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SESQUITERPENE LACTONES FROM *MICHELIA CHAMPACA**

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Key Word Index—*Michelia champaca*; Magnoliaceae; root bark; sesquiterpene lactones; cycloguaianolide; michampane; michampanolide; 8-acetoxyparthenolide; magnograndiolide.

Abstract—Five sesquiterpene lactones were isolated from *Michelia champaca* root bark. One of these, michampanolide (2,7-dihydroxy-3,7-dimethyl-11-methylene-13-oxatricyclo[8,3,0,0,3,*b*]tridecan-12-one), possessed a new skeleton, while two others, 8-acetoxyparthenolide and magnograndiolide, were isolated for the first time from *M. champaca*.

INTRODUCTION

Several sesquiterpene lactones have been isolated from the root bark of the tree *Michelia champaca* L. [1]. These include the germacrane lactones parthenolide (**1**), costunolide (**2**), dihydroparthenolide (**3**) and micheliolide (**4**) [2].

In this work we isolated compounds **1**, **2** and **5**–**7** from an extract of *M. champaca* root bark. The extract showed insecticidal activity against *Aedes egyptii* larvae. 8-Acetoxyparthenolide (**5**, also named 11,13-dehydrolanuginolide) had earlier been isolated from among others *Michelia lanuginosa* Wall [3], while magnograndiolide (**6**) had been reported as a constituent of *Magnolia grandiflora* L. [4]. Compound **7**, which had not been reported earlier, was named michampanolide.

RESULTS AND DISCUSSION

Extraction of the root bark of *M. champaca* with methylene chloride, followed by chromatography on silica gel, afforded compounds **1**, **2** and **5**–**7**. Parthenolide (**1**) and costunolide (**2**) had been isolated earlier from the root bark of this tree [1]. Our ^{1}H NMR data were in accordance with previously reported data [5]. The full assignments of the ^{1}H and ^{13}C NMR spectral data are reported in Tables 1 and 2, respectively. The assignments are based on 2D H–H and H–C correlations.

The ^{1}H and ^{13}C spectroscopic data of **5** were in accordance with those reported for 8-acetoxyparthenolide by Bruno *et al.* [6], except for the assignment of the protons in the 2-position. These assignments should be interchanged based on our interpretation of the NOESY data, which also showed interaction between H-6, H-9β, Me-14 and H-8 indicating that the acetoxyl group was in the α-position in keeping with the proposed relative configuration shown in formula **5**. This fact was further supported by the position of the chemical shift of H-8 at δ4.54 [3, 7]. By analogy with the assignment of **5**, the relative stereochemistries of the H-9 protons of **1** and the H-2 protons of **2** were assigned as shown in Table 1. The relative stereochemistries of the remaining protons of **1** and **2** were assigned from their respective NOESY spectra.

Our ^{1}H NMR data for magnograndiolide (**6**) were in accordance with those reported earlier [4]. The assignment of its ^{13}C NMR spectrum is shown in Table 2. This assignment was based on data from DEPT, HMQC [8] as well as HMBC [9]. A NOESY experiment [10] confirmed the relative configuration previously proposed [3].

The structure determination of michampanolide (**7**; $C_{15}H_{22}O_4$) was based mainly on an extensive NMR investigation. The ^{1}H and ^{13}C NMR data of **7** are presented in Table 3. The position of the vinylic pair of doublets at δ5.59 and 6.27 as well as the presence of a carbonyl carbon atom at δ169.1 made it obvious that **7** contained a γ-lactone moiety in a sesquiterpene skeleton. The ^{1}H NMR spectrum also contained two methyl singlets at δ1.32 and 1.27, two hydroxyl signals (*s* and *d*) and signals from two vicinal protons bound to oxygen-bearing carbon atoms, H-5 (δ3.59, *dd*) and H-6 (δ4.19, *dd*). Since the signal of H-5 (*dd*) was shown by H–H correlations to be coupled to H-6 and the OH-signal at δ2.58, C-4 must be a quaternary carbon atom. The phase sensitive double quantum filter (DQF) COSY spectrum showed that H-1, H-2 and H-3 as well as H-5, H-6, H-7, H-8 and H-9 formed two separate spin systems.

The assignment of the ^{13}C chemical shifts was achieved through the analysis of the DEPT and HMQC spectra. The positions of the two methyl groups and the quaternary carbon atoms could be determined from the HMBC

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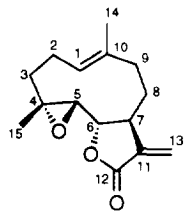

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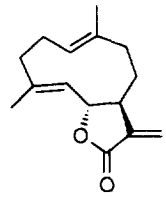

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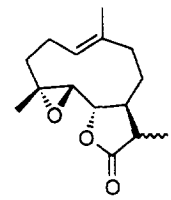

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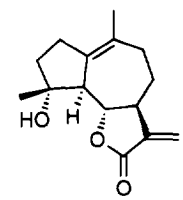

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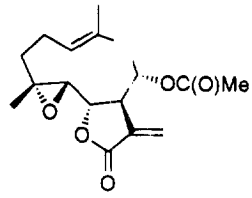

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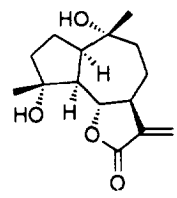

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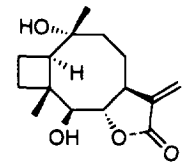

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Table 1. ^{1}H NMR spectral data (δ) of compounds **1** and **2** [the absolute values of the coupling constants (J) are given in Hz]*

H	1	2
1	5.21 *br dd* (12.5, 2.8)	4.84 *ddp* (11.3, 4.7, 1.4)
2α	2.11–2.22 *m*	1.99–2.34 *m*†
2β	2.35–2.46 *m*†	1.99–2.34 *m*†
3α	1.24 *br dt* (6.1, 13.3)	1.99–2.34 *m*†
3β	2.11–2.22 *m*	1.99–2.34 *m*†
5	2.781 *d* (8.9)	4.73 *dp* (9.9, 1.0)
6	3.86 *t* (8.6)	4.56 *dd* (9.9, 8.7)
7	2.780 *dtt* (8.6, 3.5, 1.4)‡	2.56 *dddt* (8.6, 7.6, 1.2, 3.1)‡
8α	2.11–2.22 *m*	1.99–2.34 *m*†
8β	1.72 *dddd* (15.3, 12.5, 8.5, 2.0)	1.68 *m*§
9α	2.11–2.22 *m*	1.99–2.34 *m*†
9β	2.35–2.46 *m*†	2.45 *br dd* (13.4, 6.2)
13	5.62 *d* (3.2)	5.52 *d* (3.2)
13	6.33 *d* (3.7)	6.26 *d* (3.6)
14	1.71 *t* (1.1)	1.42 *d* (0.5)
15	1.30 *d* (0.4)	1.69 *d* (1.5)

*m denotes multiplets consisting of multiline unresolvable patterns of overlapping proton signals.

†The proton signals overlap, but their positions can be distinguished with increasing frequency as follows for **1**: Hβ-9 and Hβ-2; for **2**: Hα-3, Hα-8, Hα-9, Hα-2, Hβ-2 and Hβ-3.

‡The coupling constants given are calculated values.

§Multiline unresolvable pattern.

Table 2. ^{13}C NMR spectral data (δ) of compounds **1**, **2** and **6***

C	1	2	6
1	125.3 *d*	127.1 *d*	49.9 *d*
2	24.2 *t*	26.2 *d*	25.4 *t*
3	36.4 *t*	39.5 *t*	39.4 *t*
4	61.5 *s*	141.5 *s*	80.0 *s*
5	66.4 *d*	127.3 *d*	55.4 *d*
6	82.5 *d*	82.0 *d*	82.8 *d*
7	47.7 *d*	50.5 *d*	47.3 *d*
8	30.7 *t*	28.1 *t*	25.1 *t*
9	41.3 *t*	41.0 *t*	44.0 *t*
10	134.6 *s*†	137.0 *s*	74.9 *s*
11	139.3 *s*†	140.1 *s*	138.5 *s*
12	169.3 *s*	170.5 *s*	169.5 *s*
13	121.3 *t*	119.7 *t*	120.5 *t*
14	17.0 *q*	16.1 *q*	24.3 *q*
15	17.3 *q*	17.4 *q*	23.6 *q*

*The quaternary carbon atoms were unambiguously assigned from H–C COSY long range or HMBC spectra.

†The position of the carbon atom was assigned in analogy with the position of the corresponding carbon atom in **2**.

spectrum (see Table 3). The long-range correlation between Me-15 and C-1, C-3, C-4 and C-5 observed in the HMBC spectrum suggested that the compound must have a germacrane skeleton, cyclized into a skeleton of the bicyclo[0.2.6]decane type. Consequently, **7** is a cycloguaianolide. We suggest that the novel skeleton of michampanolide be termed michampane.

The relative configuration of michampanolide (**7**) was investigated using NOE difference spectroscopy [11] as well as NOESY [10]. The correlations observed between Me-14, Me-15, 5-OH and H-6 on one hand and between 10-OH, H-1, H-5 and H-7 on the other suggest the configuration indicated in Fig. 1.

The *Michelia* species contain many germacranolides, which have most probably been formed via a common biosynthetic pathway [4]. It is likely that both magnograndiolide (**6**) and michampanolide (**7**) are biogenetically derived from parthenolide. The rupture of the epoxide ring in parthenolide (**1**) promoted transannular cyclization. Attack at C-4 and C-5 gave michampanolide (**7**) and magnograndiolide (**6**), respectively, on hydroxylation of the carbonium ion generated at C-10 (Scheme 1).

Table 3. ^1H and ^{13}C NMR spectral data (δ) of compound **7** [the absolute values of the coupling constants (J) are given in Hz]

Atom	^1H	J_{H-H}	^{13}C
1	2.53 *dd*	11.2, 8.4	48.1 *d*
2α	1.95 *ddt*	8.3, 1.6, 10.7	20.0 *t*
2β	2.08 *dq*	9.1, 10.7	
3α	1.80 *dt*	8.0, 10.6	33.4 *t*
3β	1.71 *dddd*	11.0, 9.0, 1.6, 0.6	
4			43.3 *s*
5	3.59 *dd*	9.8, 1.2	84.4 *d*
6	4.19 *dd*	9.8, 7.5	82.2 *d*
7	3.13 *m*		41.1 *d*
8α	2.41 *dddd*	16.8, 12.9, 6.2, 3.8	26.8 *t*
8β	1.49 *dddd*	14.5, 11.0, 4.6, 3.8	
9α	1.66 *dt*	15.2, 4.4	
9β	1.85 *ddd*	15.1, 12.9, 3.6	37.3 *t*
10			73.3 *s*
11			140.8 *s*
12			169.1 *s*
13	6.27 *d*	3.6	122.1 *t*
13	5.59 *d*	3.1	
14	1.32 *s*		29.3 *q*
15	1.27 *s*		14.1 *q*
OH-5	2.58 *d*	1.6	
OH-10	1.57 *s*		

EXPERIMENTAL

General. Mp: uncorr.; optical rotations: $CHCl_3$ soln at 27°; analytical TLC: Merck 5553 Kieselgel 60 aluminium foil plates; MPLC and flash chromatography (FC): Merck Kieselgel 9385. The eluents used for MPLC consisted of hexane–EtOAc mixtures, made up in gradients of increasing polarity. The mixtures were prepared by adding increasing amounts of EtOAc to hexane under stirring, before the mixture was pumped into the column [13].

^1H and ^{13}C NMR: $CDCl_3$ at 400 and 100 MHz, respectively, using the solvent as int. standard. All coupling constants reported for the ^1H NMR spectra were measured after resolution enhancement of the 1D data or from cross peaks in the phase-sensitive DQC COSY spectrum. Spectra simulations were done on a MacIntosh computer using the programme NMR", Version 1.0, from Calleo Scientific Software Publishers. The sample used in the NOE difference experiment was degassed several times using the freeze-pump-thaw cycle before vacuum-sealing the tube. The NOE difference technique was used as described by ref. [11] and the spectra were recorded with the decoupler turned off during pulse and acquisition, preceded by 2.3 sec of irradiation either in or out of resonance. The 2D experiments were performed using standard Bruker software on an Aspect 3000 computer. The NOESY and inverse experiments were carried out at 500 or 600 MHz (^1H). The mixing time in the NOESY experiment was set at 1 sec. The delay time in the HMBC experiment was 50 msec, corresponding to an average coupling constant of 10 Hz ($^2J_{CH}$ and $^3J_{CH}$).

Plant material. Michelia champaca root bark was collected from Uda Peradeniya in the Kandy district of the central province of Sri Lanka. A voucher specimen is deposited in the University Herbarium in Peradeniya.

Extraction and isolation. Dry, ground root bark of *M. champaca* (4.5 kg) was extracted with CH_2Cl_2 (*ca* 10 l) at 26° for two successive 24 hr periods and filtered. Concentration of the combined filtrates *in vacuo* below 40° gave the extract (43 g). This (40 g) was absorbed in silica gel (80 g) and subjected to MPLC on silica gel.

Parthenolide (**1**). Elution of the MPLC column with EtOAc–hexane (1:4), followed by repeated MPLC, using the same solvent mixture, gave plates of **1** (475 mg,

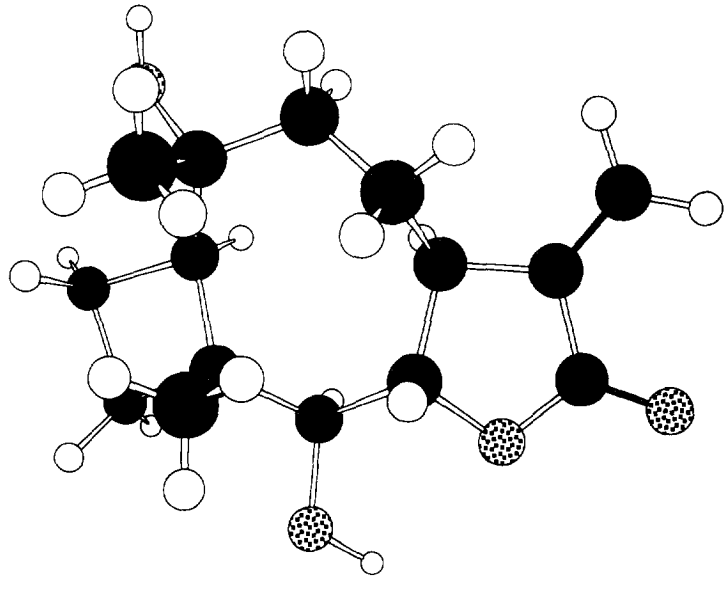
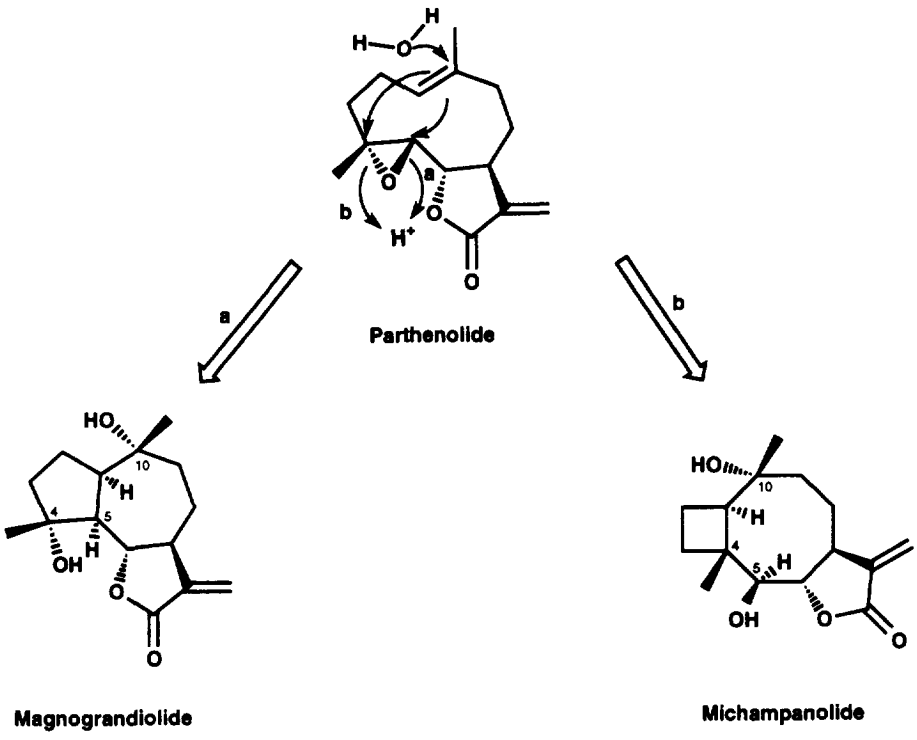


Fig. 1. Computer generated structure of michampanolide (**7**), energy minimized using Tripos force-field [12].



Scheme 1.

recrystallized from EtOAc–hexane); mp 114–115°; $[\alpha]_D$ − 80° (*c* 0.66) (lit. [1]: mp 116.7° and $[\alpha]_D$ − 81.4°); see Tables 1 and 2 for ^1H and ^{13}C NMR data.

Costunolide (**2**). Elution of the MPLC column with EtOAc–hexane (1:19), followed by repeated MPLC, using the same solvent mixture, gave **2** (80 mg); mp 104°; $[\alpha]_D$ + 128° (*c* 0.34) (lit. [1]: mp 106° and $[\alpha]_D$ + 128°); see Tables 1 and 2 for ^1H and ^{13}C NMR data.

8α-Acetoxyparthenolide (**5**). Elution of the MPLC column with EtOAc–hexane (2:3) followed by additional MPLC and FC using the same solvent mixture gave **5**. Recrystallization from EtOAc–hexane gave plates (23 mg); mp 167–168°; $[\alpha]_D$ − 90.4° (*c* 0.40) (lit. [3]: mp 168° (dec.) and $[\alpha]_D$ − 96.5°). The ^1H and ^{13}C NMR data were in accordance with those reported in ref. [6]. The assignments of the H-2 have been interchanged.

Magnograndiolide (**6**). Elution with EtOAc–hexane (3:2) followed by purification by MPLC, using the same solvent mixture, and two successive FC using MeOH–CH_2Cl_2 (1:16.5) gave **6** as a powder (57 mg); mp 173°; $[\alpha]_D$ + 4.16° (*c* 0.48) (lit. [4]: mp 176–177°); UV λ_{max}^{EtOH} nm (log ε): 216 (4.37); see Table 2 for ^{13}C NMR data.

Michampanolide (**7**). Elution of the MPLC column with EtOAc–hexane (4:1) gave a fraction, which on additional MPLC, using the same solvent system, gave **7**. Recrystallization from EtOAc–MeOH gave plates (25 mg); mp 206°; $[\alpha]_D$ − 33° (*c* 0.42); IR ν_{max}^{KBr} cm^{-1}: 3500, 3450, 2980 and 1750; UV λ_{max}^{EtOH} nm (log ε): 216 (4.41); MS *m/z* (rel. int.): 267 (1) $[M + 1]^+$, 248 (0.5), 220 (5), 149 (12), 109 (45), 71 (100) and 55 (98); see Table 3 for ^1H and ^{13}C NMR data. (Found: C, 67.5; H, 8.6. $C_{15}H_{22}O_4$ requires: C 67.6; H, 8.3%).

Opening of the epoxide ring in parthenolide. Parthenolide (200 mg) in CH_2Cl_2 (2 ml) was refluxed with 20% aq. oxalic acid soln for 6 hr. Work-up followed by chromatography gave magnograndiolide (32 mg) and michampanolide (7 mg).

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