24-Ethyl,24-methyl-29-nor-lanostanes from Leaves of *Freycinetia formosana*

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Two new 24-ethyl,24-methyl-29-nor-lanostanes (1, 2) were isolated from the MeOH extract of leaves of *Freycinetia formosana* (Pandanaceae). Their structures were elucidated based on spectroscopic evidence.

Key words *Freycinetia formosana*; Pandanaceae; 24-ethyl,24-methyl-29-nor-lanostane

Plants of the genus *Freycinetia*, belonging to the family Pandanaceae, comprise about 180 species and are distributed mainly in tropical and subtropical regions.[1] However, no phytochemical study of the plants of this genus has been studied to date. As part of our phytochemical studies on the plants of Pandanaceae,[2] the chemical components of a MeOH extract obtained from the leaves of *Freycinetia formosana* HEMSLEY (syn. *Freycinetia boninensis* NAKAI)[1,3] was investigated. We have isolated two novel 24-ethyl,24-methyl-29-nor-lanostanes from *F. formosana* and their structures were elucidated as 24-ethyl,24-methyl-29-nor-5α-lanosta-4(28),7,25-trien-3β-ol (**1**) and 24-ethyl,24-methyl-29-nor-5α-lanosta-4(28),7-dien-3β-ol (**2**), respectively. This paper describes the isolation and structural characterization of these compounds.

The $CHCl_3$ soluble part obtained from a MeOH extract was separated by a combination of silica gel chromatography and HPLC separations to isolate two new compounds (**1**, **2**). Compound **1**, a colorless glassy solid, $[\alpha]_D$ +41.5° ($CHCl_3$), gave a molecular ion peak at *m*/*z* 452.4011 in its HR-EI-MS, corresponding to the molecular formula of $C_{32}H_{52}O$. The ^{1}H-NMR spectrum of **1**, analyzed with the aids of 2D-NMR (COSY and NOESY) studies, showed the presence of five tertiary methyls (δ_H 0.65, 0.70, 0.94, 0.99, 1.64), one secondary methyl [δ_H 0.88 (3H, d, J=6.8 Hz)], one ethyl group [δ_H 1.23 and 1.44 (each 1H, m), and 0.71 (3H, t, J=7.4 Hz)], one carbinyl proton [δ_H 3.99 (1H, dd, J=11.1, 4.4 Hz)], two sets of exocyclic-methylene groups [δ_H 4.63 and 4.82 (each 1H, br s); δ_H 4.73 and 5.14 (each 1H, br s)], and one trisubstituted olefinic proton [δ_H 5.24 (1H, m)]. The ^{13}C-NMR spectrum of **1** (Table 1) showed 32 carbons. The multiplicities of each carbon were made by HMQC experiments, which revealed the presence of seven methyls, twelve methylenes, six methines, and seven quaternary carbon atoms. In the EI-MS, compound **1** exhibited important and prominent fragments at *m*/*z* 437 (M^+−Me, base peak) and at *m*/*z* 297 [M^+−$C_{11}H_{21}$ (side-chain moiety)−2H] which are characteristic fragmentation patterns of lanostane-type triterpenes.[4—6] From the above results, compound **1** is a lanostane-type triterpene having a $C_{11}H_{21}$ side chain in the molecule. The structure of the side chain in **1** was deduced from following 2D-NMR (COSY, NOESY, HMQC, and HMBC) studies. Accordingly, **1** showed signals due to a secondary methyl [H_3-21; δ_H 0.88 (3H, d, J=6.8 Hz), δ_C 19.2] coupled to a methine carbon [H-20; δ_H 1.32 (1H, m), δ_C 37.1], an isopropenyl [–C(CH_3)=CH_2] group [H_2-26; δ_H 4.63 and 4.82 (each 1H, br s), δ_C 111.3, H_3-27; δ_H 1.64 (3H, br s), δ_C 19.3, and C-25; δ_C 150.2], one tertiary methyl [H_3-33; δ_H 0.94 (3H, s), δ_C 22.2], two methylenes [H_2-22; δ_H 1.24 (2H, overlapping signals), δ_C 30.3 and H_2-23; δ_H 1.43 (2H, overlapping signals), δ_C 36.4], one ethyl group [H_2-31; δ_H 1.23 and 1.44 (each 1H, m), δ_C 32.3 and H_3-32; δ_H 0.71 (3H, t, J=7.4 Hz), δ_C 8.4], and one quaternary carbon atom (C-24; δ_C 42.2). The sequence of these functionalities in the side chain was confirmed by the HMBC experiments as shown in Fig. 1. In addition, the ^{1}H- and ^{13}C-NMR data of the side-chain moiety in **1** were very similar to those of skimmiwallinin[7] and sablacaurin B,[8] possessing the same $C_{11}H_{21}$ side chain in the molecules. The gross structure of **1** was deduced from the following HMBC experiments. Placement of the exocyclic-methylene group at C-4 was established through HMBC correlations between the exocyclic-methylene protons [H_2-28;

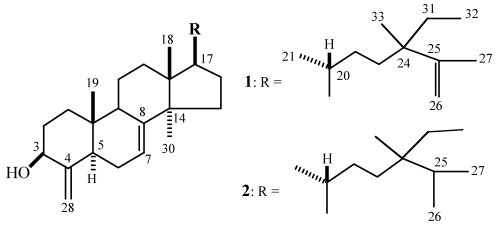

Table 1. ^{13}C- (150 MHz) NMR Spectral Data of **1** and **2** in $CDCl_3$

No.	**1**	**2**	No.	**1**	**2**
	δ_C	δ_C		δ_C	δ_C
1	37.5	37.5	17	50.6	50.8
2	32.4	32.3	18	16.0	16.0
3	73.4	73.4	19	13.4	13.4
4	152.6	152.6	20	37.1	37.4
5	45.4	45.3	21	19.2	19.1
6	25.1	25.0	22	30.3	29.5
7	115.7	115.6	23	36.4	32.6
8	144.8	144.8	24	42.2	36.9
9	45.0	45.0	25	150.2	33.3
10	37.5	37.5	26	111.3	17.1[a]
11	21.1	21.1	27	19.3	17.2[a]
12	32.1	32.1	28	103.1	103.1
13	44.5	44.5	30	24.6	24.6
14	52.0	52.0	31	32.3	28.7
15	32.9	32.9	32	8.4	8.0
16	27.6	27.7	33	22.2	20.2

a) Assignments may be interchangeable.

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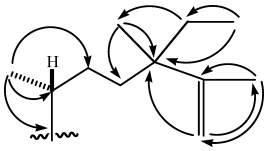

Fig. 1 Selected HMBC Correlations of **1**

δ_H 4.73 and 5.14 (each 1H, br s)] and carbons C-3 (δ_C 73.4) and C-5 (δ_C 45.4), and in turn, H-5 [δ_H 1.90 (1H, m)] showed correlations with C-3 (δ_C 73.4) and C-28 (δ_C 103.1). The signal at δ 3.99 was assigned as 3α-H due to its coupling constants (1H, dd, J=11.1, 4.4 Hz) and showed HMBC correlations with C-4 (δ_C 152.6). Finally, the trisubstituted olefinic proton signal at δ 5.24 (1H, m) was correlated with C-5 (δ_C 45.4), C-6 (δ_C 25.1), and C-14 (δ_C 52.0), suggesting the trisubstituted double bond was located at C-7(8).[4—6,9] On the basis of the above evidence as well as the precise spectral comparisons of **1** and analogous nortriterpenes,[10—12] the structure of **1** was concluded to be 24-ethyl,24-methyl-29-nor-5α-lanosta-4(28),7,25-trien-3β-ol.

Compound **2**, $[\alpha]_D$ +55.7° ($CHCl_3$), was isolated as a white amorphous powder and showed a molecular formula of $C_{32}H_{54}O$ based on the HR-EI-MS experiments. The EI-MS of **2** exhibited important fragments at *m/z* 439 (M^+−Me, base peak) and at *m/z* 297 [$M^+-C_{11}H_{23}$ (side-chain moiety)−2H][4—6] suggesting that compound **2** is a lanostanol-type triterpene having a $C_{11}H_{23}$ side chain in the molecule. The ^{13}C-NMR spectrum of **2** (Table 1) showed 32 carbons. Further a precise comparison of the ^{1}H- and ^{13}C-NMR spectra of **1** and **2** revealed that the proton and carbon chemical shifts ascribable to A, B, C, and D rings in **2** were essentially the same as those of **1**. However, the signals due to an isopropenyl [–C(CH_3)=CH_2] group in **1** was absent in **2**, being replaced by an isopropyl [–CH(CH_3)$_2$] group [H-25; δ_H 1.26 (1H, overlapping signal) δ_C 33.3, H_3-26; δ_H 0.79* (3H, d, J=6.8 Hz), δ_C 17.1** and H_3-27; δ_H 0.80* (3H, d, J=6.8 Hz), δ_C 17.2** (*,** interchangeable to each other)] in **2** to indicate that compound **2** was a 25,26-dihydrocompound of **1**. On the basis of the above evidence as well as the precise spectroscopic comparisons of **2** and analogous nortriterpenes,[10—12] the structure of **2** was concluded to be 24-ethyl,24-methyl-29-nor-5α-lanosta-4(28),7-dien-3β-ol.

Experimental

^{1}H- and ^{13}C-NMR spectra were measured on a JEOL JNM-ECA 600 spectrometer (^{1}H at 600 MHz and ^{13}C at 150 MHz in $CDCl_3$, respectively). Chemical shifts are given in δ values (ppm) relative to tetramethylsilane (TMS) as an internal standard. EI- and HR-EI-MS spectra (at 30 eV) were obtained using a JEOL JMS-700T spectrometer. Optical rotations were measured on a JASCO DIP-140 polarimeter. For column chromatography, silica gel 60 (230—400 mesh, Merck) was used. Preparative HPLC was performed on a JAI LC-908 instrument with a RI-50 differential refractometer and a JAIGEL-ODS-120T column.

Plant Material Leaves of *Freycinetia formosana* Hemsley were collected in March 2002 in Chichizima in the Bonin Islands, Japan and a voucher specimen (No. 118) was deposited in the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Setsunan University.

Extraction and Isolation Crushed leaves (360 g) were extracted with MeOH (3×7 l) at room temperature. The MeOH solution was evaporated off to give an extract (35.3 g). The MeOH extract was suspended with H_2O (700 ml) and the aqueous suspension was extracted with $CHCl_3$ (3×0.5 l). The resulting $CHCl_3$ extract (10.6 g) was chromatographed on silica gel (400 g) with hexane–EtOAc with increasing EtOAc concentration and EtOAc to give 5 fractions (Frs. A—E). Fr. B (0.22 g), eluted with hexane–EtOAc (1 : 1), was purified by repeated reversed phase preparative HPLC with CH_3CN–$CHCl_3$ (2 : 1) to afford **1** (3.5 mg) and **2** (4.4 mg), respectively.

24-Ethyl,24-methyl-29-nor-5α-lanosta-4(28),7,25-trien-3β-ol (**1**): An amorphous solid, $[\alpha]_D^{20}$ +41.5° (c=0.38, $CHCl_3$). EI- and high resolution EI-MS *m/z* (rel. int.): 452.4011 (M^+, $C_{32}H_{52}O$ requires 452.4018, 74), 438 (51), 437 (100), 419 (18), 297.2211 (M^+−side-chain−2H, $C_{21}H_{29}O$ requires 297.2218, 29), 272 (38), 257(38), 254 (42), 151 (58), 98 (56). ^{1}H-NMR δ: 0.65 (3H, s, H_3-18), 0.70 (3H, s, H_3-19), 0.71 (3H, t, J=7.4 Hz, H_3-32), 0.88 (3H, d, J=6.8 Hz, H_3-21), 0.94 (3H, s, H_3-33), 0.99 (3H, s, H_3-30), 1.23 and 1.44 (each 1H, m, H_2-31), 1.24 (2H, overlapping signals, H_2-22), 1.32 (1H, m, H-20), 1.43 (2H, overlapping signals, H_2-23), 1.64 (3H, s, H_3-27), 1.90 (1H, m, H-5α), 3.99 (1H, dd, J=11.1, 4.4 Hz, H-3α), 4.63 and 4.82 (each 1H, br s, H_2-26), 4.73 and 5.14 (each 1H, br s, H_2-28), 5.24 (1H, m, H-7). ^{13}C-NMR spectral data are shown in Table 1.

24-Ethyl,24-methyl-29-nor-5α-lanosta-4(28),7-dien-3β-ol (**2**): An amorphous solid, $[\alpha]_D^{20}$ +55.7° (c=0.39, $CHCl_3$). EI- and high resolution EI-MS *m/z* (rel. int.): 454.4176 (M^+, $C_{32}H_{54}O$ requires 454.4175, 34), 440 (36), 439 (100), 421 (33), 297.2226 (M^+−side-chain−2H, $C_{21}H_{29}O$ requires 297.2218, 10), 272 (16), 257 (22), 231 (19). ^{1}H-NMR δ: 0.66 (3H, s, H_3-18), 0.68 (3H, s, H_3-33), 0.70 (3H, s, H_3-19), 0.76 (3H, t, J=7.6 Hz, H_3-32), 0.79* (3H, d, J=6.8 Hz, H_3-26) and 0.80* (3H, d. J=6.8 Hz, H_3-27) (* interchangeable), 0.89 (3H, d, J=6.6 Hz, H_3-21), 1.00 (3H, s, H_3-30), 1.26 (1H, overlapping signal, H-25), 4.00 (1H, dd, J=11.4, 4.8 Hz, H-3α), 4.73 and 5.14 (each 1H, br s, H_2-28), 5.24 (1H, m, H-7). ^{13}C-NMR spectral data are shown in Table 1.

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