

## Lanostanoids of *Amentotaxus formosana*

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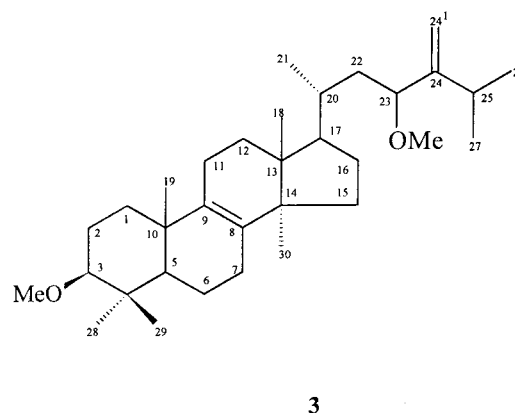
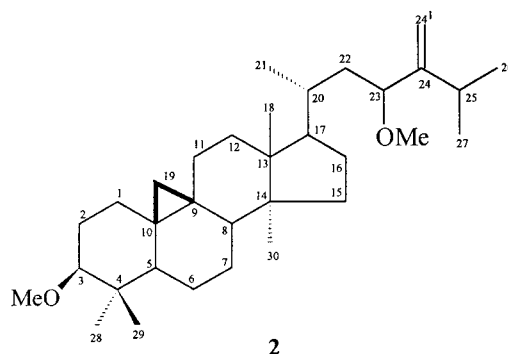
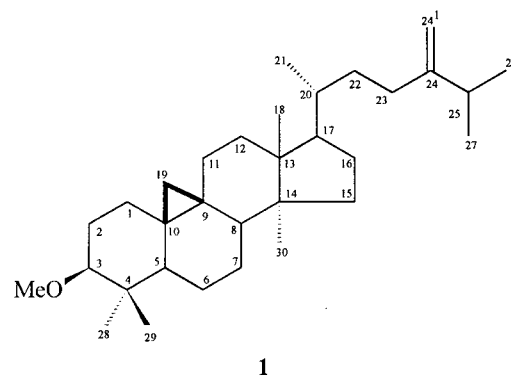
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Three lanostanoids, 3 $\beta$ -methoxycycloartan-24(24<sup>1</sup>)-ene (**1**), 3 $\beta$ ,23 $\beta$ -dimethoxycycloartan-24(24<sup>1</sup>)-ene (**2**), and 3 $\beta$ ,23 $\beta$ -dimethoxy-5 $\alpha$ -lanosta-24(24<sup>1</sup>)-ene (**3**), were isolated from the leaves of *Amentotaxus formosana*. The structures of new compounds **2** and **3** were determined by NMR and MS studies, and the structure of **3** was further confirmed by X-ray crystallographic analysis.

*Amentotaxus formosana* Li (Amentotaxaceae) is an endemic tree of southeastern Taiwan. Phytochemical or bioactive investigations of this plant have not been reported in the literature. Compound **1**, a semisynthetic compound obtained from 24-methylene cycloartanol reacted with methyl iodide,<sup>1</sup> was isolated from this plant as a new natural product. In the present paper, the <sup>13</sup>C NMR spectrum of **1** and the structure elucidation of new lanostanoids **2** and **3** are reported.

Compound **1** was characterized as 3 $\beta$ -methoxycycloartan-24(24<sup>1</sup>)-ene (**1**). The <sup>13</sup>C NMR assignments of **1** (Table 1) were made by performing <sup>1</sup>H-decoupled, DEPT, and 2D NMR correlation experiments and by comparison with the corresponding data of cycloartanol.<sup>2</sup>

The HREIMS of **2** indicated a molecular ion peak at *m/z* 484.4310, which corresponded to molecular formula C<sub>33</sub>H<sub>56</sub>O<sub>2</sub>. IR absorptions were indicative of a C=C double bond (1642 cm<sup>-1</sup>). The EIMS spectrum of **2** showed significant peaks at *m/z* 469 [M - Me]<sup>+</sup>, 452 [M - CH<sub>3</sub>-OH]<sup>+</sup>, 437 [452 - Me]<sup>+</sup>, 341 [452 - (side chain - CHMe<sub>2</sub>)]<sup>+</sup>, 309 [341 - CH<sub>3</sub>OH]<sup>+</sup>. The <sup>1</sup>H NMR spectrum of **2** showed signals for four tertiary methyl groups and three secondary methyl groups as required by the lanostane skeleton,<sup>3</sup> two geminal proton signals of a cyclopropane ring at  $\delta$  0.33 (d, *J* = 4.00 Hz) and 0.56 (d, *J* = 4.00 Hz), two methine proton signals at 2.71 (1H, dd, *J* = 10.4, 4.0 Hz, H $_{\alpha}$ -3)<sup>1</sup> and 3.60 (1H, d, *J* = 10.4 Hz), two methoxy proton signals at  $\delta$  3.21 (s) and 3.36 (s), and two olefinic proton signals at  $\delta$  4.92 (1H, s) and 4.98 (1H, s). The carbon signals of **2** were almost identical to the corresponding carbon signals of **1** except for C-20–C-27 (Table 1).<sup>2,4</sup> In addition to the above evidence, the HMBC of H $_{\alpha}$ -3/OMe-3, H $_{\alpha}$ -3/C-29, Me-28/C-3, Me-29/C-3, H-29/C-4, H-19<sub>exo</sub>/C-11, H-19<sub>exo</sub>/C-8, H-19<sub>endo</sub>/C-8, H-19<sub>endo</sub>/C-10, H-19<sub>endo</sub>/C-5, and H-19<sub>endo</sub>/C-1 confirmed that the cyclopropane ring and OMe group were linked to the C-9–C-10 bond and C-3, respectively, and H-24<sup>1</sup>/C-25, H-24<sup>1</sup>/C-23, Me-31/C-23, H-23/OMe, and H-23/C-22 confirmed the presence of a 24(24<sup>1</sup>) double bond and a OMe group linked to C-23 in **2**. Correlations between Me-28 and H-19<sub>endo</sub>, and H-23 and Me-21, in the NOESY experiment confirmed that **2** was 3 $\beta$ ,23 $\beta$ -dimethoxycycloartan-24(24<sup>1</sup>)-ene (**2**).<sup>5</sup> The <sup>13</sup>C NMR assignments of **2** (Table 1) were made by performing <sup>1</sup>H-decoupled, DEPT, and 2D NMR



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correlation experiments and by comparison with those of corresponding data of **1**.

The HREIMS of **3** indicated a molecular ion peak at *m/z* 484.4274, which corresponded to molecular formula

**Table 1.**  $^{13}\text{C}$  NMR Data ( $\delta$ ) for **1–3** ( $\text{CDCl}_3$ )<sup>a</sup>

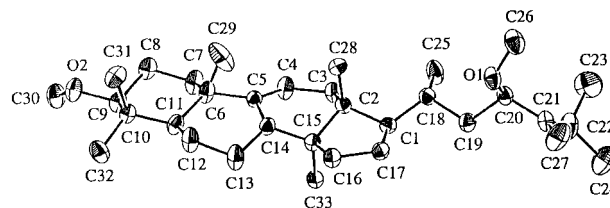
carbon	<b>1</b> <sup>b</sup>	<b>2</b> <sup>b</sup>	<b>3</b> <sup>b</sup>
1	31.8	31.6	35.6
2	25.5	25.2	22.7
3	88.5	88.3	88.8
4	40.5	40.3	37.1
5	47.7	47.5	51.3
6	21.0	20.7	18.2
7	28.1	28.0	28.3
8	48.0	47.7	134.4
9	20.0	20.0	134.6
10	26.3	26.1	38.9
11	26.0	25.7	21.1
12	32.9	32.8	26.5
13	45.3	45.2	44.7
14	48.8	48.7	49.9
15	35.5	35.3	31.2
16	26.5	26.3	30.8
17	52.3	52.8	51.0
18	18.0	18.0	15.9
19	29.8	29.5	19.2
20	35.0	32.8	33.4
21	19.3	18.0	18.6
22	36.1	42.9	43.2
23	31.3	81.5	81.8
24	157.0	156.4	156.7
24 <sup>1</sup>	105.7	107.2	107.4
25	33.8	29.6	29.9
26	21.9	23.3	22.5
27	22.0	22.3	23.5
28	25.4	25.3	28.0
29	14.8	14.6	16.2
30	19.3	19.1	24.2
3-OMe	57.6	57.4	57.5
23-OMe		56.2	56.3

<sup>a</sup> The number of protons directly attached to each carbon was verified by DEPT experiments. <sup>b</sup> Signals obtained by  $^1\text{H}$ - $^1\text{H}$  COSY, HMBC, HMQC, and NOESY techniques.

$\text{C}_{33}\text{H}_{56}\text{O}_2$ . IR absorptions were indicative of a C=C double bond ( $1643\text{ cm}^{-1}$ ). The EIMS spectrum of **3** showed significant peaks at  $m/z$  469 [ $\text{M} - \text{Me}$ ]<sup>+</sup>, 452 [ $\text{M} - \text{CH}_3\text{-OH}$ ]<sup>+</sup>, 437 [ $452 - \text{Me}$ ]<sup>+</sup>, 341 [ $452 - (\text{side chain} - \text{CHMe}_2)$ ]<sup>+</sup>, 309 [ $341 - \text{CH}_3\text{OH}$ ]<sup>+</sup>. The  $^1\text{H}$  NMR spectrum of **3** showed signals for five tertiary methyl groups and three secondary methyl groups as required by the lanostane skeleton,<sup>3</sup> two methine proton signals at  $\delta$  2.67 (1H, dd,  $J = 10.2, 4.0\text{ Hz}$ ,  $\text{H}_{\alpha-3}$ )<sup>1</sup> and 3.59 (1H, d,  $J = 10.2\text{ Hz}$ , H-23), two methoxy proton signals at  $\delta$  3.20 (3H, s) and 3.36 (3H, s), and two olefinic proton signals at  $\delta$  4.91 (1H, s) and 4.97 (1H, s). In the  $^{13}\text{C}$  NMR spectrum of **3**, the signals of C-1 to C-19 and C-28 to C-30 were almost identical to the corresponding carbon signals of lanostenol, except for C-2 to C-4 (Table 1),<sup>6,7</sup> and the signals of C-20 to C-27 were almost identical to the corresponding signals of **2**. The chemical shift values of C-3, C-2, and C-4 revealed a downfield shift of 9.8 ppm and an upfield shift of 5.2 and 1.9 ppm (Table 1) compared with those of corresponding data of lanostenol.<sup>6</sup> It was clear that a methoxy group was located at C-3.<sup>8</sup> On the basis of the above results, **3** was characterized as 3 $\beta$ ,23 $\beta$ -dimethoxy-5 $\alpha$ -lanosta-24(24<sup>1</sup>)-ene (**3**). The  $^{13}\text{C}$  NMR assignments of **3** (Table 1) were made by performing  $^1\text{H}$ -decoupled, DEPT, and 2D NMR correlation experiments and by comparison with those of corresponding data of **2** and lanostenol.<sup>6,8</sup> The characterization of **3** was further confirmed by X-ray crystallographic analysis (Figure 1).<sup>10</sup>

## Experimental Section

**General Experimental Procedures.** Melting points are reported uncorrected. The optical rotations were obtained on a JASCO model DIP-370 digital polarimeter. IR spectra were recorded on a Hitachi model 260-30 spectrophotometer.  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  (100 MHz) NMR spectra were recorded on a Varian Unity-400 spectrometer, and MS were obtained on a JMS-HX 100 mass spectrometer.

**Figure 1.** ORTEP view of **3**.

**Plant Material.** Leaves of *A. formosana* (Amentotaxaceae) were collected at Kaohsiung Hsien, Taiwan, during July 1990. A voucher specimen (9001) is deposited in the laboratory of Medicinal Chemistry.

**Extraction and Isolation.** Air-dried leaves (3.1 kg) were extracted with  $\text{CHCl}_3$ . The  $\text{CHCl}_3$  extract was chromatographed on a silica gel column, and elution with cyclohexane- $\text{CH}_2\text{Cl}_2$  (9.5:0.5) yielded **1** (5 mg). Elution with cyclohexane- $\text{CH}_2\text{Cl}_2$  (7:3) yielded **2** (6 mg) and **3** (20 mg), respectively.

**3 $\beta$ ,23 $\beta$ -Methoxycycloartan-24(24<sup>1</sup>)-ene (**1**):** colorless needles (acetone); mp 86–88 °C; IR (KBr)  $\nu_{\text{max}}$  1638  $\text{cm}^{-1}$ ;  $[\alpha]_{\text{D}}^{25}$  252° ( $c$  0.1,  $\text{CHCl}_3$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz), see Table 1; HREIMS  $m/z$  [ $\text{M}$ ]<sup>+</sup> 454.4166 (calcd for  $\text{C}_{32}\text{H}_{54}\text{O}_2$ , 454.4175).

**3 $\beta$ ,23 $\beta$ -Dimethoxycycloartan-24(24<sup>1</sup>)-ene (**2**):** colorless oil; IR (KBr)  $\nu_{\text{max}}$  1642  $\text{cm}^{-1}$ ;  $[\alpha]_{\text{D}}^{25}$  14° ( $c$  0.15,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  0.33 (1H, d,  $J = 4.0\text{ Hz}$ , H-19<sub>exo</sub>), 0.56 (1H, d,  $J = 4.0\text{ Hz}$ , H-19<sub>endo</sub>), 0.79 (3H, s, Me-29), 0.88 (3H, s, Me-30), 0.91 (3H, d,  $J = 6.4\text{ Hz}$ , Me-21), 0.95 (3H, s, Me-28), 1.01 (3H, s, Me-18), 1.05 (3H, d,  $J = 6.4\text{ Hz}$ , Me-26), 1.07 (3H, d,  $J = 6.4\text{ Hz}$ , Me-27), 2.71 (1H, dd,  $J = 11.0, 4.4\text{ Hz}$ ,  $\text{H}_{\alpha-3}$ ), 3.21 (3H, s, OMe-23), 3.36 (3H, s, OMe-3), 3.60 (1H, d,  $J = 6.4\text{ Hz}$ ,  $\text{H}_{\alpha-23}$ ), 4.91 (1H, s,  $\text{H}_2$ -24<sup>1</sup>), 4.98 (1H, s,  $\text{H}_2$ -24<sup>1</sup>);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz), see Table 1; EIMS  $m/z$  484 [ $\text{M}$ ]<sup>+</sup> (3), 469 (3), 452 (9), 437 (12), 341 (21), 309 (19), 113 (100); HREIMS  $m/z$  [ $\text{M}$ ]<sup>+</sup> 484.4275 (calcd for  $\text{C}_{33}\text{H}_{56}\text{O}_2$ , 484.4280).

**3 $\beta$ ,23 $\beta$ -Dimethoxy-5 $\alpha$ -lanosta-24(24<sup>1</sup>)-ene (**3**):** colorless prisms ( $\text{CHCl}_3$ -MeOH); mp 176–178 °C; IR (KBr)  $\nu_{\text{max}}$  1643  $\text{cm}^{-1}$ ;  $[\alpha]_{\text{D}}^{25}$  84° ( $c$  0.1,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  0.73 (3H, s, Me-18), 0.80 (3H, s, Me-29), 0.87 (3H, s, Me-30), 0.94 (3H, d,  $J = 6.4\text{ Hz}$ , Me-21), 0.99 (3H, s, Me-19, -28), 1.05 (3H, d,  $J = 6.4\text{ Hz}$ , Me-27), 1.08 (3H, d,  $J = 6.4\text{ Hz}$ , Me-26), 2.67 (1H, dd,  $J = 10.2, 4.0\text{ Hz}$ ,  $\text{H}_{\alpha-3}$ ), 3.21 (1H, s, OMe-23), 3.36 (1H, s, OMe-3), 3.59 (1H, d,  $J = 3.59\text{ Hz}$ ,  $\text{H}_{\alpha-23}$ ), 4.92 (1H, s, H-24<sup>1</sup>), 4.98 (1H, s, H-24<sup>1</sup>);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz), see Table 1; EIMS  $m/z$  484 [ $\text{M}$ ]<sup>+</sup> (3), 469 (3), 452 (9), 437 (12), 341 (21), 309 (19), 113 (100); HREIMS  $m/z$  [ $\text{M}$ ]<sup>+</sup> 484.4274 (calcd for  $\text{C}_{32}\text{H}_{54}\text{O}_2$ , 484.4280).

**X-ray Analysis.** X-ray crystal analysis was performed with a single crystal (colorless,  $0.4 \times 0.52 \times 0.58\text{ mm}$ ) obtained from  $\text{CHCl}_3$ -MeOH. X-ray diffraction data were collected on a Rigaku-AFC7S diffractometer with graphite-monochromated Mo K $\alpha$  radiation. The structure was solved by direct methods and expanded with Fourier techniques.<sup>9</sup> All non-H atoms were refined anisotropically using full-matrix least-squares techniques. All calculations were performed with the TeXsan crystallographic software package of Molecular Structure Corporation. The crystal data were as follows:  $\text{C}_{33}\text{H}_{56}\text{O}_2$ , monoclinic,  $P2_1$  (No. 4):  $a = 7.494(3)\text{ \AA}$ ,  $b = 19.015(3)\text{ \AA}$ ,  $c = 10.758(3)\text{ \AA}$ ,  $\beta = 98.2(3)^\circ$ , and  $V = 1517.3(8)\text{ \AA}^3$ ;  $Z = 2$ ;  $R = 5.6\%$ ,  $R_w = 5.8\%$  for 2976 independent reflections.<sup>10</sup>

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## References and Notes

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- (10) Crystallographic data (including structure factor) for **3** have been deposited with the Cambridge Crystallographic Data Center (CCDC 173147). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax +44 (1223) 336033; e-mail deposit@ccdc, cam.ac.uk).

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