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## 3 $\beta$ -METHOXYCYCLOARTAN-27-OIC ACID AND OTHER CONSTITUENTS FROM THE LEAVES OF *PSEUDOTSUGA JAPONICA*

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ABSTRACT.—A new saturated tetracyclic triterpene acid was isolated from the leaves of *Pseudotsuga japonica*. The structure was established as 3 $\beta$ -methoxycycloartan-27-oic acid [**1**] on the basis of chemical and spectral evidence.

The genus *Pseudotsuga* (Pinaceae) consists of eight species including one each in Japan and Formosa, three species in China, and two species in western North America (1,2). As a part of our work on utilization of chemical constituents in the leaves and the bark of coniferous trees which have been considered to be waste products in the forest industry, we investigated constituents of the leaves of *Pseudotsuga japonica* (Shirasawa) Beissn. [Japanese name: Togasawara].

The literature survey on this genus revealed that several 9-oxobisaborane type sesquiterpenes (3,4) involving pseudotsugonal, a diterpene alcohol, thunbergol (5,6), and a triterpene alcohol, 24*R*-cycloeuocalanol (7) had been isolated from the timber of *Pseudotsuga menziesii* (Mirb.) Franco (Douglas fir). A sesquiterpene hydrocarbon, germacrene D, had also been isolated from the essential oil of *P. japonica*, although the plant part used for this experiment was not described (8).

*P. japonica* grows only in restricted regions in the Kii Mountain Range between Wakayama and Nara Prefectures and the eastern part of the mountains in Kohchi Prefecture, Japan (2,9). Repeated cc of the Et<sub>2</sub>O extract of the leaves led to the isolation of a new triterpene acid **1** along with the known  $\beta$ -eudesmol and rheosmin.

### RESULTS AND DISCUSSION

Compound **1** is one of the abundant constituents in the leaf extracts. The molecular formula was assigned as C<sub>31</sub>H<sub>52</sub>O<sub>3</sub> from the hreims spectrum. It showed a positive color with the Liebermann-Burchard reagent. The ir, <sup>1</sup>H-nmr, and <sup>13</sup>C-nmr spectra (Table 1) indicated the presence of a methylene group between two quaternary sp<sup>3</sup> carbons in a cyclopropane ring as a pair of AB doublets at  $\delta_H$  0.33 and 0.56, four quaternary methyl groups, two secondary methyl groups, a secondary methoxy group attributable to the C-3 $\beta$  (equatorial) position similar to the usual triterpene ether [ $\delta_H$  3.37 (OMe) and 2.73

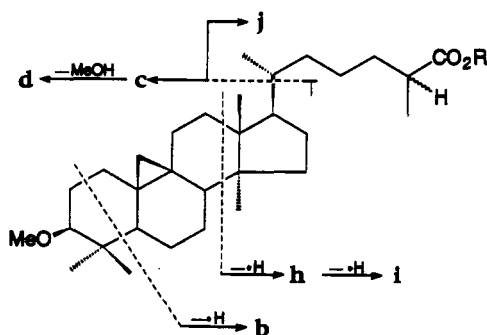


TABLE 1.  $^1\text{H}$ - (300 MHz) and  $^{13}\text{C}$ -nmr (74.5 MHz) Chemical Shifts of Compounds **1** and **2** in  $\text{CDCl}_3$  (TMS=0).

Position	$\delta_{\text{H}}$ Compound		$\delta_{\text{C}}$ Compound	
	<b>1</b>	<b>2</b>	<b>1</b>	<b>2</b>
1 .....			31.38	31.67
2 .....			33.94	34.08
3 .....	2.73 dd $J=11.2, 4.3$	2.70 dd $J=11.2, 4.3$	88.57	88.90
4 .....			40.48	40.36
5 .....			52.37	52.25
6 .....			20.96	20.81
7 .....			28.15	28.01
8 .....			47.67	47.57
9 .....			19.95	19.78
10 .....			26.26	26.10
11 .....			25.98	25.85
12 .....			35.54	35.41
13 .....			45.29	45.16
14 .....			48.80	48.71
15 .....			32.92	32.75
16 .....			26.51	26.34
17 .....			47.99	47.92
18 .....	0.89	0.85	19.32	19.14
19 .....	0.33 d $J=4.0$	0.32 d $J=4.0$	29.70	29.58
.....	0.56 d $J=4.0$	0.55 d $J=4.0$		
20 .....			35.93	35.81
21 .....	0.86 d $J=7.0$	0.79 d $J=7.0$	18.26	18.66
22 .....			36.01	35.88
23 .....			23.91	23.83
24 .....			25.43	25.27
25 .....			39.26	39.36
26 .....	1.18 d $J=7.0$	1.03 d $J=7.0$	16.73	16.79
27 .....			182.46	172.82
28 .....	0.95	0.89	25.53	25.37
29 .....	0.79	0.73	14.78	14.61
30 .....	0.95	0.89	18.03	17.86
OMe .....	3.37	3.31	57.63	57.61
CO <sub>2</sub> Me .....	—	3.61	—	51.44

( $\text{H}-\text{C}-\text{OMe}$ ), and a carboxyl group ( $\nu$  max 3550–3250, 1738 w, and 1700  $\text{cm}^{-1}$ ;  $\delta_{\text{C}}$  182.46). No olefin signal was observed. Together with the DEPT spectrum, these data indicated that compound **1** was a saturated methoxytetracyclic triterpene acid bearing a cyclopropane ring. Treatment of **1** with  $\text{CH}_2\text{N}_2$  in  $\text{Et}_2\text{O}$  afforded a methyl ester **2**, in which the ester methyl signal was observed at  $\delta_{\text{H}}$  3.61 and  $\delta_{\text{C}}$  51.44 in the  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr spectra, in addition to the methoxy signal ( $\delta_{\text{H}}$  3.31 and  $\delta_{\text{C}}$  57.61). As shown in Table 2, the 2D long range  $^1\text{H}$ - $^{13}\text{C}$  COSY of **1** indicated cross correlations among the signals of the methyl protons and the related carbons. One of two secondary methyl proton signals observed at  $\delta$  0.86 in the  $^1\text{H}$ -nmr spectrum of **1** was attributed to the methyl group on C-21 in the side chain. Therefore, the other signal appearing at low field ( $\delta_{\text{H}}$  1.18) was assigned to the methyl group at C-26, geminal to the 27-carboxyl at the C-25 position in the side chain of **1**.

Conclusive evidence for structure **1** was obtained by detailed eims analysis. In the hreims spectrum, **1** showed two predominant fragment ion peaks at  $m/z$  397.3104 [ $\text{C}_{27}\text{H}_{41}\text{O}_2$ ] $^+$  and 371.2950 [ $\text{C}_{25}\text{H}_{39}\text{O}_2$ ] $^+$ . The former was attributed to ion **a** arising from

TABLE 2. 2D Long Range  $^1\text{H}$ - $^{13}\text{C}$  COSY  
Data of Compound **1**.

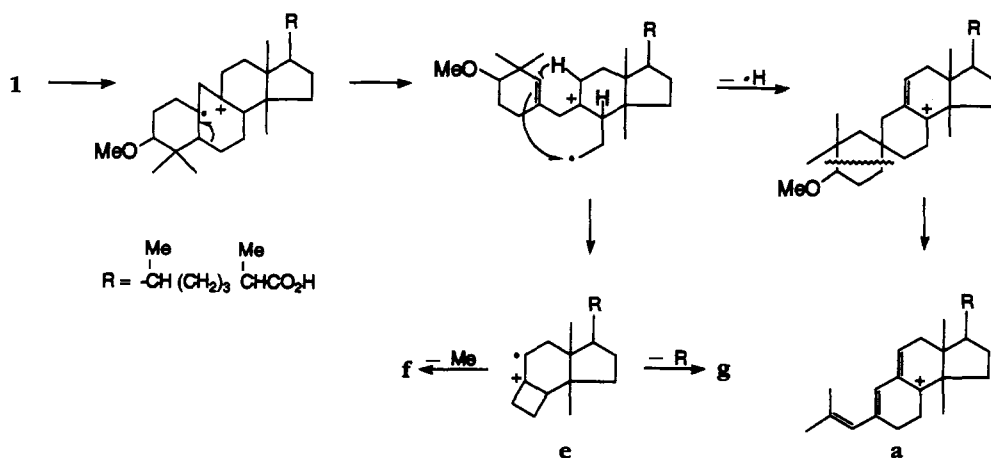
Me	Correlated C
18 .....	12, 13, 14, 17
21 .....	17, 20, 22
26 .....	24, 25, 26
28 .....	3, 4, 5, 29
29 .....	3, 4, 5, 28
30 .....	8, 13, 14, 15
OMe .....	3

fission of the C-5–C-6 bond from the  $[\text{M}]^+$  ion, formation of the spiro B ring by migration of H-11 to C-5, elimination of H-8, and subsequent cleavage of the ring A involving the loss of two hydrogen atoms. The latter peak was assigned to ion **b**,  $[\text{M}-\text{C}_6\text{H}_{13}\text{O}]^+$ , due to cleavage of C-1–C-2 and C-4–C-5 bonds of the A ring. Eight other predominant fragment peaks indicating **1** to be a cycloartane triterpene (10) were observed together with peaks at  $m/z$  457  $[\text{M}-\text{Me}]^+$ , 440  $[\text{M}-\text{MeOH}]^+$  (100%), and 425  $[\text{M}-\text{Me}-\text{MeOH}]^+$ . Fragments appearing at  $m/z$  329.2843  $[\text{C}_{23}\text{H}_{37}\text{O}]^+$  and 297.2582  $[\text{C}_{22}\text{H}_{33}]^+$  in **1** were assigned to ions **c** and **d** caused by loss of the side chain from the  $[\text{M}]^+$  ion and subsequent loss of a methanol from ion **c**, respectively. A peak observed at  $m/z$  318.2558  $[\text{C}_{21}\text{H}_{34}\text{O}_2]^+$  (**e**) was also typical for this ring system, and it was accompanied by two satellite peaks due to the further loss of a methyl and the side chain at  $m/z$  303.2323  $[\text{C}_{20}\text{H}_{31}\text{O}_2]^+$  (**f**) and 175.1486  $[\text{C}_{13}\text{H}_{19}]^+$  (**g**) (Scheme 1). Fragment peaks due to cleavage of the ring C were observed at  $m/z$  237.1854  $[\text{C}_{15}\text{H}_{25}\text{O}_2]^+$  (**h**) and 236.1776  $[\text{C}_{15}\text{H}_{24}\text{O}_2]^+$  (**i**), respectively, along with a peak for the side chain moiety at  $m/z$  143 (**j**). All corresponding fragment ions were also detected in the eims of **2** (see Experimental). Consequently, the structure of **1** was established to be 3 $\beta$ -methoxycycloartan-27-oic acid except for the stereochemistry of C-25.

This is the first report of a methoxycycloartane bearing a carboxyl group in the terminal position of the side chain from natural sources.

### EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were measured on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were taken in  $\text{CHCl}_3$  using a Jasco DIP-140

SCHEME 1. Mass spectral fragmentation of compound **1**.

polarimeter. Ir spectra were recorded as KBr disks with a Perkin-Elmer 1720X FT ir spectrophotometer.  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr spectra were obtained in  $\text{CDCl}_3$  on a Varian XL-300 instrument at 300 MHz and 74.5 MHz, respectively, using TMS as internal standard. Eims were recorded at 70 eV (probe) on a Hitachi M-80 double focusing mass spectrometer equipped with an M-003 data processor. Si gel 60 and alumina 90 (each Merck, 70–230 mesh) were used for cc. Si gel HF<sub>234</sub> (0.25 mm) and PF<sub>234</sub> (2 mm) were employed for tlc and preparative tlc.

**PLANT MATERIAL.**—The leaves of *P. japonica* were collected around the top of Mt. Nachi, Wakayama Prefecture, Japan, in July 1989. The plant was identified by Dr. Gen Murata, Department of Botany, Faculty of Science, Kyoto University. A voucher specimen is deposited at the Laboratory of Chemistry Herbarium in Osaka University of Pharmaceutical Sciences.

**ISOLATION OF COMPOUNDS.**—The air-dried leaves of *P. japonica* (2.02 kg) were extracted with  $\text{Et}_2\text{O}$  (3 liters $\times$ 5) using an automatic glass percolator. The resulting solutions were combined and evaporated in vacuo to give a dark green extract (71.4 g), which was subjected to cc on Si gel (3.5 kg). Elution of the column with  $\text{CHCl}_3$  afforded a yellow and oily eluate (A) (33.65 g) from fractions 25–103 (each 500 ml). Further cc yielded a crude crystalline mass (B) (4.3 g) from fractions 104–113 (each 500 ml) eluted with  $\text{CHCl}_3$ - $\text{EtOAc}$  (10:1). Repeated cc of A on alumina (1.5 kg) afforded a viscous oil (C) (976 mg) from fractions 64–92 (each 200 ml) eluted with  $\text{C}_6\text{H}_6$ - $\text{CHCl}_3$  (10:1). Further elution with  $\text{C}_6\text{H}_6$ - $\text{CHCl}_3$  (2:1) furnished the known 4-(4'-hydroxyphenyl)butan-3-one (rheosmin) (11,12) (167 mg), mp 80–82° (MeOH) [lit. (15) mp 81–82°], from fractions 197–216 (each 200 ml). Repeated cc ( $\times$ 2) of C on alumina (50 g) afforded a solid, 43 mg, from fractions 14–21 (each 100 ml) eluted with *n*-hexane- $\text{C}_6\text{H}_6$  (5:1). It was sublimed to give the known  $\beta$ -eudesmol (13–15) (31 mg), mp 73–75° (MeOH),  $[\alpha]^{23}_D + 63.6^\circ$  ( $c=0.44$ ) [lit. (12) mp 73–76°,  $[\alpha] + 65.1^\circ$  ( $c=2.84$ )]. Recrystallization of solid B from MeOH/ $\text{CHCl}_3$  furnished compound 1 (3.6 g).

**Compound 1.**—Prisms: mp 168–169.5°;  $[\alpha]^{23}_D + 33.2^\circ$  ( $c=0.34$ ); hreims  $m/z$   $[\text{M}]^+$  472.3917 ( $\text{C}_{31}\text{H}_{52}\text{O}_3$ , requires 472.3914); ir  $\nu$  max  $\text{cm}^{-1}$  3550–3250 br, 2917, 2850, 1738 sh, 1700 (COOH), 1466, 1420, 1376, 1362, 1188, 1135, 1102, 968, 930;  $^1\text{H}$  and  $^{13}\text{C}$  nmr see Table 1; eims  $m/z$   $[\text{M}]^+$  472 (25%),  $[\text{M}-\text{Me}]^+$  457 (21),  $[\text{M}-\text{MeOH}]^+$  440 (100),  $[\text{M}-\text{Me}-\text{MeOH}]^+$  425 (77),  $[\text{a}]^+$  397 (43),  $[\text{b}]^+$  371 (52),  $[\text{c}]^+$  329 (11),  $[\text{e}]^+$  318 (51),  $[\text{f}]^+$  303 (19),  $[\text{d}]^+$  297 (22),  $[\text{h}]^+$  237 (20),  $[\text{i}]^+$  236 (16), 203 (35),  $[\text{g}]^+$  175 (59),  $[\text{j}]^+$  143 (10).

**Methyl ester of compound 1.**—To a solution of compound 1 (35 mg) in  $\text{Et}_2\text{O}$  (3 ml) was added 30 ml of fresh  $\text{CH}_2\text{N}_2/\text{Et}_2\text{O}$  (1.1%) at room temperature for 1 h. Treatment of the mixture as usual afforded a residue (37 mg), which was purified by preparative tlc [*n*-hexane- $\text{CHCl}_3$  (2:1)] to give a methyl ester 2 as needles (29 mg): mp 105.5–108° (MeOH/ $\text{CHCl}_3$ );  $[\alpha]^{23}_D + 48.6^\circ$  ( $c=0.29$ ); hreims  $[\text{M}]^+ m/z$  486.4068 ( $\text{C}_{32}\text{H}_{54}\text{O}_3$ , requires 486.4072); ir  $\nu$  max  $\text{cm}^{-1}$  3041 (cyclopropane- $\text{CH}_2$ ), 2967, 2948, 2927, 2877, 1742 ( $\text{CO}_2\text{Me}$ ), 1465, 1377, 1341, 1201, 1164, 1154, 1143, 1099, 1010, 983;  $^1\text{H}$  and  $^{13}\text{C}$  nmr see Table 1; eims  $m/z$   $[\text{M}]^+$  486 (10%),  $[\text{M}-\text{Me}]^+$  471 (100),  $[\text{M}-\text{MeOH}]^+$  454 (19),  $[\text{M}-\text{Me}-\text{MeOH}]^+$  439 (69),  $[\text{a}]^+$  411 (52),  $[\text{b}]^+$  385 (66),  $[\text{e}]^+$  332 (65),  $[\text{c}]^+$  329 (17),  $[\text{f}]^+$  317 (23),  $[\text{d}]^+$  297 (30), 290 (11),  $[\text{h}]^+$  251 (23),  $[\text{i}]^+$  250 (25), 203 (46),  $[\text{g}]^+$  175 (81),  $[\text{j}]^+$  157 (14), 135 (51).

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