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Lanostanes and friedolanostanes from the pericarp of *Garcinia hombroniana*

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Abstract

The CH_2Cl_2 extract from the pericarp of *Garcinia hombroniana* yielded three 17,14-friedolanostanes [(24*E*)-3α-hydroxy-17,14-friedolanostan-8,14,24-trien-26-oic acid, methyl (24*E*)-3α,23-dihydroxy-17,14-friedolanostan-8,14,24-trien-26-oate and methyl (24*E*)-3α,9,23-trihydroxy-17,14-friedolanostan-14,24-dien-26-oate] and two lanostanes [3β- and 3α-hydroxy-23-oxo-9,16-lanostadien-26-oic acid]. The structure of (24*E*)-3α-hydroxy-17,14-friedolanostan-8,14,24-trien-26-oic acid was determined using spectroscopic and X-ray analyses, while the structures of the other compounds were elucidated solely from analysis of spectroscopic data.
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Keywords: *Garcinia hombroniana*; Guttiferae; Pericarp; Lanostane; 17,14-Friedolanostane

1. Introduction

Garcinia hombroniana (Guttiferae; local name "Waa") is widely distributed in the southern part of Thailand. Bronianone (**1**) is the sole compound isolated from the stem wood (Ollis et al., 1969; Rao et al., 1973). In this paper, we describe the isolation and structural determination of compounds from the pericarp of *G. hombroniana*, which was collected on our campus.

2. Results and discussion

Ground pericarp of *G. hombroniana* was consecutively extracted with hexane, CH_2Cl_2, Me_2CO and MeOH. The CH_2Cl_2 concentrate was mixed with EtOAc. The soluble fraction was chromatographed on silica gel and the crude fractions obtained were further purified by treatment with organic solvent and/or acetylation to afford three new 7,14-friedolanostanes (**2**, **3** and **4**) and two new lanostanes (**5** and **6**). The insoluble fraction yielded additional amounts of **5**. Lanostane **6** was characterized as the corresponding acetate **6a** after acetylation with Ac_2O in pyridine.

For structural elucidation, all ^1H NMR spectra were assigned by ^1H–^1H COSY and/or decoupling experiments and the ^{13}C NMR signals were assigned from DEPT, HMQC and HMBC spectra.

Acid **2** (molecular formula $C_{30}H_{46}O_3$ by HR–MS) exhibited IR absorption bands at 3400 (O–H) and 1695 cm^{-1} (C=O of α,β-unsaturated carboxylic acid). The ^1H NMR spectrum of **2** indicated the presence of one secondary methyl (δ 0.90, *d*, $J = 6.6$ Hz), five tertiary methyls (δ 0.77, 0.84, 0.89, 1.00 and 1.02) and one oxymethine proton (δ 3.46, *brs*). These signals were regarded as being due to a tetracyclic triterpene having a 3α-hydroxy group (Lin et al., 1988; Shiao et al., 1988a,b). The peak at *m/z* 313 (M^+-$C_8H_{13}O_2$) in the EIMS spectrum, together with the signals of the vinylic and methyl protons at δ 6.94 (*qt*, $J = 7.2$ and 1.1 Hz) and δ 1.86 (*d*, $J = 1.1$ Hz), respectively, suggested the structure of the side chain to be [-CH(Me)CH_2CH_2CH=C(Me)COOH)]. The multiplicity of signals in the ^{13}C NMR spectrum as determined by DEPT experiments indicated the presence of 7 methyl carbons (δ 11.7, 14.8, 15.1, 16.1, 18.4, 21.7 and 27.7), 9 methylene carbons (δ 17.1, 22.1, 25.2,

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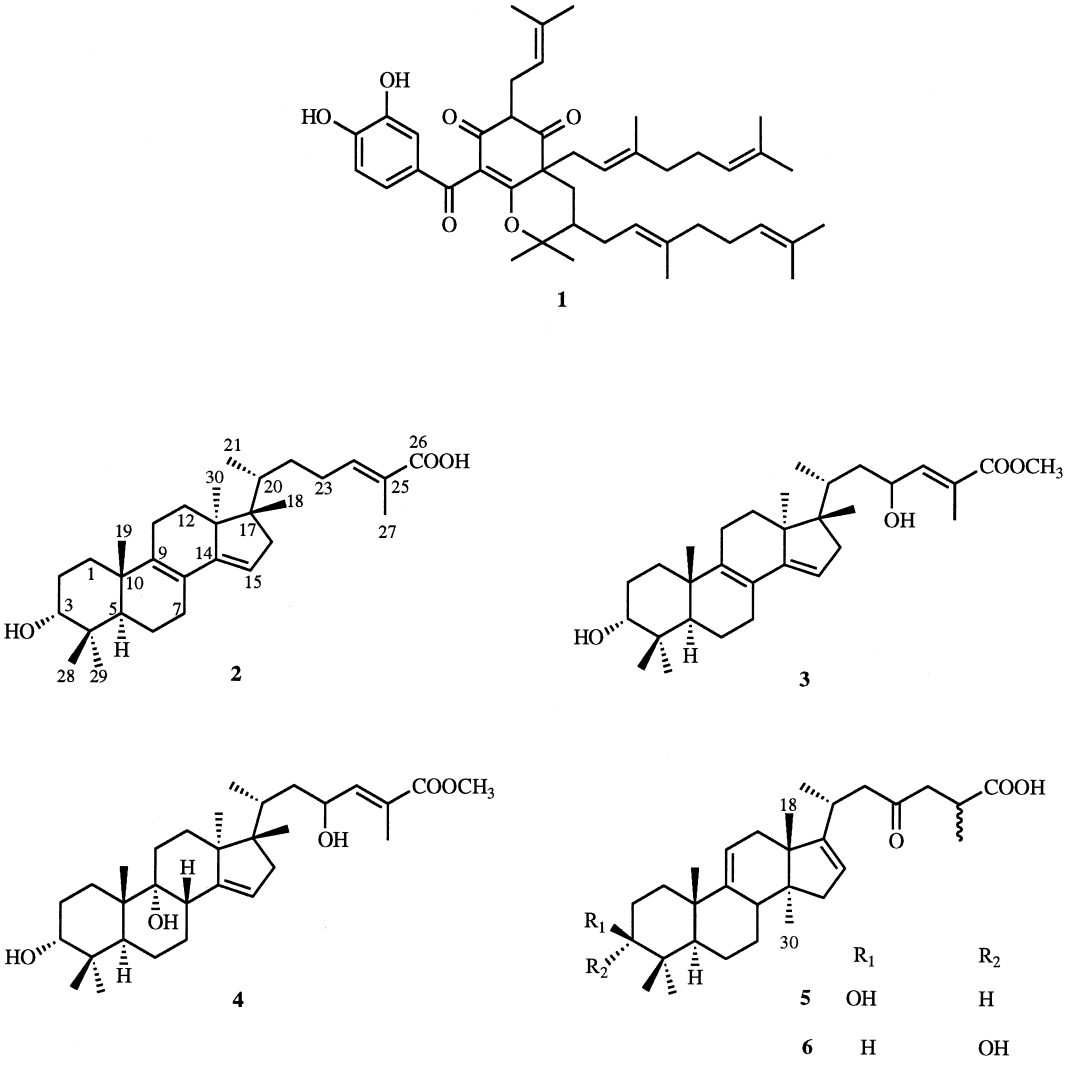
26.1, 26.7, 28.7, 29.5, 31.0 and 44.8), 5 methine carbons (δ 37.4, 43.7, 74.5, 115.0 and 141.8) and 9 quaternary carbons (δ 37.0, 37.2, 47.4, 49.8, 122.1, 127.1, 142.0, 148.0 and 169.6). These NMR spectral data demonstrated that **2** contained one tetrasubstituted double bond and two trisubstituted double bonds, one of which was located in the side chain. Two remaining double bonds were shown to be conjugated based on the strong absorptions at 250 and 262 nm in the UV spectrum. In the HMBC correlation spectrum, Me-21 (δ 0.90, *d*, *J*=6.6 Hz) exhibited correlation peaks with C-13 (δ 47.4), C-16 (δ 44.8) and C-17 (δ 49.8). Moreover, the carbon resonance for Me-21 showed correlations with two methyl proton signals at δ 0.77 (Me-18) and δ 0.84 (Me-30). The vinylic proton at δ 5.27 (*s*, H-15) correlated with C-8 (δ 122.1), C-13 (δ 47.4), C-14 (δ 148.0), C-16 (δ 44.8) and C-17 (δ 49.8), suggesting that the conjugated trisubstituted and tetrasubstituted double bonds were located at C-14/C-15 and C-8/C-9. The β-methine proton signal (H-3) at δ 3.46 demonstrated long-range polarization transfer to carbon signals at δ 37.0 (C-4), 43.7 (C-5), 27.7 (Me-28) and 21.7 (Me-29). Thus, structure **2**, having the same unusual skeleton as found in mariesiic acid A (Hasegawa et al., 1985), was established. Final confirmation of the structure **2** as (24*E*)-3α-hydroxy-17,14-friedolanostan-8,14,24-trien-26-oic acid came from X-ray diffraction analysis. The compound crystallised in the space group C2 (#5) with unit cell dimensions of $a=28.254(1)$ Å, $b=7.241(2)$ Å, $c=13.343(2)$ Å and $\beta=94.580(7)^{o}$, with $Z=4$ and $V=2721.1(6)$ Å^3. The calculated crystal density is 1.110 gcm^{-3}. The absolute structure was not determined; an ORTEP view is shown in Fig. 1. Crystallographic details and data have been deposited with the Cambridge Crystallographic Data Centre.

The methyl ester **3** analysed for $C_{31}H_{48}O_4$ by HR-MS. In the UV spectrum, strong absorptions at 228, 250 and 262 nm indicated that **3** possessed the same chromophore



Scheme 1.

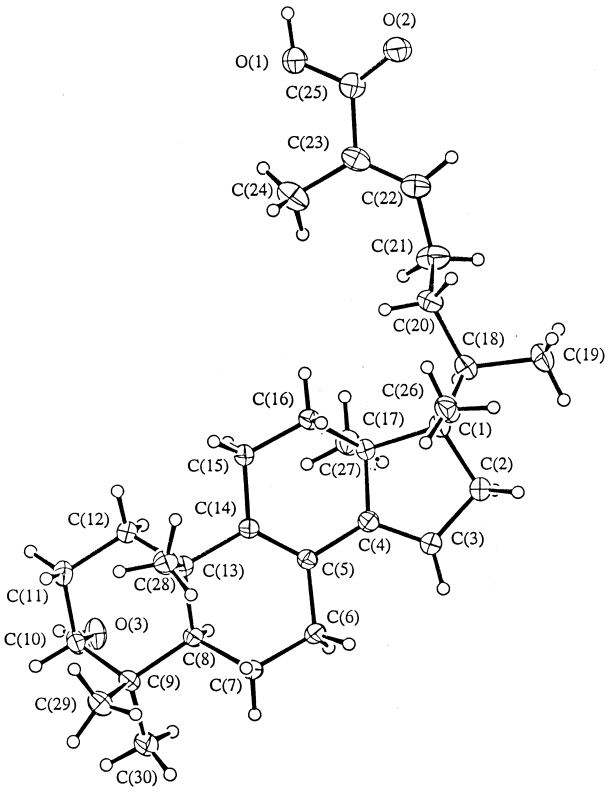


Fig. 1. ORTEP view of **2**.

as **2**. The IR spectrum showed the presence of a hydroxyl group (3400 cm^{-1}) and an α,β-unsaturated ester (1705 cm^{-1}). The ^1H NMR spectrum demonstrated the presence of five angular methyls (δ 0.76, 0.89, 0.91, 0.99 and 1.01), one vinylic methyl (δ 1.87, *d*, $J=1.1$ Hz) and one secondary methyl (δ 0.95, *d*, $J=7.0$ Hz), one olefinic proton (δ 5.27, *s*) and one oxymethine proton (δ 3.45, *brs*). These data were similar to those of **2** except that the signal of the olefinic proton in the side chain appeared as a *qd* (δ 6.72, $J=7.2$ and 1.1 Hz) in **3** but as a *qt* (δ 6.94, $J=7.2$ and 1.1 Hz) in **2**. Two additional signals of an oxymethine proton at δ 4.60 (*ddd*, $J=10.7$, 7.2 and 2.5 Hz) and the methoxy signal at 3.77 (*s*) were observed. Decoupling experiments demonstrated that irradiation of the oxymethine proton at δ 4.60 changed the splitting pattern of olefinic proton at δ 6.72 from *qd* to *q* ($J=1.1$ Hz). These data suggested that **3** had the second hydroxy group at C-23. **3** was, therefore, determined as methyl (24*E*)-3α,23-dihydroxy-17,14-friedolanostan-8,14,24-trien-26-oate.

The initial EIMS of the methyl ester **4** suggested the molecular formula ($C_{31}H_{48}O_4$, M_r 484). Later, it was realized that this result was due to the facile loss of water because of the presence of a tertiary alcohol. However, the electrospray MS indicated that the correct molecular formula was $C_{31}H_{50}O_5$. An IR absorption band at 1710 cm^{-1} and a carbonyl carbon resonance (δ 168.4) indicated the presence of an α,β-unsaturated ester functionality. The ^{13}C NMR spectrum together with data from DEPT experiments showed four sp^2 carbon signals (δ 120.3, 126.9, 144.6 and 153.6) due to two trisubstituted double bonds, two secondary oxygenated carbons (δ 66.7 and 76.0) and one oxygenated quaternary carbon (δ 75.3). The ^1H NMR spectrum revealed signals of two oxymethine protons (δ 3.40, *brs* and δ 4.56, *ddd*, $J=10.8$, 8.0 and 2.2 Hz), two trisubstituted olefinic protons (δ 5.33, *brm* and δ 6.70, *qd*, $J=8.0$ and 1.4 Hz) and seven methyl groups ascribed to five tertiary methyls (δ 0.75, 0.85, 0.91, 0.96 and 1.23), one secondary methyl (δ 0.92, *d*, $J=7.0$ Hz) and one olefinic methyl (δ 1.86, *d*, $J=1.4$ Hz). The above proton signals were similar to those of **3**. Compound **4** was initially assigned to have a structure similar to **3** with a 3α-hydroxy group, one trisubstituted double bond at C-14/C-15 and the same side chain as that of **3**. The position of this trisubstituted double bond was confirmed by analysis of HMBC spectra, which exhibited cross peaks between the olefinic proton, H-15 (δ 5.33, *brm*) and C-8 (δ 39.2), C-13 (δ 49.1), C-16 (δ 44.7) and C-17 (δ 54.0). It was found during acquisition of the NMR spectroscopic data that **4** was transformed to **3** in acidic $CDCl_3$ solution. This indicated that the third hydroxyl group would be located either at C-8 or C-9. The HMBC spectrum suggested that C-9, not C-8, carries this hydroxyl group, due to the cross peaks between the Me-19 (δ 0.91, *s*) and H-8 (δ 2.50–2.40, *m*) signals and the C-9 signal (δ 75.3). In addition, enhancement of the H-8 signal by the irradiation at δ 0.91 (Me-19) in the NOE difference spectrum indicated that the relative stereochemistry between the H-8 and the Me-19 group to be *cis*. The hydroxyl group at C-9 was therefore *trans* to Me-19. Thus triol **4** was elucidated as methyl (24*E*)-3α,9,23-trihydroxy-17,14-friedolanostan-14,24-dien-26-oate.

Acid **5** showed the molecular formula $C_{30}H_{46}O_4$ by HR-MS. The ^1H NMR spectrum exhibited 5 tertiary methyls (δ 0.75, 0.79, 0.79, 0.99 and 1.04), two secondary methyls (δ 1.02, *d*, $J=6.0$ Hz; δ 1.16, *d*, $J=7.0$ Hz) and two olefinic protons at δ 5.29 (*s*) and δ 5.30 (*d*, $J=6.4$ Hz). Moreover, the absence of an olefinic proton signal at δ 6.70–6.90 suggested that **5** was a tetracyclic triterpene carrying a saturated side chain. The presence of a doublet signal of a secondary methyl (Me-27) at δ 1.16 ($J=7.0$ Hz), and the proton signals at δ 2.80–2.75 (*m*, H-25), δ 2.46 (*dd*, $J=20.0$ and 10.0 Hz, H-24) and 2.85 (*dd*, $J=20.0$ and 8.0 Hz, H-24), suggested the presence of a carbonyl functionality at C-23 (Chairul et al., 1990; Barrero et al., 1991). The DEPT experiment demonstrated that both olefinic protons belonged to two trisubstituted double bonds which were shown by the UV spectrum to be unconjugated. The ^{13}C NMR spectrum also exhibited two carbonyl carbon signals (δ 177.1 and 207.8), 6 quaternary carbon signals (sp^3C; δ 39.0, 39.4, 46.4 and 50.7: sp^2C; δ 149.5 and 155.4), 7 methine carbon signals (sp^3C; δ 27.7, 34.3, 39.7, 52.4 and 77.4: sp^2C; δ 113.9 and 120.1), 8 methylene carbon signals (δ 21.0,

27.6, 27.8, 31.0, 36.1, 40.5, 46.3 and 49.2) and seven methyl carbon signals (δ 15.9, 17.0, 19.2, 19.8, 20.9, 22.1 and 28.4). The splitting pattern of 3-H as *dd* with coupling constants of 9.6 and 4.0 Hz implied that the 3-OH was in the equatorial β position (Lin et al., 1988; Shiao et al., 1988a,b). The position of the two double bonds was determined by 2D HMBC correlations (Table 1). The olefinic proton (H-16, δ 5.29, *s*) exhibited cross peaks with the carbon signals of Me-18 and Me-21 while the other olefinic proton signal (H-11, δ 5.30, *d*, $J=6.4$ Hz) demonstrated long-range polarization transfer to the carbon signal of Me-19. On the basis of these spectral studies, the structure of **5** was elucidated as 3β-hydroxy-23-oxo-9,16-lanostadien-26-oic acid, a new lanostane.

The ^{1}H NMR spectrum of the acetate of **6** (**6a**) was almost identical to that of the acetate of **5** (**5a**). However, the signal at δ 4.50 (*dd*, $J=9.6$ and 4.0 Hz, axial H-3) was replaced by a narrow signal at δ 4.68 (equatorial H). **6a** was, therefore, the 3α isomer of **5a**.

Although 17,13- and 17,14-friedolanostanes have been isolated from *Abies mariesii* (Hasegawa et al., 1985, 1987) and *A. firma* (Hasegawa et al., 1987), this is the first isolation of 17,14-friedolanostanes from *Garcinia* species. These rearranged lanostane skeletons are considered to be biosynthesized from a lanostane skeleton (Hasegawa et al., 1987).

3. Experimental

3.1. General

^{1}H NMR: 400 MHz, $CDCl_3$ unless otherwise stated, with TMS as int. standard; ^{13}C NMR: 100 MHz. Acetylation was performed in Ac_2O in pyridine at room temp. overnight.

Table 1
Major HMBC correlations of compound **5**

Proton	HMBC
H-3	38.97 (C-4), 36.06 (C-1), 28.35 (Me-28), 15.91 (Me-29)
H-5	39.66/39.41 (C-8/C-10), 38.97 (C-4), 28.35 (Me-28), 22.05 (Me-19), 15.91 (Me-29)
H-8	149.50 (C-9), 113.91 (C-11)
H-11	50.66 (C-13), 39.66 (C-18), 39.41 (C-10), 30.96 (C-12), 22.05 (Me-19)
H-16	50.66 (C-13), 40.54 (C-15), 20.92 (Me-21), 19.22 (Me-18)
H-20	207.80 (C-23), 155.39 (C-17), 120.14 (C-16)
Me-18	50.66 (C-13), 30.96 (C-12)
Me-19	52.38 (C-5), 39.41 (C-10), 36.06 (C-1)
Me-21	49.15 (C-22)
Me-27	46.30 (C-24), 34.33 (C-25)
Me-28	52.38 (C-5), 38.97 (C-4), 15.91 (Me-29)
Me-29, Me-30	52.38 (C-5), 50.66 (C-13), 46.41 (C-14), 40.54 (C-15), 39.66 (C-8), 39.41 (C-10), 38.97 (C-4), 28.35 (Me-28)

3.2. Plant material

Pericarp of *G. hombroniana* was collected at Prince of Songkla University, Hat Yai campus, Thailand, in 1993. A voucher specimen is deposited in the Herbarium of the Department of Biology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

3.3. Extraction and isolation

Dried and ground pericarps (4.7 kg) were extracted successively with hexane, CH_2Cl_2, Me_2CO and MeOH to give hexane (53 g), CH_2Cl_2 (274 g), Me_2CO (205 g) and MeOH (9570 g) extracts after concentration. The CH_2Cl_2 extract (11.5 g) was partially dissolved in EtOAc. The EtOAc-insoluble fraction afforded **5** (20 mg). The soluble fraction was subjected to CC on silica gel using a stepwise gradient system (hexane–CH_2Cl_2, CH_2Cl_2–EtOAc and EtOAc–MeOH) to give 8 frs. (fr. 1–8). Fr. 5 (565 mg) was separated into two portions with EtOAc. **2** (21 mg) was obtained from the EtOAc-insoluble portion. The soluble portion was fractionated by CC on silica gel using the above gradient system to afford 4 frs. (fr. 5.1–5.4). Fr. 5.2 (247 mg) was further subjected to CC on silica gel using a stepwise gradient system (hexane–CH_2Cl_2, CH_2Cl_2–EtOAc and EtOAc–MeOH) to yield **3** (129 mg). Fr. 5.3 (232 mg) was dissolved in Et_2O. **2** (46 mg) was obtained from the insoluble portion. Fr. 6 (182 mg) was further fractionated into two portions by dissolving in Et_2O. **5** (79 mg) was obtained from the Et_2O- insoluble fr. The soluble fr. was further dissolved in EtOAc to afford the EtOAc-soluble part which was then subjected to acetylation reaction. Upon PLC of the reaction mixture, **6a** (10 mg) was obtained. Fr. 7 (1.134 g) was dissolved in EtOAc; the insoluble fr. (75 mg), upon acetylation and subsequent purification of the mixture by PLC, yielded **5a** (5 mg). Fr. 8 (4.589 g) was separated into two portions with EtOAc. The EtOAc-soluble portion was further dissolved in Et_2O to yield **4** (310 mg) from the insoluble fraction.

3.4. (24E)-3α-Hydroxy-17,14-friedolanostan-8,14,24-trien-26-oic acid (2)

White solid, mp 231–232°C; $[\alpha]_D^{29}$ −59° ($c=0.84$, MeOH); HR–MS *m/z* 454.34598 for $C_{30}H_{46}O_3$ (calcd. 454.34470); MS *m/z* (rel. int.): 454 $[M]^+$ (100%), 439 (26%), 421 (85%), 313 (15%); IR $[\nu]_{KBr}$ cm^{-1}: 3400, 2960, 1695; UV $[\lambda]_{max}^{MeOH}$ nm: 228 (ε 11,000), 244 (ε 11,615), 250 (ε 12,000), 262 (ε 7,692); ^{1}H NMR spectral data: δ 6.94 (1H, *qt*, $J=7.2$ and 1.1 Hz, H-24), 5.27 (1H, *s*, H-15), 3.46 (1H, *brs*, H-3), 2.40–2.23 (3H, *m*, 2xH-7,

H-16), 2.20–1.90 (6H, *m*, 2xH-11, H-12, H-16, 2xH-22), 1.90–1.85 (1H, *m*, H-20), 1.86 (3H, *d*, $J=1.1$ Hz, Me-27), 1.70–1.50 (8H, *m*, 2xH-1, 2xH-2, H-5, 2xH-6, H-12), 1.22–1.10 (2H, *m*, 2xH-23), 1.02 (3H, *s*, Me-19), 1.00 (3H, *s*, Me-28), 0.90 (3H, *d*, $J=6.6$ Hz, Me-21), 0.89 (3H, *s*, Me-29), 0.84 (3H, *s*, Me-30), 0.77 (3H, *s*, Me-18); ^{13}C NMR spectral data ($CDCl_3+d_6$ DMSO): δ 169.6 (C-26), 148.0 (C-14), 142.0 (C-9), 141.8 (C-24), 127.1 (C-25), 122.1 (C-8), 115.0 (C-15), 74.5 (C-3), 49.8 (C-17), 47.4 (C-13), 44.8 (C-16), 43.7 (C-5), 37.4 (C-20), 37.2 (C-10), 37.0 (C-4), 31.0 (C-23), 29.5 (C-1), 28.7 (C-2), 27.7 (C-28), 26.7 (C-22), 26.1 (C-7), 25.2 (C-12), 22.1 (C-11), 21.7 (C-29), 18.4 (C-19), 17.1 (C-6), 16.1 (C-30), 15.1 (C-18), 14.8 (C-21), 11.7 (C-27).

3.5. Methyl (24E)-3α,23-dihydroxy-17,14-friedolanstan-8,14,24-trien-26-oate (**3**)

White powder, mp 112–113°C; $[\alpha]_D^{29}-35°$ (c = 0.28, MeOH); HR–MS *m*/*z* 484.35614 for $C_{31}H_{48}O_4$ (calcd. 484.35526). MS *m*/*z* (rel. int.): 484 $[M]^+$ (42%), 469 (18%), 451 (34%), 313 (35%), 246 (58%), 41 (100%); IR $[\nu]_{KBr}$ cm^{-1}: 3400, 2960, 1705; UV $[\lambda]_{max}^{MeOH}$ nm: 228 (ε 13,583), 250 (ε 10,416), 260 (ε 9,583); ^{1}H NMR spectral data: δ 6.72 (1H, *qd*, $J=7.2$ and 1.1 Hz, H-24), 5.27 (1H, *s*, H-15), 4.60 (1H, *ddd*, $J=10.7$, 7.2 and 2.5 Hz, H-23), 3.77 (3H, *s*, OMe), 3.45 (1H, *brs*, H-3), 2.40–2.30 (3H, *m*, 2xH-7, H-16), 2.25–2.15 (1H, *m*, H-20), 2.15–2.00 (2H, *m*, 2xH-22), 2.00–1.90 (1H, *m*, H-16), 1.87 (3H, *d*, $J=1.1$ Hz, Me-27), 1.80–1.40 (9H, *m*, 2xH-1, H-2, H-5, 2xH-6, 2xH-12, H-11), 1.30–1.05 (2H, *m*, H-2, H-11), 1.01 (3H, *s*, Me-30), 0.99 (3H, *s*, Me-28), 0.95 (3H, *d*, $J=7.0$ Hz, Me-21), 0.91 (3H, *s*, Me-19), 0.89 (3H, *s*, Me-29), 0.76 (3H, *s*, Me-18); ^{13}C NMR spectral data: δ 168.5 (C-26), 148.8 (C-14), 144.5 (C-9), 142.3 (C-24), 127.0 (C-25), 122.8 (C-8), 115.8 (C-15), 75.8 (C-3), 65.8 (C-23), 51.9 (OMe), 50.0 (C-17), 48.0 (C-13), 45.5 (C-16), 44.4 (C-5), 39.2 (C-11), 37.8 (C-10), 37.6 (C-4), 33.4 (C-20), 30.1 (C-1), 29.2 (C-2), 28.0 (C-28), 26.7 (C-7), 25.6 (C-12), 22.7 (C-22), 22.2 (C-29), 18.9 (C-30), 18.1 (C-6), 17.1 (C-19), 15.6 (C-18), 15.2 (C-21), 12.7 (C-27).

3.6. Methyl (24E)-3α,9,23-trihydroxy-17,14-friedolanostan-14,24-dien-26-oate (**4**)

White solid; mp 128–130°C; $[\alpha]_D^{29}-48°$ ($c=0.42$, MeOH); HR–MS *m*/*z* 484.35441 $[M\text{-}H_2O]^+$ for $C_{31}H_{48}O_4$ (calcd. 484.35526); EIMS *m*/*z* (rel. int.): 502 $[M]^+$ (14%), 484 $[M\text{-}H_2O]^+$ (28%), 451 (8%), 313 (31%), 297 (13%), 43 (100%); Electrospray MS *m*/*z* (rel. int.): 1530 $[3M+Na]^+$ (85%), 1027 $[2M+Na]^+$ (100%), 525 $[M+Na]^+$ (6%), 485 $[MH\text{-}H_2O]^+$ (25%); IR $[\nu]_{KBr}$ cm^{-1}: 3495, 2965, 1710; UV $[\lambda]_{max}^{MeOH}$ nm: 228 (ε 13,720); ^{1}H NMR spectral data ($CDCl_3+C_6D_6$): δ 6.70 (1H, *qd*, $J=8.0$ and 1.4 Hz, H-24), 5.33 (1H, *brm*, H-15), 4.56 (1H, *ddd*, $J=10.8$, 8.0 and 2.2 Hz, H-23), 3.75 (3H, *s*, OMe), 3.40 (1H, *brs*, H-3), 2.50-2.40 (1H, *m*, H-8), 2.40–2.30 (1H, *m*, H-16), 2.30–2.20 (1H, *m*, H-20), 2.20–1.92 (2H, *m*, H-5, H-7), 1.92–1.85 (2H, *m*, H-1, H-2), 1.86 (3H, *d*, $J=1.4$ Hz, Me-27), 1.83–1.75 (2H, *m*, H-11, H-16), 1.75–1.65 (1H, *m*, H-22), 1.65–1.55 (3H, *m*, H-2, H-11, H-12), 1.54–1.47 (2H, *m*, H-6, H-12), 1.45-1.35 (2H, *m*, H-6, H-7), 1.23 (3H, *s*, Me-30), 1.15–1.05 (2H, *m*, H-1, H-22), 0.96 (3H, *s*, Me-28), 0.92 (3H, *d*, $J=7.0$ Hz, Me-21), 0.91 (3H, *s*, Me-19), 0.85 (3H, *s*, Me-29), 0.75 (3H, *s*, Me-18); ^{13}C NMR spectral data ($CDCl_3+C_6D_6$): δ 168.4 (C-26), 153.6 (C-14), 144.6 (C-24), 126.9 (C-25), 120.3 (C-15), 76.0 (C-3), 75.3 (C-9), 66.7 (C-23), 54.0 (C-17), 51.8 (OMe), 49.1 (C-13), 44.7 (C-16), 42.1 (C-10), 39.2 (C-8), 39.1 (C-22), 39.0 (C-5), 37.5 (C-4), 33.0 (C-20), 29.6 (C-11), 29.0 (C-12), 28.5 (C-28), 25.7 (C-7), 25.1 (C-2), 23.6 (C-1), 22.0 (C-29), 20.8 (C-6), 19.5 (C-30), 16.3 (C-19), 15.3 (C-18), 15.1 (C-21), 12.7 (C-27).

3.7. 3β-Hydroxy-23-oxo-9,16-lanostadien-26-oic acid (**5**)

White powder, mp 218–220°C; $[\alpha]_D^{29}+58°$ ($c=0.34$, MeOH); HR–MS *m*/*z* 470.34061 for $C_{30}H_{46}O_4$ (calcd. 470.33960). MS *m*/*z* (rel. int.): 470 $[M]^+$ (17%), 437 (15%), 313 (79%), 43 (100%); IR $[\nu]_{KBr}$ cm^{-1}: 3560, 3350, 2940, 1710; ^{1}H NMR spectral data: δ 5.30 (1H, *d*, $J=6.4$ Hz, H-11), 5.29 (1H *s*, H-16), 3.20 (1H, *dd*, $J=9.6$ and 4.0 Hz, H-3), 2.85 (1H, *dd*, $J=20.0$ and 8.0 Hz, H-24), 2.80–2.75 (1H, *m*, H-25), 2.68 (1H, *dd*, $J=18.0$ and 6.0 Hz, H-22), 2.65–2.62 (1H, *m*, H-20), 2.49 (1H, *dd*, $J=$ 18.0 and 10.0 Hz, H-22), 2.46 (1H, *dd*, $J=20.0$ and 10.0 Hz, H-24), 2.40–2.28 (2H, *m*, H-8, H-12), 2.07 (1H, *d*, $J=$ 15.2 Hz, H-15), 1.82 (1H, *dd*, $J=15.2$ and 3.6 Hz, H-15), 1.78–1.57 (5H, *m*, 2xH-2, H-6, H-7, H-12), 1.57–1.28 (4H, *m*, 2xH-1, H-6, H-7), 1.16 (3H, *d*, $J=7.0$ Hz, Me-27), 1.04 (3H, *s*, Me-19), 1.02 (3H, *d*, $J=6.0$ Hz, Me-21), 0.99 (3H, *s*, Me-28), 0.82 (1H, *dd*, $J=6.0$ and 2.0 Hz, H-5), 0.79 (6H, *s*, Me-29, Me-30), 0.75 (3H, *s*, Me-18); ^{13}C NMR spectral data: δ 207.8 (C-23), 177.1 (C-26), 155.4 (C-17), 149.5 (C-9), 120.1 (C-16), 113.9 (C-11), 77.4 (C-3), 52.4 (C-5), 50.7 (C-13), 49.2 (C-22), 46.4 (C-14), 46.3 (C-24), 40.5 (C-15), 39.7 (C-8), 39.4 (C-10), 39.0 (C-4), 36.1 (C-1), 34.3 (C-25), 31.0 (C-12), 28.4 (C-28), 27.8 (C-7), 27.7 (C-20), 27.6 (C-2), 22.1 (C-19), 21.0 (C-6), 20.9 (C-21), 19.8 (C-30), 19.2 (C-18), 17.0 (C-27), 15.9 (C-29).

3.8. 3β-Acetoxy-23-oxo-9,16-lanostadien-26-oic acid (**5a**)

White solid; mp 144–145°C; HR–MS *m*/*z* 512.35090 for $C_{32}H_{48}O_5$ (calcd. 512.35016); MS *m*/*z* (rel. int.): 512 $[M]^+$ (4%), 480 (2%), 355 (12%), 307 (4%), 43 (100%); ^{1}H NMR spectral data: δ 5.29 (1H, *d*, $J=6.4$ Hz, H-11), 5.21 (1H, *s*, H-16), 4.50 (1H, *dd*, $J=9.6$ and 4.0 Hz, H-3), 3.01–2.90 (1H, *m*, H-25), 2.85 (1H, *dd*, $J=18.0$ and 8.0

Hz, H-24), 2.73–2.63 (1H, *m*), 2.65 (1H, *dd*, $J=18.0$ and 6.0 Hz, H-22), 2.50 (1H, *dd*, $J=18.0$ and 10.0 Hz, H-22), 2.46 (1H, *dd*, $J=18.0$ and 6.0 Hz, H-24), 2.39–2.31 (2H, *m*), 2.06 (3H, *s*, OCMe), 1.88–1.40 (10H, *m*), 1.04–1.25 (3H, *m*), 1.20 (3H, *d*, $J=7.0$ Hz, Me-27), 1.08 (3H, *s*, Me-19), 1.04 (3H, *d*, $J=8.0$ Hz, Me-21), 0.90 (3H, *s*, Me-28), 0.87 (3H, *s*, Me-29), 0.79 (3H, *s*, Me-30), 0.75 (3H, *s*, Me-18).

3.9. 3α-Acetoxy-23-oxo-9,16-lanostadien-26-oic acid (**6a**)

White solid; mp 140–141°C; HR–MS *m*/*z* 512.35131 for $C_{32}H_{48}O_5$ (calcd. 512.35016); MS *m*/*z* (rel. int.): 512 $[M]^+$ (4%), 480 (2%), 355 (12%), 43 (100%); IR$[\nu]_{KBr}$ cm^{-1}: 3570, 2780, 1730, 1690; ^1H NMR spectral data: δ 5.29 (1H, *d*, $J=6.4$ Hz, H-11), 5.21 (1H, *s*, H-16), 4.68 (1H, *s*, H-3), 3.01–2.90 (1H, *m*, H-25), 2.85 (1H, *dd*, $J=18.0$ and 8.0 Hz, H-24), 2.73–2.63 (1H, *m*), 2.65 (1H, *dd*, $J=18.0$ and 6.0 Hz, H-22), 2.50 (1H, *dd*, $J=18.0$ and 10.0 Hz, H-22), 2.46 (1H, *dd*, $J=18.0$ and 6.0 Hz, H-24), 2.41–2.30 (2H, *m*), 2.07 (3H, *s*, OCMe), 2.00–1.40 (10H, *m*), 1.04–1.23 (3H, *m*), 1.20 (3H, *d*, $J=7.0$ Hz, Me-27), 1.08 (3H, *s*, Me-19), 1.04 (3H, *d*, $J=8.0$ Hz, Me-21), 0.90 (3H, *s*, Me-28), 0.87 (3H, *s*, Me-29), 0.79 (3H, *s*, Me-30), 0.75 (3H, *s*, Me-18).

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References

Barrero, A.F., Sanchez, J.F., Alvarez-Manzaneda, E.J., Dorado, M.M., Haidour, A., 1991. Endoperoxide diterpenoids and other constituents from *Abies marocana*. Phytochemistry 30, 593–597.

Chairul, T., Tokuyama, T., Nishizawa, M., Shiro, M., Tokuda, H., Hayashi, Y., 1990. Malonate half-esters of homolanostanoid from an Asian *Ganoderma* fungus. Phytochemistry 29, 923–928.

Hasegawa, S., Miura, T., Hirose, Y., Iitaka, Y., 1985. A new rearranged lanostanoid, mariesii acid A, from the seeds of *Abies mariesii*. Chemistry Letters 1589–1592.

Hasegawa, S., Miura, T., Kaneko, N., Hirose, Y., Iitaka, Y., 1987. Further new rearranged lanostanoids from the seeds of *Abies mariesii* and *A. firma*. Tetrahedron 43, 1775–1784.

Lin, L.-J., Shiao, M.-S., Yeh, S.-F., 1988. Triterpenes from *Ganoderma lucidum*. Phytochemistry 27, 2269–2271.

Ollis, W.D., Redmen, B.T., Sutherland, I.O., Jewers, K., 1969. Constituent of bronianone. Journal of Chemical Society (D) 15, 879–880.

Rao, A.V.R., Ventakaraman, K., Yemul, S.S., 1973. Structure of Bronianone from *Garcinia hombroniana*. Tetrahedron Letters 50, 4981–4982.

Shiao, M.-S., Lin, L.-J., Yeh, S.-F., 1988a. Triterpenes in *Ganoderma lucidum*. Phytochemistry 27, 873–875.

Shiao, M.-S., Lin, L.-J., Yeh, S.-F., 1988b. Triterpenes from *Ganoderma lucidum*. Phytochemistry 27, 2911–2914.