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Lanostanes and friedolanostanes from the bark of *Garcinia speciosa*

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Abstract

The $CHCl_3$ extract of the bark of *Garcinia speciosa* contained four 17,14-friedolanostanes and five lanostanes as well as friedelin and common plant constituents. The friedolanostanes were the previously known methyl ester of (24*E*)-3α,23α-dihydroxy-17,14-friedolanostan-8,14,24-trien-26-oic acid and the methyl esters of three hitherto unknown acids, 3α-hydroxy-16α,23α-epoxy-17,14-friedolanostan-8,14,24-trien-26-oic acid, 3α,23α-dihydroxy-8α,9α-epoxy-17,14-friedolanostan-15-oxo-24-en-26-oic acid and 3α,23α-dihydroxy-17,14-friedolanostan-15-oxo-8(14),24-dien-26-oic acid. New lanostanes were 3β,9α-dihydroxylanost-24-en-26-al and the methyl ester of 3β-hydroxy-23-oxo-9,16-lanostadien-26-oic acid. Structures were established by analysis of spectroscopic data. In the case of the lanostanes the previously unassigned C-25 stereochemistry was shown to be 25*R* by X-ray analysis of 3β-hydroxy-23-oxo-9,16-lanostadien-26-oic acid. In the case of the friedolanostanes the configuration at C-23 was established as 23*R*, identical with the absolute configuration at C-23 of mariesiic acids A and B.

Keywords: *Garcinia speciosa*; Guttiferae; 17,14-Friedolanostanes; Lanostanes

1. Introduction

Characteristic constituents of *Garcinia* species (Guttiferae, Clusioideae) are prenylated xanthones; however, a recent report (Rukachaisirikul et al., 2000) described the isolation of two new lanostanes and three new friedolanostanes from the pericarp of *Garcinia hombroniana* Pierre. We now report isolation from the bark of *Garcinia speciosa* Wall., a tree indigenous to Thailand and Myanmar, of four friedolanostanes **1–4** and five lanostanes **5a–d** and 6. Stigmasterol, β-sitosterol, their 3-0-acetates and friedelin were also found.

2. Results and discussion

Extraction of the bark of *Garcinia speciosa* Wall. with $CHCl_3$, chromatography of the concentrate over Si gel and rechromatography of the various fractions or purification by TLC afforded four friedolanostanes **1–4** and five lanostanes **5a–d** and **6** in addition to friedelin, sitosterol and its acetate, stigmasterol and its acetate, 24-ethyl-3-oxocholest-4-ene and 24-ethyl-3-oxocholesta-4,22-diene. Friedolanostane **1** as well as lanostanes **5a** and **5d**, the latter characterized only in the form of its acetate **5c**, had been reported earlier from *Garcinia hombroniana* (Rukachaisirikul et al., 2000) but without specification of the stereochemistry at C-23 (in the case of **1**) and C-25 (in the case of **5a** and **5d**).

Structures were established by ^1H and ^{13}C NMR spectrometry using ^1H-^1H COSY, NOESY and/or decoupling experiments with the assignments in Table 1 deduced from DEPT, HSQC and HMBC. In the case of

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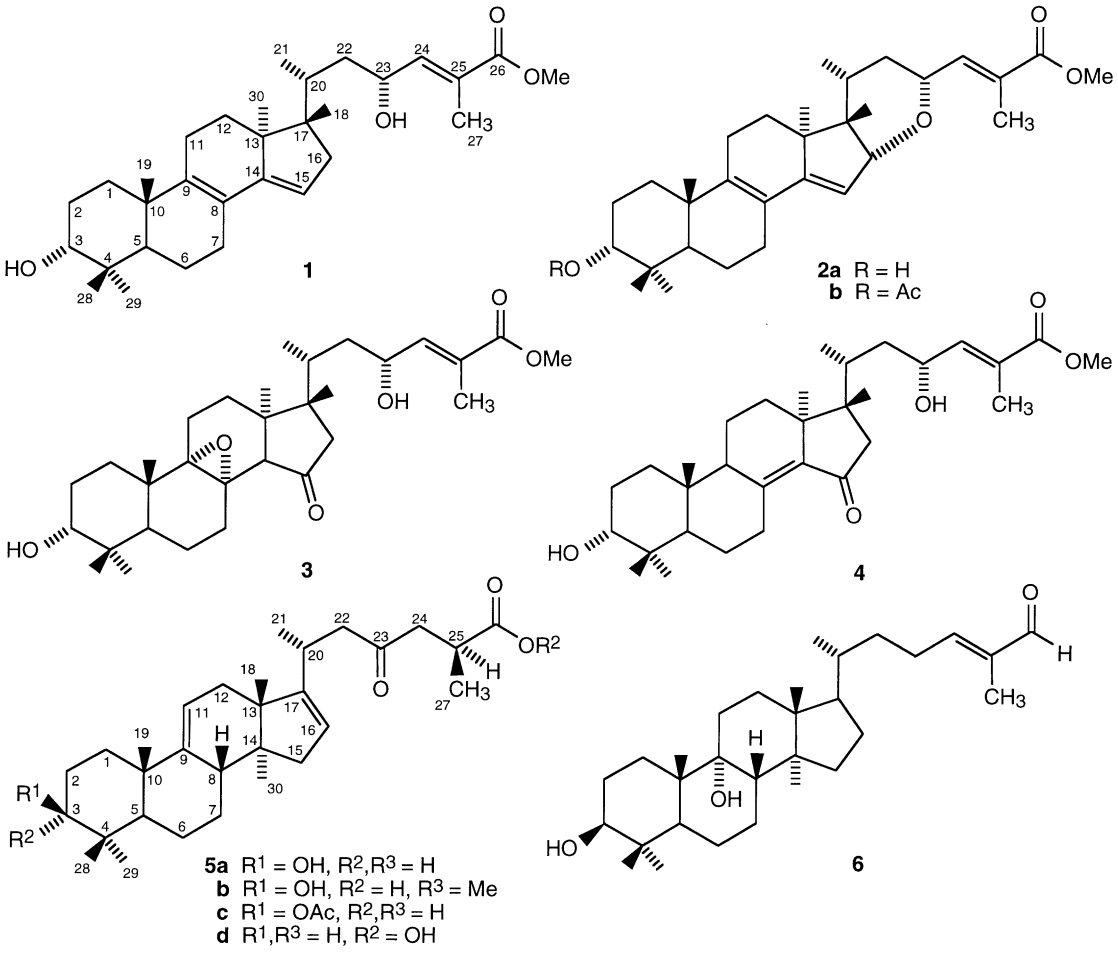

1 the ^{1}H and ^{13}C NMR spectrum data corresponded to those reported earlier (Rukachaisirikul et al., 2000), and since the coupling constants involving H-23 were essentially identical with those of **2**–**4** (*vide infra*) the previously unspecified C-23 stereochemistry was assigned as 23*R*.

Its ^{1}H and ^{13}C NMR spectra, the latter listed in Table 1, established that friedolanostane **2a**, further characterized as a monoacetate **2b**, had the same carbon skeleton as friedolanostane **1**, with conjugated double bonds joining C-8 and C-9, and C-14 and C-15, respectively. However, C-16 in ring D was now oxygenated, the C-16 triplet of **1** near δ 45 having been replaced by a doublet at δ 89.29 and, as further evidenced by the presence of a new doublet at δ 3.89 indicating the presence of a hydroxyl function, correlated with it. Simultaneously the signals attributed to H-23 resp C-23 of **1** had downfield shifts from δ 4.53 and δ 66.6 to δ 4.25 and δ 74.25. This, together with the empirical formula $C_{31}H_{48}O_4$ from mass spectrometry, indicated the presence of an oxygen bridge linking C-16 and C-23. Coupling constants and NOESY experiments demonstrated that the new tetrahydropyran ring was in the chair conformation with H-16 and H-23 both axial, β-orientated and *cis*, i.e. 16*R*, 23*R*. In this conformation the coupling constants involving H-23β (10.9, 8.1 and 2.4 Hz) did not differ significantly from those observed earlier by Rukachaisirikul et al. and by us for H-23 of **1** (10.7, 7.5, 2.5 Hz), thus leading to the conclusion that the C-23 stereochemistry of **1** was identical with that of **2a**,**b** or 23*R* and also identical with the 23*R* configuration deduced earlier for the friedolanostanes methyl maresiates A and B from the seeds of *Abies maresii* (Hasegawa et al., 1985, 1987).

In friedolanostane **3** which had the same C-20–C-27 side chain as **1** and **2a**,**b** and the same stereochemistry at C-23, the C-9, C-10 double bond of **1** and **2a**,**b** was epoxidized, with C-8 and C-9 upfield (compared with 1 and 2a,b) at δ 80.23 and 64.76 and the neighboring carbon atoms C-7, C-10 and C-11 experiencing the shifts (Table 1) expected from epoxidation of the double bond. Ring D was a cyclopentanone, the carbonyl group (C=O at δ 220.00) being located on C-15 because of the presence of three relatively low field signals caused by three adjacent protons in the ^{1}H NMR spectrum. Two of these were due to mutually coupled H-16a and H-16b at δ 1.66 and δ 1.91 (J's = 18.3 Hz) attached to C-16 represented in the ^{13}C NMR spectrum by a

Table 1
^{13}C NMR spectral data of compounds **2a,b**, **3**, **4**, **5b,d** and **6** ($CDCl_3$)

C	2a[a]	2b[a]	3[b]	4[b]	5b[a]	5d[a]	6[a]
1	29.06	29.91	29.98	32.14	36.09	30.49	29.50
2	25.41	23.09	26.23	25.58	27.72	25.60	27.31
3	75.60	77.66	74.74	75.73	78.86	76.27	78.61
4	37.52	36.71	37.43	37.60	39.11	37.86	38.79
5	44.09	45.33	43.02	47.88	52.43	46.66	45.17
6	17.85	17.80	21.95	22.58	21.20	21.10	21.38
7	26.36	26.43	33.90	28.92	27.96	27.92	23.72
8	122.50	122.65	80.23	152.90	39.88	39.99	40.49
9	145.91	145.90	64.76	49.00	149.43	149.44	77.57
10	37.98	37.97	50.88	41.56	39.59	39.57	42.62
11	22.24	22.24	40.27	17.86	114.41	114.15	27.92
12	29.85	29.88	30.88	30.07	31.13	31.13	29.12
13	48.02	48.07	46.71	45.66	50.93	50.92	45.76
14	155.59	155.55	58.25	138.28	46.59	46.71	47.48
15	115.32	115.63	220.00	207.59	40.76	40.75	33.76
16	89.29	89.27	42.07	52.28	120.27	120.32	28.02
17	45.81	45.87	43.91	44.41	155.67	155.65	50.25
18	25.02	24.69	20.77	16.33	19.34	19.37	14.61
19	18.91	18.85	14.48	21.05	22.13	21.93	16.74
20	39.06	39.05	34.94	33.39	27.96	28.04	35.90
21	16.37	16.42	14.78	15.36	21.09	21.10	18.44
22	36.32	36.35	39.19	39.18	49.47	49.45	34.80
23	74.25	74.27	66.65	66.54	208.16	208.22	25.96
24	142.08	142.11	144.37	144.30	46.61	46.36	155.58
25	127.69	127.71	126.91	127.11	34.46	34.33	139.09
26	168.38	168.40	168.49	168.40	176.33	180.54	195.46
27	12.95	13.00	12.75	12.70	17.06	16.86	9.16
28	22.10	21.74	28.45	28.47	15.65	22.53	15.40
29	27.93	27.55	27.36	22.22	28.22	28.34	28.27
30	22.37	22.47	22.36	16.21	19.89	19.92	18.28
OMe	51.72	51.78	51.99	51.98	51.85		
Acetate		170.84, 21.30					

[a] 125 MHz.
[b] 75 MHz.

signal at δ 42.07; the third was the singlet of a proton (H-14) at δ 3.46 attached to C-14 at δ 58.25 and *cis* to the α-oriented C-30 methyl at δ 0.93 (carbon signal at δ 22.36) as shown by the NOESY spectrum. The unusual chemical shift of the H-14 signal might be rationalized if the C-9,10 epoxide were α-orientated as depicted in formula **3** as the result of the favored approach (model) of an oxidizing agent from the *alpha* side. If this were so, however, it is difficult to explain the chemical shift and coupling constants of H-11α, with a *ddd* (*J*'s ~ 11, 11 and 10 Hz) at δ 3.05 clearly coupled to H-11β at δ 2.3 (*J*'s = 11, 6 Hz), H-12β at 2.44 (*J*'s = 12.5, 10) and H-12α near δ 1.5, unless ring C were somewhat distorted from the half-chair conformation. Since the substance could not be obtained in crystalline form verification of this supposition by X-ray analysis was not possible.

Friedolanostane **4**, which contained the usual side chain on C-17 and five methyl singlets was an α β-unsaturated cyclopentenone (C=O signal at δ 207.59) containing no vinylic hydrogen other than H-24 and a conjugated double bond linking C-8 at δ 152.90 and C-14 at δ 138.28. The mutually coupled (*J* = 18.3 Hz) signals of H-16a and H-16b on C-16 at δ 52.28 appeared at δ 2.36 and δ 2.04, respectively. Since the molecular formula was $C_{31}H_{48}O_5$, and the ^{13}C NMR spectrum contained no frequencies in the C-O region other than those of C-3 at δ 75.73 and C-23 at δ 66.54, and since the H-3 and H-23 signals appeared at δ 3.43 and δ 4.56 as usual, a *ddd* (*J* = 14, 3.8 and 2.2 Hz) at δ 4.22 attached to a carbon at δ 28.92 initially seemed anomalous, but could eventually be traced (model) to H-7β in the plane of the double bond and the oxygen of the α,β-unsaturated ketone which was geminally coupled to H-7α at 1.76 and vicinally coupled to H-6α,β within a five proton multiplet near δ 1.4.

As mentioned earlier lanostanes **5a** and **5d**, the latter characterized only in the form of its acetate 5c, had been reported previously from *Garcinia hombroniana* (Rukachaisirikul et al., 2000) but the configuration at C-25 was not specified. Isolated from *Garcinia speciosa* were not only **5a** and **5d**, but also **5b** and **5c**. ^{13}C NMR spectra of **5b–d** are listed in Table 1. The results of an X-ray analysis illustrated in Fig. 1 demonstrated that its C-25 stereochemistry, and by implication the C-25 stereochemistry of its congeners was 25*R*. The remaining new lanostane was aldehyde **6**, whose structure was deduced from the empirical formula and the spectroscopic data listed in the Experimental section and in Table 1.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded at ambient temperature on a Bruker AMC instrument operating at 300.13 and 75.47 MHz, or on a Bruker DRX instrument operating at 500 and 125 MHz, respectively. EI mass spectra were measured on a Hitachi Perkin-Elmer RMV-6M instrument. HRMS spectra were acquired using + FAB ionization with Xe gas at GKV on a KRATOS Concept III 2 sector mass spectrometer. The accelerating voltage was 8 KV. Rotations were determined using a Polarotronic Universal Schmidt and Haensch polarimeter. Si gel for chromatograpy was Si gel 60 (0.2–0.5 mm) Merck, for analytical and preparative TLC Si gel G 60 GF 254 Merck.

3.2. Plant material

Garcinia speciosa Wall. (Guttiferae) was collected in Narathiwat Province, Southern Thailand, in August 2002. A voucher specimen was deposited at the Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

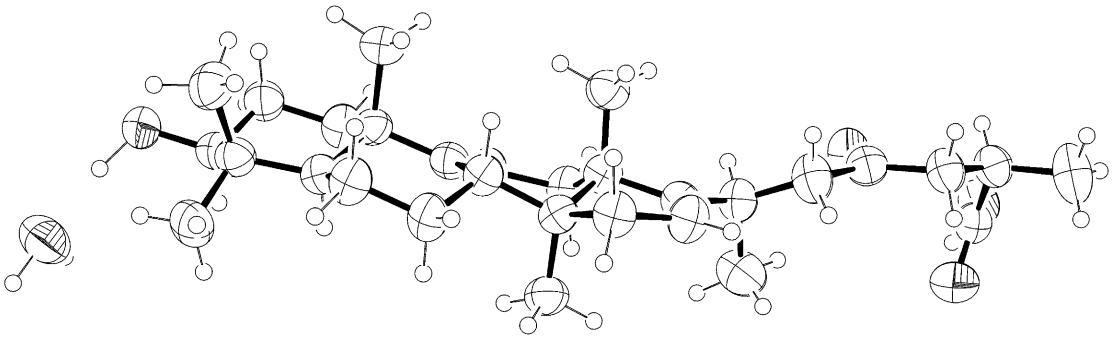

Fig. 1. ORTEP view of lanostane **5a**.

3.3. Extraction and isolation

Dried and powdered bark of *G. speciosa* (2.3 kg) was percolated by $CHCl_3$ to exhaustion (12 l). Evaporation of the $CHCl_3$ solution at reduced pressure gave a crude viscous $CHCl_3$ extract (77 g) which was applied to a silica gel 60 column (500 g) and eluted with petrol-$CHCl_3$, $CHCl_3$ and $CHCl_3$–$(CH_3)_2O$, with 500 ml fractions being collected as follows: Frs. 1–60 (petrol–$CHCl_3$, 7:3), 61–100 (petrol–$CHCl_3$, 1:1), 101–228 (petrol–$CHCl_3$, 3:7), 230–344 (petrol–$CHCl_3$, 1:9), 345–414 ($CHCl_3$), 415–468 ($CHCl_3$–$(CH_3)_2O$, 9:1), 469–489 ($CHCl_3$–$(CH_3)_2O$, 4:1), 490–503 ($CHCl_3$–$(CH_3)_2O$, 7:3), 504–551 ($CHCl_3$–$(CH_3)_2O$, 1:1), 552–561 ($CHCl_3$–$(CH_3)_2O$, 3:7), 562–563 ($(CH_3)_2O$).

Fr. 2, on recrystallization from MeOH, afforded a mixture (20 mg) of sitosterol acetate and stigmasterol acetate identified by ^1H and ^{13}C NMR and spectroscopic analysis MS. Frs. 12–14 were combined and recrystallized from *n*-hexane and $CHCl_3$ to give friedelin (75 mg) identified by ^1H and ^{13}C NMR and MS. Frs. 23–24 on combination and crystallization from hexane–$CHCl_3$ gave a mixture (112 mg) of β-sitosterol and stigmasterol identified by ^1H and ^{13}C NMR and MS. Frs. 20–26 on combination and purification by TLC (Si gel, petrol–$CHCl_3$–HCO_2H, 1:1:0.1) gave a mixture (122 mg) of 24-ethyl-3-oxocholest-4-ene and 24-ethyl-3-oxocholest-4,22-diene identified by ^1H and ^{13}C NMR and MS. Frs. 91–104 (187 mg) were combined and purified by TLC (Si gel, toluene–EtOAc–$(CH_3)_2O$–HCO_2H, 88:10:2:1) to give **5b** (26 mg). Frs. 105–111 (2 g) were combined, placed on a Si gel 60 column (50 g) and eluted with petrol–$CHCl_3$ and $CHCl_3$–$(CH_3)_2O$, 250 ml subfractions being collected as follows: Sbfrs. 1–9 (petrol–$CHCl_3$, 1:1), 10–21 (petrol–$CHCl_3$, 3:7), 22–28 (petrol–$CHCl_3$, 1:9), 29–43 ($CHCl_3$), 44–49 ($CHCl_3$–$(CH_3)_2O$, 9:1), 50–59 ($CHCl_3$–$(CH_3)_2O$, 7:3) and 60–61 ($CHCl_3$–$(CH_3)_2O$, 1:1). Purification of sbfrs. 13–15 (230 mg) by TLC (Si gel, toluene–EtOAc–$(CH_3)_2O$–HCO_2H, 84:14:2:1) gave **5c** (27 mg). Frs 112–121 (2.2 g) were combined, placed on a Si gel 60 column (50 g) and eluted with petrol–$CHCl_3$ and $CHCl_3$–$(CH_3)_2O$, 250 ml subfractions (sbfrs) being collected as follows: Sbfrs 1–5 (petrol $CHCl_3$, 7:3), 6–16 (petrol–$CHCl_3$, 1:1), 17-62 (petrol–$CHCl_3$, 3:7), 63–75 (petrol–$CHCl_3$, 1:9), 76–83 ($CHCl_3$), 84–95 ($CHCl_3$–$(CH_3)_2O$, 9:1), 96–102 ($CHCl_3$–$(CH_3)_2O$, 7:3). Purification of sbfr. 20 (251 mg) by TLC (RP-18 $F_{254}S$, Merck 5434, MeOH–H_2O, 9:1) gave **6** (10 mg). Frs. 122–146 (3.7 g) were combined and applied to a Si gel 60 column (80 g) and eluted with petrol–$CHCl_3$ and $CHCl_3$–$(CH_3)_2O$, with 250 ml sbfrs being collected as follows: Sbfrs 1–7 (petrol–$CHCl_3$, 3:7), 8–14 (petrol–$CHCl_3$, 1:4), sbfrs 15–20 (petrol–$CHCl_3$, 1:9), 21–27 ($CHCl_3$), 28–73 ($CHCl_3$–$(CH_3)_2O$, 9:1), 74–79 ($CHCl_3$–$(CH_3)_2O$, 4:1), 80–89 ($CHCl_3$–$(CH_3)_2O$, 7:3), 90–104 ($CHCl_3$–$(CH_3)_2O$, 1:1). Purification of sbfr 30 (213 mg) by TLC (Si gel, toluene–EtOAc–$CHCl_3$–HCO_2H, 70:10:20:1) furnished 17 mg more of **5c**. Purification of sbfrs 39–41 (198 mg) by TLC (Si gel, toluene–EtOAc–$CHCl_3$–HCO_2H, 50:10:20:1) gave **5d** (14 mg). Combination of frs. 153–174 (3.6 g) and recrystallization from MeOH gave a mixture (129 mg) of **5a** and **5d**. Crystallization of a 250 mg portion of frs. 230–258 (4.6 g) from MeOH furnished **5a** (104 mg). Frs. 259–414 (21 g) were combined, applied over Si gel 60 (300 g) and eluted with petrol–$CHCl_3$ and $CHCl_3$–$(CH_3)_2O$, 500 ml sbfrs being collected as follows: Sbfrs 1–36 (petrol–$CHCl_3$, 2:3), and 37–128 (petrol–$CHCl_3$, 1:4). Purification of sbfrs 7–20 (208 mg) by TLC (Si gel, toluene–EtOAc–$CHCl_3$–HCO_2H, 80:10:10:1) gave **2a** (60 mg). Purification of sbfr. 37 (932 mg) by TLC (Si gel 60, toluene–EtOAc, $CHCl_3$–HCO_2H 70:20:10:1) gave 97.2 mg of 1. Sbfrs 49–53 (760 mg) were combined and purified by TLC (Si gel, petrol–EtOAc–$CHCl_3$–HCO_2H, 10:10:10:1) to give **3** (36 mg) and **4** (44 mg).

3.4. Methyl(24E)-3α,23α(=R)-dihydroxy-17,14-friedolanostan-8,14,24-trien-26-oate (1)

Physical properties, ^1H and ^{13}C NMR spectra corresponded to those reported earlier (Rukachaisirikul et al., 2000). The coupling constants involving H-23 were essentially identical with those of **2**–**4**.

3.5. Methyl)(24E)-3α,16α,23α (=6R,23R)-epoxy-17,4-friedolan-8,14,24-trien-26-oate (**2a**)

Obtained only as a gum; $[\alpha]_D^{25}$ 7.5 °C ($CHCl_3$, 6.4 g/100 ml); +FAB-HRMS *m/z* 483.34742, for $C_{31}H_{48}O_4+H^+$ calcd. 483.34744. EI MS *m/z* (rel. int.) 482$[M]^+$ (5%), 382 (5%), 288 (100%), 255 (32%), 213 (10%), 201 (15%), 187 (60%), 175 (17%), 161 (12%), 149 (25%), 135 (15%), 111 (15%), 97 (25%), 83 (92%), 69 (41%), 55(44%); IR $[\nu]_{NaCl}$ cm^{-1} 3600-3300, 2944, 2874, 1720, 1656, 1626, 1453, 1440, 1376, 1347, 1299, 1255, 1213, 1155, 1104, 1094, 1064, 1025, 997, 752, ^1H NMR ($CDCl_3$ 500 MHz): δ 6.74 (1H, *dd*, *J*=7.9, 1.2 Hz, H-24), 5.50 (1H, *d*, *J*=3.0 Hz, H-15), 4.25 (1H, *ddd*, *J*=10.9, 8.1, 2.4 Hz, H-23 β), 3.89 (1H, *d*, *J*=3.1 Hz, H-16 β), 3.71 (3H, *s*, OMe), 3.45 (1H, *m*, H-3 β), 2.25–2.4, 2.0–2.2 (each *m*, 1H, H-7), 2.0–2.2 (1H, *m*, H-11), 1.8–2.0 (2H, *m*, H-2a or b, H-20 β), 1.88 (3H, *d*, *J*,=1.2 Hz, H-27), 1.76 (1H, *dd*, *J*=13.1, 12.5 Hz, H-22 α), 1.5–1.8 (5H, *c*, H-1, 2b or a, 5 α, 6, 12),1.48 (1H, *m*, H-6), 1.39 (1H, *ddd*, *J*=13.4, 5.0, 2.4 Hz, H-22 β), 1.27 (3H, *s*, H-30), 1.16 (3H, *d*, *J*=7.2 Hz, H-21), 1.02 (*s*, 3H, H-19), 0.99 (*s*, 3p, H-29), 0.89 (*s*, 3p, H-18), 0.89 (*s*, 3p, H-28); ^{13}C NMR spectrum in Table 1.

Acylation of **2a** with Ac_2O–pyridine in the usual fashion afforded monoacetate **2b** as a gum which could not be induced to solidify; $[\alpha]_D^{25}$ −13° ($CHCl_3$, 0.93 g/100 ml); ^1H NMR ($CDCl_3$, 300 MHz): δ 6.74 (1H, *dd*, *J*=7.9, 1.1 Hz, H-24), 5.53 (1H, *d*, *J*=3.2 Hz, H-15), 4.69 (1H, *brs*, H-3 β), 4.28 (1H, *m*, H-23 β), 3.90 (1H, *d*, *J*=3.1 Hz, H-16), 3.72 (3H, *s*, OMe), 2.3-2.1 (4H, *c*, H-7, H-11), 2.07 (3H, *s*, OAc), 2.0-1.8 (3H, *c*, H-2, H-20), 1.88 (3H, *d*, *J*=1.2 Hz, H-27), 1.17 (3H, *d*, *J*=7 Hz, H-21), 1.03 (3H, *s*, H-19), 0.93 (3H, *s*, H-28), 0.88 (3H, *s*, H-18), 0.87 (3H, *s*, H-29), ^{13}C NMR spectrum in Table 1.

3.6. Methyl(24E)-3α,23α-dihydroxy-8α,9α-epoxy-15-oxo-17,14-friedolanostan-24-en-26-oate(**3**)

Obtained as a gum; $[\alpha]_D^{25}$ −3° ($CHCl_3$, 1.8 g/100 ml); HREIMS *m/z* 516.34511; for $C_{31}H_{48}O_6$, calcd. 516.34509; ^1H NMR (300 MHz, $CDCl_3$): δ 6.66 (1H, *dq*, *J*=8.3, 1.4 Hz, H-24), 4.49 (1H, *ddd*, *J*=10.5, 8.3, 2.1 Hz, H-23), 3.73 (3H, *s*, OMe), 3.46 (1H, *brs*, H-14), 3.35 (1H, *t*, *J*=2.4 Hz, H-3β), 3.05 (1H, *ddd*, *J*=11.3, 11.3, 10.4 Hz, H-11α), 2.44 (1H, *dd*, *J*=12.6, 10.1, H-12β), 2.29 (1H, *dd*, *J*=11.3, 6.2 Hz, H-11β), 2.31–2.20 (1H, H-7a), 1.96 (1H, *dd*, *J*=12.6, 7.1 Hz, H-5), 1.91 (1H, *d*, *J*=14 Hz, H-16β), 1.95–1.86 (1H, *c*, H-20), 1.82 (3H, *d*, *J*=1.3 Hz, H-27), 1.82–1.56 (1H, *c*, H-2a), 1.76 (1H, *ddd*, *J*=12.5, 2.9, 2.9 Hz, H-1a), 1.66 (1H, *d*, *J*=14 Hz, H-16α), 1.64–1.57 (2H, *c*, H-6a, H-7a), 1.59 (1H, *dd*, *J*=11.9, 2 Hz, H-2b), 1.61–1.52 (1H, *c*, H-7b), 1.53–1.42 (2H, *c*, H-1b, H-12α), 1.49-1.39 (1H, *c*, H-6b), 1.42 (1H, *ddd*, *J*=10, 10, 2.6 Hz, H-22a), 1.05–0.95 (1H, *c*, H-22b), 1.05 (3H, *s*, H-18), 0.96 (3H, *s*, H-19), 0.93 (3H, *s*, H-30), 0.90 (3H, *s*, H-28), 0.88 (3H, *d*, *J*=7.0 Hz, H-21), 0.87 (3H, *s*, H-29), ^{13}C NMR spectrum in Table 1.

3.7. Methyl (24E)-3α,23α-dihydroxy-15-oxo-17,15-friedolanostan-8(14),24-dien-26-oate (**4**)

Obtained as a gum; $[\alpha]_D^{28}$ −34° ($CHCl_3$, 2.1 g/100 ml); HREIMS *m/z* 500.35009; for $C_{31}H_{48}O_5$, calcd. 500.35018; ^1H NMR (500 MHz, $CDCl_3$): δ 6.70 (1H, *dq*, *J*=8.2, 1.4 Hz, H-24), 4.56 (1H, *ddd*, *J*=9.4, 9.4, 2.1 Hz, H-23), 4.22 (1H, *ddd*, *J*=14, 3.8, 2.2 Hz, H-7β), 3.75 (*s*, 3H, OMe), 3.41 (1H, *t*, *J*=2.4 Hz, H-3), 2.36 (1H, *d*, *J*=18.3 Hz, H-16α), 2.04 (1H, *d*, *J*=18.3 Hz, H-16β), 2.3–2.1 (1H, *c*, H-20), 1.96-1.90 (2H, *c*, H-2a, H-9), 1.86 (3H, *d*, *J*=1.4 Hz, H-27), 1.76 (1H, *ddd*, *J*=14, 14, 4.9 Hz, H-7α)∼1.8–1.58 (6H, *c*, H-1a,b, 2b, 11a, 12a,b), 1.64 (1H, *t*, *J*=9.4 Hz, H-5), 1.45-1.3 (5H, *c*, H-6a, b, 11b, 12b, 22a), 1.09 (3H, *s*, H-30), 0.97 (3H, *s*, H-28), 0.93 (3H, *d*, *J*=7 Hz, H-21), 0.83 (3H, *s*, H-29), 0.81 (3H, *s*, H-19), 0.82 (3H, *s*, H-18), ^{13}C NMR spectrum in Table 1.

3.8. X-ray analysis of (25R)-3β-hydroxy-23-oxo-9,16-lanostandien-26-oic acid (**5a**)

The physical properties as well as the ^1H and ^{13}C NMR spectra of this substance corresponded to the properties reported earlier (Rukachaisirikul et al., 2000); mp 220–222°, $[\alpha]_D^{20}$ −61° (MeOH, 0.13 g/100 ml). Crystals suitable for X-ray diffraction were obtained by slow evaporation of a saturated MeOH solution and belonged to space group $P2_12_12_1$ with cell dimensions *a*=6.298(2) Å, *b*=14.278(5) Å, *c*=30.609(13) Å (uncertainties in parentheses), *Z*=4, calcd density 1.18 g cm^3. Analysis of the X-ray diffraction data revealed that one water molecule per compound molecule was retained in the crystal. Data were collected at room temperature with a Stoe IPDS image plate and Mo Kα radiation. A total of 18025 reflections were measured of which 5244 were independent and 3389 were observed ($I>2\sigma$ (I). The structure was solved using SHELXS-97 (Sheldrick, 1997a) and refined with SHELXL-97 (Sheldrick, 1997b). Non-hydrogen atoms were refined anisotropically; the refinement converged to *R* (all data)=9.24% and WR^2 (all data)=11.63%. All hydrogen atoms except those of one methyl group (C-27) were found in the difference map. Therefore, the hydrogen positions around C-27 were calculated assuming a tetrahedral arrangement for the carbon atom and refined using the riding model. A network of hydrogen bonds involving the water molecule and molecule **5a** was observed; relevant hydrogen bond distances are given in Table 2.

3.9. Methyl (25R)-3β-hydroxy-23-oxo-9,16-lanostadien-26-oate (**5b**)

White powder, mp 180-182°, $[\alpha]_D^{25}$ −124.2° ($CHCl_3$, $c = 0.54$ g/100 ml), HR EI MS 484.35519, for $C_{31}H_{48}O_4$ calcd. 484.35526; EI MS *m/z* (rel. int.) 484 (M^+,88), 469 (13), 451 (17), 340 (17), 325 (30), 313 (100), 295 (25), 173 (40), 171 (32), 159 (26), 145 (21); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.28 (1H, *d*, *J* = 6 Hz, H-11), 5.21 (1H, *t*, *J* = 1.3 Hz, H-16), 3.67 (3H, *s*, OMe), 3.23 (1H, *dd*, *J* = 11.6, 4.3 Hz, H-3α), 2.94 (1H, *dq*, 6.9, 6.1 Hz, H-25?α), 2.87 (1H, *dd*, *J* = 17.5, 8.1 Hz, H-24a), 2.75-2.60 (2H, *m*, H-20, 22a), 2.46 (1H, *dd*, *J* = 17.5, 5.4 Hz, H-24b), 2.55–2.40 (*m*, H-22b), 2.35–2.30 (2H, *m*, H-8, H-12α), 2.07 (1H, *brd*, *J* = 14.5 Hz, H-15β), 1.84 (1H, *brd*, *J* = 14.5, H-15α), 1.9–1.6 (7H, *c*, H-1β, 2a,b,6a,7a,b,12β)1.6-1.4 (2H, m, H-1α, H-6b), 1.16 (3H, *d*, *J* = 7.1 Hz, H-27), 1.06 (3H, *s*, H-19), 1.03 (3H, *d*, *J* = 6.7 Hz, H-21), 0.99 (3H, *s*, H-29), 0.95-0.85 (1H, *m*, H-5), 0.83 (3H, *s*, H-28), 0.79 (3H, *s*, H-30), 0.75 (3H, *s*, H-18); ^{13}C NMR spectrum in Table 1.

3.10. (25R)-3α-Hydroxy-23-oxo-9,16-lanostadien-26-oic-acid (**5d**)

Gum; $[\alpha]_D^{25}$ 17.5 °C ($CHCl_3$, $c = 1.3$ g/100 ml), EI MS *m/z* (rel. int.) 470 (M^+,100), 455 (20), 437 (32), 340 (27), 325 (35), 313 (98), 295 (55), 241 (30), 225 (25), 187 (28), 185 (34) 173 (55, 159 (51), 145 (45), 133 (56) 121 (69), ^{1}H NMR ($CDCl_3$, 500 MHz,) δ 5.32 (1H, *d*, *J* = 5.9 Hz, H-11), 5.21 (1H, *d*, *J* = 1.3 Hz), H-16), 3.45 (1H, *t*, *J* = 2.6 Hz, H-3β), 2.97 (1H, *quint*, *J* = 7 Hz, H-25), 2.86 (1H, *dd*, 17.8, 7.9 Hz, H-24a), 2.67 (2H, *m*, H-20, 22a), 2.49 (1H, *dd*, *J* = 17.8, 5.6 Hz, H-24b), 2.49 (1H, *m*, H-22b), 2.35 (2H, *m*, H-8, 12a), 2.07 (1H, *brd*, *J* = 15.1 Hz, H-15β), 2.00 (1H, *dddd*, *J* = 14, 14, 2.7, 2.7, H-2β), 1.83 (1H, *dd*, *J* = 14.9, 3.2 Hz, H-15β), 1.20 (3H, *d*, *J* = 7.1 Hz, H-27), 1.08 (3H, *s*, H-19), 1.03 (3H, *d*, *J* = 6.7 Hz, H-21), 0.97 (3H, *s*, H-29), 0.89 (3H, *s*, H-28), 0.81 (3H, *s*, H-30), 0.75 (3H, *s*, H-18); ^{13}C NMR spectrum in Table 1.

3.11. 3β,9α-Dihydroxylanost-24-en-26-ol (**6**)

White amorphous powder; $[\alpha]_D^{27}$ 5.0° ($CHCl_3$, 0.3 g/100 ml), HR EI MS *m/z* 458.37596; for $C_{30}H_{50}O_3$ calcd. 458.37600; ^{1}H NMR ($CDCl_3$, 500 MHz,) δ 9.39 (1H, *s*, H-26), 6.49 (1H, *ddq*, *J* = 7.4, 7.3, 1.2 Hz, H-24), 3.21 (1H, *dd*, *J* = 11, 4.4 Hz, H-3α), 2.45–2.2 (2H, *c*, H-23a,b), 2.0-1.8 (5H, *c*, H-8, 11a,b, 16a,b), 1.75 (3H, *brs*, H-27), 1.7–1.45 (11H, *c*, H-2a,b, 5, 6a, 7a, 12a,b, 17, 20, 22a, b), 1.4–1.1 (6H, *c*, H-1a,b, 6b, 7b, 15a,b), 1.04 (3H, *s*, H-19), 1.00 (3H, *s*, H-29), 0.96 (3H, *d*, *J* = 6.3 Hz, H-21), 0.93 (3H, *s*, H-30), 0.81 (3H, *s*, H-28), 0.80 (3H, *s*, H-18); ^{13}C NMR spectrum in Table 1.

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