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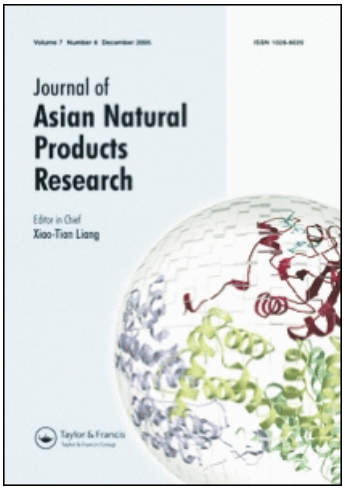

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Wei-Han Zhang[a]; Hong-Mao Zhong[b]; Chun-Tao Che[c]
[a] Hutchison Medipharma Ltd., Shanghai, China [b] Chinese Academy of Sciences, South China Sea Institute of Oceanography, Guangzhou, China [c] School of Chinese Medicine, The Chinese University of Hong Kong, Hong Kong, China

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Cycloartanes from the red alga *Galaxaura* sp.

WEI-HAN ZHANG†, HONG-MAO ZHONG‡ and CHUN-TAO CHE¶*

†Hutchison Medipharma Ltd., Shanghai, China
‡South China Sea Institute of Oceanography, Chinese Academy of Sciences, Guangzhou, China
¶School of Chinese Medicine, The Chinese University of Hong Kong, Hong Kong, China

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Six cycloartane triterpenes have been isolated from the red alga *Galaxaura* sp. The new structures (galaxaurols A–E) were determined to be methyl 3β-hydroxy-23-oxocycloart-24-en-29-oate (**2**), methyl 23(*E*)-3β-hydroxy-25-methoxycycloart-23-en-29-oate (**3**), methyl 23(*E*)-3β-hydroxycycloarta-23,25-dien-29-oate (**4**), 23(*E*)-25-methoxycycloart-23-en-3β,29-diol (**5**), and cycloart-24-en-3β,23α,29-triol (**6**), respectively.

Keywords: *Galaxaura* sp.; Galaxaurol; Cycloartane triterpenes

1. Introduction

Red algae (Rhodophyta) are a rich source of biologically active metabolites from the marine environment [1]. During our search for new chemical entities from marine organisms [2–4], the tropical red alga *Galaxaura* sp. (Family Chaetangiaceae) were collected from the South China Sea and studied for its chemical composition. Since not much attention has been paid to the chemistry of this genus, the chemical constitution of this genus is poorly understood, except that polysaccharides [5] and sterol derivatives [6–8] have been reported from three species of *Galaxaura*. Some of the desmosterols isolated from *Galaxaura marginata* display cytotoxic activities toward cancer cell lines [7,8]. This report describes the isolation and structural elucidation of six triterpenoid metabolites together with three known compounds from a *Galaxaura* sp.

2. Results and discussion

A 95% EtOH extract of the dried samples of *Galaxaura* sp. was concentrated to yield a dark-green residue, which was partitioned between EtOAc and H_2O. Work-up of

*Corresponding author. Tel.: +852-2609-8130. Fax: +852-2603-7203. E-mail: chect@cuhk.edu.hk

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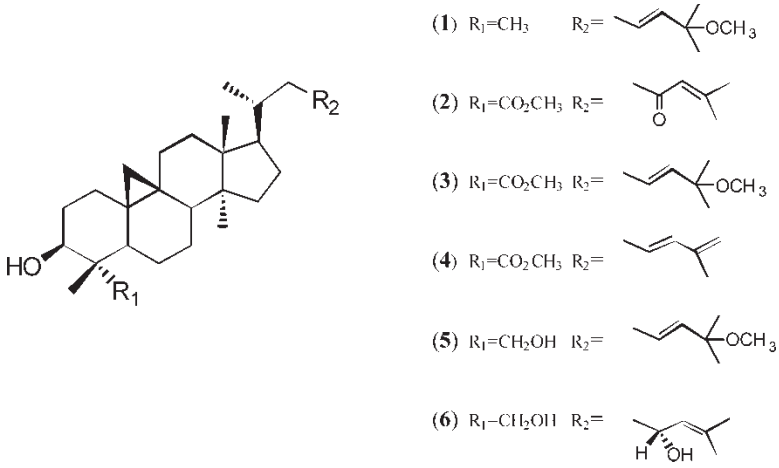
the EtOAc-soluble portion by repeated chromatography on silica gel and RP-18 gel resulted in the purification of six triterpenes (**1**–**6**).



The ^{1}H NMR spectra of **1**–**6** display characteristic signals for tetrasubstituted cyclopropyl methylene at about δ_H 0.4 and 0.6 (d, *J* values of about 4.5 Hz) [9,10]. A doublet of doublets arising from the 3α-H of triterpene also appears at about δ_H 4.0 (*J* values of ca. 4.5 and 11 Hz) in the spectra of all compounds. These isolates are therefore cycloartanes bearing a 3β-OH functional group.

Compound **1** ($C_{31}H_{52}O_2$) displayed NMR and MS properties in good agreement with those reported for 25-methoxycycloart-23-en-3β-ol [10]. A large coupling constant ($J = 15.6$ Hz) observed for H-24 indicated a *trans* orientation of the double bond. Compound **1** was thus identified as 23(*E*)-25-methoxycycloart-23-en-3β-ol.

Galaxaurol A (**2**) showed a molecular ion at *m/z* 484 $[M]^+$ in its EIMS and 31 carbon signals in the ^{13}C NMR spectrum (table1), leading to the assignment of a molecular formula $C_{31}H_{48}O_4$. The presence of an α,β-unsaturated ketone was inferred by UV absorption at 252 nm and the IR absorption band at ν_{max} 1700 cm^{-1}. This assignment was also supported by the HMBC results (figure 1), which showed long-range coupling between δ_H 6.06 (H-24) and δ_C 201.6 (C-23). Comparison of the ^{1}H and ^{13}C NMR data of **2** with those reported for 23-oxocycloart-24-en-3β-ol [11] indicated an identical side-chain structure. A major difference was noted in that the C-29 methyl signal was missing in the spectrum of **2**. Instead, carboxyl (δ_C 177.6) and methoxyl signals [δ_C 51.8; δ_H 3.71 (3H, s)] were observed. This was accompanied by an upfield shift of the C-30 chemical shift to δ_C 9.2, presumably due to the effect of the oxidation state of C-29 [12]. The C-29 signal (δ_C 177.6) showed HMBC long-range correlations with 30-CH_3 (δ_H 1.14) and H-3 (δ_H 4.10, dd, $J = 4.4$, 11 Hz). All available evidence suggested the presence of a methyl ester group on C-29. The relative stereochemistry of **2** was then determined by interpretation of the DIFNOE data. Thus, NOE enhancement was observed between H-3/H-5 (6.5%), confirming the 3β-OH orientation. The NOE observed between H-17/28-CH_3 (3.2%) and H-19/30-CH_3 (2.4%) further led to the assignments of 17α-H and 30β-CH_3, respectively. Hence the structure of galaxaurol A (**2**) was elucidated to be methyl 3β-hydroxy-23-oxocycloart-24-en-29-oate.

The molecular formula of galaxaurol B (**3**) was established as $C_{32}H_{52}O_4$ based on its EIMS and ^{13}C NMR data (table 1). The ^{1}H and ^{13}C NMR results indicated that **3** differs from **2** only

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Table 1. ^{13}C NMR data of **1**–**6**.

Carbon	***1***	***2***	***3***	***4***	***5***	***6***
1	31.9	32.6	31.4	31.4	31.8	33.7
2	30.4	29.7	29.5	29.5	27.9	32.5
3	78.8	77.3	75.4	75.4	80.0	79.4
4	40.5	48.9	54.8	54.8	49.6	46.1
5	47.1	47.6	44.3	44.3	40.1	43.2
6	21.1	23.0	23.0	23.0	20.4	21.2
7	26.4	26.3	25.5	25.5	25.1	27.1
8	47.9	44.4	47.7	47.7	47.8	49.2
9	20.0	19.9	19.9	19.9	19.8	20.6
10	26.1	25.5	25.0	25.0	25.2	25.6
11	26.0	28.3	26.4	26.4	26.3	27.8
12	32.8	31.4	32.6	32.6	32.6	28.8
13	45.3	45.4	45.3	45.3	44.3	46.1
14	48.8	48.6	48.8	48.8	45.1	49.6
15	35.6	35.4	35.4	35.4	35.4	36.1
16	28.0	29.5	28.0	28.1	26.9	26.3
17	51.9	52.6	51.9	52.1	51.8	53.6
18	18.1	18.0	18.0	18.0	18.0	18.4
19	29.9	29.8	29.8	29.8	30.0	30.3
20	36.3	36.3	36.2	36.7	36.1	40.1
21	18.3	19.2	18.3	18.4	18.2	18.7
22	39.3	51.7	39.3	39.7	39.2	45.2
23	128.7	201.6	128.7	129.5	129.0	65.9
24	136.5	124.3	136.6	134.0	136.0	130.7
25	74.9	154.7	74.9	142.2	75.1	132.2
26	26.2	19.4	26.2	114.0	25.9	18.0
27	25.7	27.6	25.7	18.8	25.9	25.8
28	19.3	19.3	19.2	19.3	18.9	19.5
29	25.4	177.6	177.6	177.6	63.0	63.7
30	14.0	9.2	9.2	9.2	10.6	11.4
OCH_3	50.3		50.2		50.0	
$COOCH_3$		51.8	51.9	51.8		

Spectra were recorded at 100 MHz NMR in $CDCl_3$ for **1**–**4**, in $CDCl_3$–CD_3OD for **5**, in $CDCl_3$–DMSO-d_6 for **6**. Values are expressed in ppm downfield from TMS.

in the structure of the side chain between C-22 to C-25. The side chain of **3** was determined to contain Δ^{23} and 25-OCH_3 functional groups by a direct comparison of the NMR data with those of **1** (confirmed by 2D NMR experiments). The HMBC experiment showed long-range correlation signals between the olefinic carbon at δ_C 136.6 (C-24) and two methyl groups at

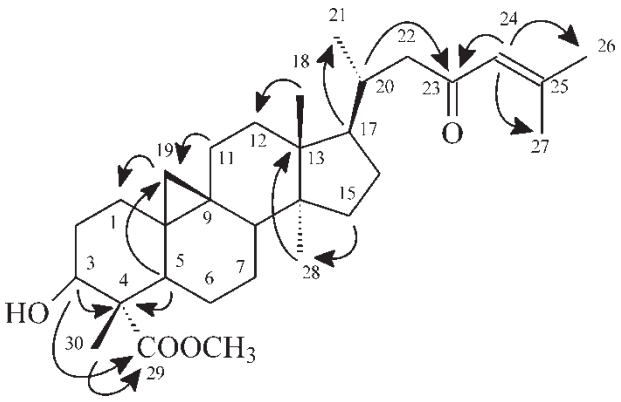

Figure 1. Key HMBC correlations of **2**.

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δ_H 1.26 (26-CH_3) and δ_H 1.27 (27-CH_3). HMBC cross peaks were also observed between the oxygenated carbon at δ_C 74.9 (C-25) and δ_H 5.53 (H-23)/5.39 (H-24). All available evidence thus led to the assignment of methyl 23(*E*)-3β-hydroxy-25-methoxycycloart-23-en-29-oate for galaxaurol B (**3**).

Galaxaurol C (**4**) was assigned a molecular formula $C_{31}H_{48}O_3$ based on EIMS (*m/z* 468) and ^{13}C NMR results. Comparison of the ^{13}C NMR data (table 1) of **4** with those of **3** readily revealed that the two compounds have the same skeleton. The ^1H NMR spectrum of **4** is virtually identical to that of **3**, except that the 26-CH_3 and OCH_3 signals are missing in the former. Instead, a terminal methylene signal (δ_H 4.86) is observed in **4**, which is attributed to 26-CH_2 based on the HMBC results. Thus, long-range coupling is observed between C-24 and H-26/H-27, and between C-25 and H-23/H-24/H-26/H-27. These results led to the assignment of a conjugated diene system on the side chain. Therefore, galaxaurol C (**4**) was determined to be methyl 23(*E*)-3β-hydroxycycloarta-23,25-dien-29-oate.

Galaxaurol D (**5**) has a molecular ion at *m/z* 472 in its EIMS, which is consistent with a molecular formula $C_{31}H_{52}O_3$. The ^1H and ^{13}C NMR spectra (table1) are similar to those of **3**, but some differences include the absence of methoxycarbonyl signals and an upfield shift of 30-CH_3 (δ_H 1.15 in **3** and 0.75 in **5**), and the presence of two geminal carbinol doublet signals at δ_H 3.65 and 3.75 ($J = 11.7$ Hz) and a methylene carbon at δ_C 63.0. The oxygenated methylene group was assigned to C-29 based on the observation of an HMBC cross-peak between C-3 and H-29/30-CH_3, as well as between C-29 and H-3/30-CH_3. The absence of a carbonyl absorption in the IR spectrum of **5** supports this assignment. The relative stereochemistry of **5** was then determined by interpretation of the DIFNOE data. The NOE enhancement (5.8%) between H-3 and H-5 confirmed the 3β-OH orientation. In addition, NOE between H-17/28-CH_3 (3.2%) and H-19/30-CH_3 (1.8%) led to the assignments of 17α-H and 30β-CH_3, respectively. The large coupling constant ($J = 15.6$ Hz) for H-24 indicated a trans orientation of the C-23/C-24 double bond. Based on the above evidence, the structure of galaxaurol D (**5**) was elucidated as 23(*E*)-25-methoxycycloart-23-en-3β,29-diol.

The EIMS of galaxaurol E (**6**) shows a molecular ion at *m/z* 458, which is consistent with a molecular formula $C_{30}H_{50}O_3$. A comparison of the ^{13}C NMR data (table 1) between **6** and **5** revealed that the two compounds have the same skeletal structure. However, the side chain of **6** showed similar NMR characteristics to those of cycloart-24-en-3β,23(*R*),28-triol-3-sulfate [13], suggesting the presence of 23α-OH and Δ^{24} functional groups. This was supported by the observation of HMBC long-range correlations between δ_H 1.80 (H-20) and δ_C 65.9 (C-23), between δ_H 1.65 (26-CH_3)/1.69 (27-CH_3) and δ_C 130.7 (C-24), and between δ_H 4.36 (dt, H-23)/1.65 (26-CH_3)/1.69 (27-CH_3) and δ_C 132.2 (C-25). In addition, the DQF COSY exhibits correlations between δ_H 0.98 (H-22) and 4.36 (dt, H-23). All available evidence led to the structural assignment of cycloart-24-en-3β,23α,29-triol for galaxaurol E (**6**). The relative stereochemistry was determined by DIFNOE results as in other compounds.

During the course of isolation, three known structures were also obtained. They were determined to be (24*R*)-stigmast-5-en-3β-ol [14], 3-*O*-α-L-arabinopyranosyl-28-*O*-[α-L-rhamnopyranosyl(1→4)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl]-hederagenin [15], and palmitic acid, by comparison of their spectral data with published values.

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3. Experimental

3.1 General experimental procedures

NMR spectra were recorded on a JEOL JNM-EX-400-FT-NMR spectrometer. IR spectra were obtained on a Perkin Elmer 16 PC FT-IR spectrometer and mass spectra on a Finnigan TSQ 7000 mass spectrometer. Optical rotations were measured on a Perkin Elmer 241 polarimeter. UV spectra were recorded on a Milton Roy 3000 Array spectrophotometer. Melting points were measured on a Leica Galen III melting point apparatus and are uncorrected.

3.2 Animal material

The red alga *Galaxaura* sp. was collected in 1995 in Xisha Island in the South China Sea. The sample was identified by Professor Liu Xijin (South China Sea Institute of Oceanography, the Chinese Academy of Sciences) at the genus level. A voucher specimen has been deposited in the Research Center of Organic Natural Products Chemistry, Zhongshan University, Guangzhou, China.

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3.3 Extraction and isolation

A dried sample of *Galaxaura* sp. (2.5 kg) was steeped in 95% EtOH (3 × 5 L). The resultant extract was then concentrated and partitioned between EtOAc and H_2O. The EtOAc-soluble fraction (30 g) was subjected to vacuum liquid chromatography, eluting with hexane–ethyl acetate, and then acetone–methanol, to afford 11 fractions. Repeated chromatography of these fractions led to the isolation of **1**–**6**, (24*R*)-stigmast-5-en-3β-ol [14], and palmitic acid. From the water-soluble fraction, 3-*O*-α-L-arabinopyranosyl-28-*O*-[α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl]hederagenin [15] was obtained. The identities of the known compounds were determined by comparison of spectral data with values reported in the literature.

3.3.1 23(*E*)-25-Methoxycycloart-23-en-3β-ol (1). White powder (7 mg); IR (KBr) ν_{max} (cm^{-1}): 3350, 2980, 1620, 1020; ^1H NMR ($CDCl_3$), δ (ppm): 0.33 (1H, d, $J = 3.8$ Hz, H-19b), 0.55 (1H, d, $J = 3.8$ Hz, H-19a), 0.80 (3H, s, Me-30), 0.88 (6H, s, Me-21, Me-28), 0.96 (6H, s, Me-18, Me-29), 1.23 (6H, s, Me-26, Me-27), 3.16 (3H, s, OMe), 3.30 (1H, m, H-3α), 5.30 (1H, m, H-23), 5.39 (1H, d, $J = 15.6$ Hz, H-24); ^{13}C NMR, see table 1; EIMS *m/z* 456 $[M]^+$ (8), 438 (32), 424 (28), 395 (32), 391 (22), 363 (81), 343 (12), 315 (10), 297 (20), 284 (40), 255 (40), 215 (20), 147 (88), 109 (100).

3.3.2 Methyl 3β-hydroxy-23-oxocycloart-24-en-29-oate (galaxaurol A, 2). White powder (26 mg), mp 165–166°C; $[\alpha]_D^{25} + 26$ (*c* 0.038, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 252 nm (2.8); IR (KBr) ν_{max} (cm^{-1}): 3450, 2930, 2870, 1730, 1700, 1620, 1280; ^1H NMR ($CDCl_3$), δ (ppm): 0.38 (1H, d, $J = 4.4$ Hz, H-19b), 0.61 (1H, d, $J = 4.4$ Hz, H-19a), 0.86 (3H, d, $J = 5.8$ Hz, Me-21), 0.89 (3H, s, Me-28), 1.02 (3H, s, Me-18), 1.14 (3H, s, Me-30), 1.88 (3H, s, Me-27), 2.18 (3H, s, Me-26), 3.71 (3H, s, COOMe), 4.10 (1H, dd, $J = 4.4$, 11.2 Hz, H-3α),

6.06 (1H, s, H-24); ^{13}C NMR, see table 1; EIMS *m/z* 484 $[M]^+$ (5), 468 (8), 436 (8), 401 (6), 386 (18), 359 (12), 300 (8), 246 (9), 187 (42), 147 (72), 125 (58), 83 (100); elemental analysis (%): C 76.76, H 10.12; calcd for $C_{31}H_{48}O_4$, C 76.80, H 9.99.

3.3.3 Methyl 23(*E*)-3β-hydroxy-25-methoxycycloart-23-en-29-oate (galaxaurol B, 3). White powder (15 mg), mp 174–175°C; $[\alpha]_D^{25}$ + 34.2 (*c* 0.023, $CHCl_3$); IR (KBr) ν_{max} (cm^{-1}): 3450, 2930, 2870, 1730, 1270, 1080; ^{1}H NMR ($CDCl_3$), δ (ppm): 0.38 (1H, d, J = 3.9 Hz, H-19b), 0.60 (1H, d, J = 3.9 Hz, H-19a), 0.88 (3H, d, J = 6.8 Hz, Me-21), 0.89 (3H, s, Me-28), 0.96 (3H, s, Me-18), 1.15 (3H, s, Me-30), 1.26 (3H, s, Me-26), 1.27 (3H, s, Me-27), 3.15 (3H, s, OMe), 3.71 (3H, s, COOMe), 4.10 (1H, dd, J = 4.4, 11.2 Hz, H-3α), 5.39 (1H, d, J = 15.6 Hz, H-24), 5.53 (1H, m, H-23); ^{13}C NMR, see table 1; EIMS *m/z* 500 $[M]^+$ (5), 485 (3), 482 (3), 468 (16), 450 (36), 359 (8), 300 (12), 284 (40), 147 (77), 109 (100).

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3.3.4 Methyl 23(*E*)-3β-hydroxycycloarta-23,25-dien-29-oate (galaxaurol C, 4). White powder (9 mg), mp 157–158°C; $[\alpha]_D^{25}$ +32.8 (*c* 0.024, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 248 nm (2.5); IR (KBr) ν_{max} (cm^{-1}): 3450, 2940, 2870, 1730, 1440, 1090; ^{1}H NMR ($CDCl_3$), δ (ppm): 0.38 (1H, d, J = 4.4 Hz, H-19b), 0.61 (1H, d, J = 4.4 Hz, H-19a), 0.88 (3H, d, J = 4.3 Hz, Me-21), 0.89 (3H, s, Me-28), 0.96 (3H, s, Me-18), 1.14 (3H, s, Me-30), 1.84 (3H, s, Me-27), 3.71 (3H, s, COOMe), 4.10 (1H, dd, J = 4.8, 12.8 Hz, H-3α), 4.86 (2H, s, H-26), 5.64 (1H, m, H-23), 6.12 (1H, d, J = 15.6 Hz, H-24); ^{13}C NMR, see table 1; EIMS *m/z* 468 $[M]^+$ (8), 450 (16), 386 (6), 359 (8), 300 (4), 281 (16), 147 (56), 109 (100).

3.3.5 23(*E*)-25-Methoxycycloart-23-en-3β,29-diol (galaxaurol D, 5). White powder (20 mg), mp 225–226°C; $[\alpha]_D^{25}$ +30.3 (*c* 0.026, CH_3OH); IR (KBr) ν_{max} (cm^{-1}): 3440, 2940, 1640, 1250, 1070; ^{1}H NMR ($CDCl_3$/CD_3OD), δ (ppm): 0.41 (1H, d, J = 3.8 Hz, H-19b), 0.58 (1H, d, J = 3.8 Hz, H-19a), 0.75 (3H, s, Me-30), 0.86 (3H, s, Me-28), 0.88 (3H, d, J = 8.8 Hz, Me-21), 0.97 (3H, s, Me-18), 1.26 (6H, s, Me-26, Me-27), 3.16 (3H, s, OMe), 3.65 (1H, d, J = 11.7 Hz, H-29a), 3.75 (1H, d, J = 11.7 Hz, H-29b), 4.47 (1H, dd, J = 4.4, 11.2 Hz, H-3α), 5.38 (1H, d, J = 15.6 Hz, H-24), 5.52 (1H, m, H-23); ^{13}C NMR, see table 1; EIMS *m/z* 472 $[M]^+$ (1), 454 (5), 436 (3), 408 (22), 359 (10), 331 (12), 311 (68), 147 (42), 109 (100); elemental analysis (%): C 78.68, H 11.16; calcd for $C_{31}H_{52}O_3$, C 78.75, H 11.09.

3.3.6 Cycloart-24-en-3β,23α,29-triol (galaxaurol E, *6*). White powder (56 mg), mp 213–214°C; $[\alpha]_D^{25}$ +62.2 (*c* 0.037, CH_3OH); IR (KBr) ν_{max} (cm^{-1}): 3430, 2940, 2870, 1638, 1220, 1070; ^{1}H NMR ($CDCl_3$–DMSO-d_6), δ (ppm): 0.38 (1H, d, J = 3.8 Hz, H-19b), 0.57 (1H, d, J = 3.8 Hz, H-19a), 0.69 (3H, s, Me-30), 0.92 (3H, s, Me-28), 0.93 (3H, d, J = 8.2 Hz, Me-21), 1.00 (3H, s, Me-18), 1.65 (3H, s, Me-26), 1.69 (3H, s, Me-27), 3.25 (1H, d, J = 11.8 Hz, H-29a), 3.48 (1H, d, J = 11.8 Hz, H-29b), 4.28 (1H, dd, J = 4.4, 11.2 Hz, H-3α), 4.36 (1H, dt, J = 3.0, 9.3 Hz, H-23), 5.15 (1H, d, J = 9.6 Hz, H-24); ^{13}C NMR, see table 1; EIMS *m/z* 458 $[M]^+$ (1), 440 (2), 422 (6), 404 (6), 389 (12), 331 (10), 311 (20), 295 (20), 255 (18), 147 (32), 109 (100); anal. (%): C 78.44, H 10.87; calcd for $C_{30}H_{50}O_3$, C 78.54, H 10.99.

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