (br s)	128.3 CH	C-3, 4, 7, 15, 17,
6	-	145.5 qC	-
7	-	208.4 qC	-
8	2.53 (dd, 12.6, 13.3), 2.91 (dd, 1.52, 12.60)	38.0 CH_2	C-6, 7, 9, 10, 13
9	2.49 (t, 13.4)	52.9 CH	C-7, 8, 11, 14, 18, 19, 20
10	-	37.6 qC	-
11	2.36 (d, 17.4)[b], 2.43 (d, 17.4)	55.5 CH_2	C-13, 18, 19
12	-	215.5 qC	-
13	-	65.7 qC	-
14	-	217.2 qC	-
15	3.62 (d, 9.41)	46.6 CH	C-2, 3, 4, 14
16	1.13 (d, 7.51)	16.1 CH_3	C-1, 3
17	1.92 (d, 1.62)[a]	20.5 CH_3	C-5, 6, 7
18	0.94 (s)	23.7 CH_3	C-9, 10, 19
19	1.24 (s)	28.3 CH_3	C-9, 10, 11, 18
20	1.41 (s)	14.6 CH_3	C-9, 12, 13, 14

Table 3.6. ^{1}H and ^{13}C NMR Spectroscopic Data of Compound **3.9** ($CDCl_3$)

Position	δ_H (ppm, *J* in Hz)	δ_C (ppm)	HMBC
1	1.99 (m)[a], 1.67 (dd, 4.0, 13.6)	37.1 CH_2	C-2, 3, 5, 8, 9,10, 11, 19
2	2.53 (d, 4.85), 2.37 (t 3.25)	34.3 CH_2	C-1, 3, 10
3	-	200.4 qC	-
4	5.79 (s)	126.4 CH	C-2, 5, 6, 10, 19
5	-	168.5 qC	-
6	4.32 (t, 2.55)	73.2 CH	C-4, 8, 10
7	1.22 (m), 1.975 (m)[a]	38.6 CH_2	C-5, 6, 9, 14
8	0.995 (m)[b]	29.8 CH	C-6, 11, 13, 15
9	0.89 (t, 4.4)	53.6 CH	C-1, 5, 7, 12, 14, 19
10	-	38.0 qC	-
11	0.98 (m)[b]	21.0 CH_2	C-8, 10, 13
12	1.11 (m), 1.16 (m)	39.6 CH_2	C-9, 14, 17, 18
13	-	42.5 qC	-
14	1.14 (m)	55.9 CH	C-7, 9 12, 13, 16, 18
15	0.95 (m), 1.480 (m)	24.2 CH_2	C-8, 13
16	1.10 (m)[c], 1.829 (m)	28.2 CH_2	C-13, 14, 15
17	1.11 (m)[c]	56.1 CH	C-12, 13, 14, 15, 18, 20, 21, 22
18	0.71 (s)	12.0 CH_3	C-12, 13, 14, 17
19	1.35 (s)	19.5 CH_3	C-1, 5, 9, 10, 11
20	0.995 (m)[b]	36.1 CH	C-13, 16, 23
21	0.90 (d 6.4)[d]	18.8 CH_3	C-17, 20, 22, 23
22	1.27 (m), 0.99 (m)[b]	33.9 CH_2	C-17, 20, 21, 23, 24
23	0.89 (m)[d], 1.15 (m)	26.2 CH_2	C-20, 28, 25
24	0.90 (m)	45.9 CH	C-22, 23, 26, 27, 29
25	1.64 (m)	29.2 CH	C-23, 24, 26, 27, 28
26	0.81 (d, 7.2)	19.8 CH_3	C-24, 25, 27
27	0.79 (d, 7.2)	19.0 CH_3	C-24, 25, 26
28	1.08 (m) 1.25 (m)	23.1 CH_2	C-23, 25
29	0.84 (t, 7.6)	12.2 CH_3	C-24, 28

[a-d]Overlapped signals

Compounds **3.1**-**3.2**, **3.10**-**3.11**, caniojane, and 1,11-*bisepi*-caniojane were evaluated for their *in vitro* activity against *Plasmodium falciparum*, K-1 strain.[3.11] Caniojane exhibited the greatest inhibitory activity with an IC_{50} value of 3.3±0.6 μg/mL, compounds **3.1** and **3.10** showed weaker activity with IC_{50} values 4.1±0.2 and 5.4±1.7μg/mL, respectively (Table 3.7). Compound **3.2**, the C-2 epimer of **3.1,** and compound **3.11** were not active at 10 μg/mL. Only caniojane showed moderate inhibitory activity against *Mycobacterium tuberculosis* H37Ra[3.12] with an MIC value of 25 μg/mL. Compound **3.2** and caniojane showed mild to marginal cytotoxicity against Vero cells[3.13], whereas compounds **3.1**, **3.10** and **3.11** were non-cytotoxic at 50 μg/mL.

Table 3.7. Antiplasmodial, Antituberculosis and Cytotoxic Activities of the Isolates

Compound	antiplasmodial[a]	anti-TB[b]	cytotoxicity[a]
compound **3.1**	4.1±0.2	inactive[d]	non-cytotoxic[f]
compound **3.2**	inactive[c]	inactive[d]	49.4
caniojane	3.3±0.6	25	12.9
1,11-*bisepi*-caniojane	7.9	nd[e]	nd[e]
jatropholone A (**3.10**)	5.4±1.7	inactive[d]	non-cytotoxic[f]
jatropholone B (**3.11**)	inactive[e]	inactive[d]	non-cytotoxic[f]
dihydroartemisinine[f]	(4.0 nM)	-	-
isoniazide[g]	-	0.1	-
kanamycin[g]	-	2.5	-
ellipticine[g]	-	-	0.7±0.2

[a]IC_{50} in μg/mL. [b]MIC in μg/mL. [c]inactive at 10 μg/mL. [d]inactive at 200 μg/mL. [e]nd = not determined. [f]non-cytotoxic at 50 μg/mL. [g]Positive control substance.

References

(3.1) Sutthivaiyakit, S.; Mongkolvisut, W.; Ponsitipiboon, P.; Prabpai, S.; Kongsaeree, P.; Ruchirawat, S. Mahidol, C. *Tetrahedron Lett.* **2003**, *44*, 3637-3640.
(3.2) Mongkolvisut, W.; Sutthivaiyakit, S.; Leutbecher, H.; Mika, S.; Klaiber, I.; Moeller, W.; Roesner, H.; Beifuss, U.; Conrad, J. *J. Nat. Prod.* **2006**, *69*, 1435-1441.
(3.3) Bohlmann, F.; Zdero, C.; King, R. M.; Robinson, H. *Phytochemistry* **1983**, *22*, 1201-1206. (b) Xu, F.; Morikawa, T.; Matsuda, H.; Ninomiya, K.; Yoshikawa, M. *J. Nat. Prod.* **2004**, *67*, 569-576.
(3.4) Achenbach, H.; Benirschke, G. *Phytochemistry* **1997**, *45*, 149-157.
(3.5) Villarreal, A. M.; Dominguez, X. A.; Williams, H. J.; Scott, A. I.; Reibenspies, J. *J. Nat. Prod.* **1988**, *51*, 749-753.
(3.6) Greca, M. D.; Monaco, P.; Previtera, L. *J. Nat. Prod.* **1990**, *53*, 1430-1435.
(3.7) Purushoothaman, K. K.; Chandrasekharan, S.; Cameron, A. F.; Connolly, J. D.; Labbe, C.; Maltz, A.; Rycroft, D. S. *Tetrahedron Lett.* **1979**, *11*, 979-980.
(3.8) Jakupovic, J.; Grenz, M.; Schmeda-Hirschmann, G. *Phytochemistry* **1988**, *27*, 2997-2998.
(3.9) Naengchomnong, W.; Thebtaranonth, Y.; Wiriyachitra, P.; Okamoto, K. T.; Clardy, J. *Tetrahedron Lett.* **1986**, *27*, 2439-2442.
(3.10) (a) Bohlmann, F.; Zdero, C.; King, R. M.; Robinson, H. *Phytochemistry* **1983**, *22*, 1201-1206. (b) Xu, F.; Morikawa, T.; Matsuda, H.; Minomiya, K.; Yoshikawa, M. *J. Nat. Prod.* **2004**, *67*, 569-576.
(3.11) (a) Trager, W.; Jensen, J. B. *Science* **1976**, *193*, 673-675. (b) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother*. **1979**, *16*, 710-718.
(3.12) Collins, L. A.; Franzblau, S. G. *Antimicrob. Agents Chemother*. **1997**, *41*, 1004-1009.
(3.13) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107-1112.