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SATURATED GAMMACERANES FROM THE STEM BARK OF *ABIES MARIESII*

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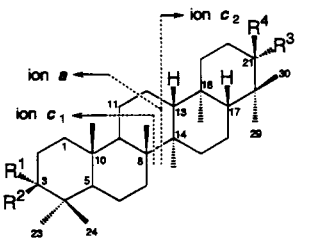
ABSTRACT.—Three previously unidentified gammaceranes, **1–3**, were isolated together with three known triterpenes, gammaceran-3β,21α-diol, hopan-3α,22-diol, and hopan-3β,22-diol, from the stem bark of *Abies mariesii*. The structures of these novel compounds were established as gammaceran-3,21-dione [**1**], gammaceran-3α,21β-diol [**2**], and 3α-hydroxygammaceran-21-one [**3**], respectively, on the basis of chemical and spectral evidence.

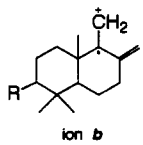
Previously, we have reported that the leaves and the stem bark of *Abies mariesii* Mast. (Pinaceae) (1) contain a considerable amount of abieslactone [3α-methoxy-9β*H*-lanosta-7,24-dien-26,23*R*-olide] (2,3). Further study on the chemical constituents of another *Abies* species led to the isolation of a considerable number of abieslactone analogues (4–7) from the stem bark of *A. firma* Sieb. et Zucc. and *A. veitchii* Lindl. Recently, we have isolated from the bark of the latter tree an unusually migrated lanostane derivative named spiroveitchionolide [(3*R*,7*S*,9*R*,23*R*)-7-hydroxy-3-methoxy-8-oxo-7(8→9)-*abeo*-lanost-24-eno-26,23-lactone] (8), together with gammaceran-3β,21α-diol [**3**] and hopan-3α,22-diol [**4**] (9). In addition, several γ-keto acids and their methyl esters prepared from abieslactone and its analogues, as well as the 25,26,27-trisnor-α-hydroxy acid derived from abieslactone, have been found to strongly suppress tumor promoter-induced phenomena in vitro and in vivo without any toxicity (10). These results prompted us to reexamine the stem bark constituents of *A. mariesii*. This paper deals with the characterization of three novel gammaceranes, **1–3**, isolated from the stem bark of this plant together with three known triterpene diols.

RESULTS AND DISCUSSION

The three known triterpene diols were confirmed as gammaceran-3β,21α-diol [**4**] (9), hopan-3α,22-diol [**5**] (9), and hopan-3β,22-diol [**6**] (11,12) by comparison with literature data.

Compound **1** was assigned the molecular formula $C_{30}H_{48}O_2$ by hreims. The ir spectrum exhibited the presence of a six-membered ring ketone (ν max 1708 cm^{-1}), while no ir absorption band was observed due to an ethylene bond. The ^1H- and ^{13}C-nmr data of **1** (Tables 1 and 2), determined with a DEPT experiment, showed signals of four





1 R^1,R^2=O, R^3,R^4=O
2 R^1,R^4=OH, R^2,R^3=H
2a R^1,R^4=OAc, R^2,R^3=H
3 R^1=OH, R^2=H, R^3,R^4=O
3a R^1=OAc, R^2=H, R^3,R^4=O
4 R^2,R^3=OH, R^1,R^4=H

TABLE 1. ^{1}H-Nmr Chemical Shifts of Compounds **1**, **2**, **2a**, **3**, **3a**, and **4** in $CDCl_3$ (300 MHz, TMS=0).[a]

Proton(s)	Compound					
	1	**2**	**2a**	**3**	**3a**	**4**[b]
Me-23	1.08	0.94	0.84	0.94	0.84	0.97
Me-24	1.03	0.83	0.87	0.83	0.87	0.76
Me-25	0.94	0.83	0.85	0.84	0.85	0.82
Me-26	1.01	0.97	1.02	0.97	0.98	0.96
Me-27	1.01	0.97	1.02	1.02	1.05	0.96
Me-28	0.94	0.83	0.85	0.93	0.93	0.82
Me-29	1.03	0.83	0.87	1.01	1.03	0.76
Me-30	1.08	0.94	0.84	1.07	1.08	0.97
H-3	—	3.39 t J=2.7 Hz	4.62 t J=2.7 Hz	3.40 t J=2.7 Hz	4.62 t J=2.7 Hz	3.20 dd J=11.6 and 5.7 Hz
H-21	—	3.39 t J=2.7 Hz	4.62 t J=2.7 Hz	—	—	3.20 dd J=11.6 and 5.7 Hz
OAc	—	—	2.08	—	2.08	—
OAc	—	—	2.08	—	—	—

[a]Assignments were made by 2D ^{1}H-^{1}H COSY, 2D ^{1}H-^{13}C COSY and 2D long-range ^{1}H-^{13}C COSY experiments.

[b]Data cited from Tanaka and Matsunaga (9).

quaternary methyl groups, five methylene groups, two methine groups, three sp^3 carbons and a carbonyl carbon (δ_C 218.08), corresponding to one half of the molecular formula. In the eims spectrum, compound **1** showed only two predominant fragment ion peaks at *m/z* 220.1826 $[C_{15}H_{24}O]^+$ (ion **a**) and 205.1586 $[C_{14}H_{21}O]^+$ (100%, ion **c**) between 200 and 400 mass units, together with peaks at *m/z* 440 $[M]^+$ and 425.3420 $[M-Me]^+$. Ion **a** corresponded to a half moiety of a symmetrical gammacerane system and ion **c** was assigned to the moiety of the genuine A/B or D/E rings arising from the cleavage between the C-8–C-14 and C-9–C-11 or C-12–C-13 bonds of the system (9), indicating **1** to be a symmetrical gammaceranedione. Thus, ^2J and ^3J C/H correlations obtained from the 2D long-range ^{1}H-^{13}C COSY data of **1** could be magnified as shown in Table 3 and the two keto groups were located at the C-3 and C-21 positions of the gammacerane skeleton. The complete structure was determined by synthesis. Oxidation of gammaceran-3β,21α-diol [**4**] with CrO_3 in pyridine furnished gammaceran-3,21-dione, identical in all respects with compound **1**.

Compound **2** was shown to have the molecular formula $C_{30}H_{52}O_2$ by hreims. The ir spectrum exhibited only one characteristic absorption band for a hydroxy group (ν max 3465 cm^{-1}, br). Its ^{1}H- and ^{13}C-nmr spectra (Tables 1 and 2) also revealed signals corresponding to a half of the molecular formula and apparent in the eims spectrum were fragment ion peaks typical for cleavage of gammaceranediol at *m/z* 426.3832 $[M-H_2O]^+$, 411 $[M-Me-H_2O]^+$, 408.3753 $[M-2H_2O]^+$, 220 (ion **b**), 207.1746 $[C_{14}H_{23}O]^+$ (ion **c**), and 189.1642 (ion **c**$-H_2O$, 100%). Although the signal patterns of **2** in the ^{1}H- and ^{13}C-nmr spectra resembled those of **4**, the signals due to the hydroxy methine group in the former at δ_H 3.39 (1H, narrow t, J=2.7 Hz) and δ_C 72.28 (d, C-3) were different from those of the latter. Acetylation of **2** gave a diacetate [**2a**], *m/z* 528 $[M]^+$, in which the methine proton signal geminal to the hydroxy group was shifted to δ 4.62 (1H, narrow t, J=2.7 Hz) (4), indicating the two hydroxy groups in **2** to have the same axial symmetry. Oxidation of **2** with CrO_3 in pyridine gave the dione identical to **1**. All the above data clearly proved the structure of **2** to be gammaceran-3α,21β-diol.

TABLE 2. ^{13}C-Nmr Chemical Shifts of Compounds **1**, **2**, **2a**, **3**, **3a**, and **4** in $CDCl_3$ (74.5 MHz, TMS=0).[a]

Carbon	Compound					
	1	**2**	**2a**	**3**	**3a**	**4**[b]
1	39.54	33.21	33.85	33.23	33.89	38.66
2	34.14	25.35	22.86	25.34	22.85	27.36
3	218.08	72.28	78.34	76.20	78.31	79.00
4	47.34	37.50	36.63	37.51	36.64	38.83
5	54.80	48.87	49.92	48.83	50.04	55.10
6	19.70	18.32	18.18	18.29	18.16	18.37
7	32.38	32.87	32.82	32.93	32.85	33.06
8	41.69	41.95	41.94	41.94	41.95	41.66
9	49.67	49.98	50.02	50.10	50.04	50.21
10	36.75	37.11	37.03	37.14	37.04	37.03
11	21.69	21.04	20.99	21.09	21.05	21.15
12	21.69	21.04	20.99	21.69	21.69	21.15
13	49.67	49.98	50.02	49.54	49.54	50.21
14	41.69	41.95	41.94	41.69	41.72	41.66
15	32.38	32.87	32.82	32.34	32.40	33.06
16	19.70	18.32	18.18	19.72	19.76	18.37
17	54.80	48.87	49.92	54.87	54.85	55.10
18	36.75	37.11	37.03	36.75	36.77	37.03
19	39.54	33.21	33.85	39.56	39.54	38.66
20	34.14	25.35	22.86	34.20	34.17	27.36
21	218.08	72.28	78.34	218.27	218.12	79.00
22	47.34	37.50	36.63	47.37	47.35	38.83
23	26.57	28.27	27.84	28.26	27.82	28.00
24	21.08	22.14	21.77	22.13	21.69	15.36
25	15.87	15.77	15.76	15.84	15.83	15.95
26	16.17	16.57	16.65	16.38	16.44	16.45
27	16.17	16.57	16.65	16.38	16.44	16.45
28	15.87	15.77	15.76	15.84	15.83	15.95
29	21.08	22.14	21.77	21.09	21.10	15.36
30	26.57	28.27	27.84	26.55	26.61	28.00
OCO*Me*	—	—	21.43	—	21.41	—
OCO*Me*	—	—	21.43	—	—	—
OCOMe	—	—	170.87	—	170.82	—
OCOMe	—	—	170.87	—	—	—

[a]Assignments were made by 2D ^{1}H-^{1}H COSY, 2D ^{1}H-^{13}C COSY and 2D long-range ^{1}H-^{13}C COSY experiments.

[b]Data cited from Tanaka and Matsunaga (9).

Compound **3** was accorded the molecular formula $C_{30}H_{50}O_2$ by hreims. The ir, ^{1}H-, and ^{13}C-nmr spectra (Tables 1 and 2), obtained with a DEPT experiment, indicated that **3** was composed of eight quaternary methyls, ten methylenes, four methines, six quaternary sp^3 carbons, a secondary hydroxy group [ν max 3542 cm^{-1}; δ_H 3.40 (1H, narrow t, J=2.7 Hz); δ_C 76.20] which can be located at the C-3 position as in typical triterpenols from a biogenetic view-point, and a saturated six-membered ring ketone (ν max 1701 cm^{-1}; δ_C 218.27). In the eims spectrum, **3** showed a pair of predominant fragment peaks arising from ion **c** at *m/z* 207.1746 $[C_{14}H_{22}OH]^+$ (ion $\mathbf{c}_1$) and 205.1583 $[C_{13}H_{21}C{=}O]^+$ (ion $\mathbf{c}_2$), along with peaks at *m/z* 424 $[M-H_2O]^+$, 409 $[M-Me-H_2O]^+$, 222 (ion **a**), 218 (ion **b**), and 189.1645 (ion $\mathbf{c}_1-H_2O$, base peak), suggesting it was a gammaceranolone. Oxidation of **3** with CrO_3 in pyridine afforded the dione identical with **1**. Upon acetylation, it gave a keto-acetate [**3a**], *m/z* 484 $[M]^+$, in which the carbinolic methine proton signal observed at δ 3.40 in **3** was shifted to δ 4.62 (1H,

TABLE 3. ^2J and ^3J H/C Correlations of Methyl Proton Signals in the 2D Long-range ^{1}H-^{13}C COSY Nmr Spectrum of Compound **1**.

Compound **1**			
Proton		Correlated Carbon	
Number	δ_H	δ_C	Cn
Me-23	1.08	218.08	3, 21
		47.34	4, 22
Me-30		54.80	5, 17
		21.08	24, 29
Me-24	1.03	218.08	3, 21
		47.34	4, 22
Me-29		54.80	5, 17
		26.57	23, 30
Me-25	0.94	39.54	1, 19
		54.80	5, 17
Me-28		49.67	9, 13
		36.75	10, 18
Me-26	1.01	32.38	7, 15
		41.69	8, 14
Me-27		49.67	9, 13

narrow t, J=2.7 Hz). Signal patterns of the methine protons in **3** and **3a** were in good agreement with those of **2** and **2a**. Thus, the hydroxy group of **3** was confirmed to have 3α-orientation and the structure was unambiguously determined as 3α-hydroxygammaceran-21-one.

Compounds **1** through **3** are novel, and this report represents only the second instance of the isolation of hopane and gammacerane derivatives from *Abies* species (9). Accordingly, these compounds seem to be chemotaxonomically important.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were measured on a Yanagimoto micro melting-point apparatus and are uncorrected. Optical rotations were taken using a Jasco DIP-140 polarimeter. Ir spectra were run as KBr disks with a Perkin-Elmer model 1720 Ft-ir spectrophotometer. ^{1}H- and ^{13}C-nmr spectra were obtained in $CDCl_3$ 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 a M-003 data processor. Si gel 60 (Merck, 70–230 mesh) and alumina 90 (Merck, 70–230 mesh) were used for cc. Si gel PF_{254} plates (Merck, 2 mm) were employed for prep. tlc.

PLANT MATERIAL.—The stem bark of *Abies mariesii* Mast. was collected in mountainous terrain under the control of the National Yamaguchi Forestry Office, Fukushima Prefecture, Japan, in July 1991. A voucher specimen (AM-9107-3) is deposited at the Herbarium of the Laboratory of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences.

EXTRACTION AND ISOLATION.—The air-dried and finely chopped stem bark of *A. mariesii* (12 kg) was extracted with CH_2Cl_2 (15 liters×5) employing an automatic glass percolator. The resulting CH_2Cl_2 solutions were combined and concentrated to give a tarry extract (564.8 g), which was subjected to Si gel (7 kg) cc. Elution of the column with $CHCl_3$ afforded residues **A** (38.88 g) and **B** (23.97 g) from fractions 42–48 and 57–98 (each fraction=1 liter). Repeated cc of **A** on Si gel (1.1 kg) gave a viscous residue (7.62 g) from the fractions eluted with $CHCl_3$, which was rechromatographed using alumina (280 g) and C_6H_6 to give compounds **1** (28 mg) and **2** (315 mg) and a solid mixture (276 mg) from fractions 29–37, 46–64, and 65–106 (each fraction=100 ml), respectively. Repeated cc of the solid mixture over 10% $AgNO_3$-impregnated alumina furnished compound **3** (23 mg) from the fractions eluted with *n*-hexane-C_6H_6 (1:1). Repeated cc of **B** on Si gel successively afforded solid mixture [**C**] (10.57 g) and the known gammaceran-

3β,21α-diol [**4**], 232 mg, mp 312–315° (MeOH/$CHCl_3$), $[\alpha]^{23}D$ +21.6° (*c*=0.25, $CHCl_3$) [lit. (9) mp >310°, $[\alpha]D$ +21°] from fractions 36–49 and 50–72 (each fraction=200 ml) eluted with $CHCl_3$-EtOAc (20:1), respectively. Repeated cc of **C** over alumina (800 g), consecutively afforded two known compounds, hopan-3α,22-diol [**5**], 151 mg, mp 275–278° (MeOH/$CHCl_3$), $[\alpha]^{23}D$ +30.6° (*c*=0.22, $CHCl_3$) [lit. (9) mp 278–280.5° (MeOH/$CHCl_3$), $[\alpha]^{23}D$ +31°], and hopan-3β,22-diol [**6**], 232 mg, mp 270–273° (MeOH/$CHCl_3$), $[\alpha]^{23}D$ +51.8° (*c*=0.23, $CHCl_3$) [lit. (11) mp 284–285°, $[\alpha]D$ +53.0°], from the fractions eluted with C_6H_6-$CHCl_3$ (2:1). Compounds **4** and **5** were identified by direct comparison (mmp, co-tlc, ir, ^1H- and ^{13}C-nmr and eims) with authentic samples and **6** was confirmed by physical and spectral correlation with data previously reported (11,12).

COMPOUND **1**.—Prisms, mp 308–311° (MeOH/$CHCl_3$), $[\alpha]^{23}D$ +91.6° (*c*=0.36, $CHCl_3$); ir ν max 2946, 2890, 1708 (six-membered-ring ketone), 1456, 1425, 1387, 1378, 1226, 1142, 1114, 1019, 1006, and 976 cm^{-1}; ^1H and ^{13}C nmr, see Tables 1 and 2; hreims $[M]^+$ *m/z* 440.3655 ($C_{30}H_{48}O_2$ requires 440.3652); eims *m/z* $[M]^+$ 440 (31%), 425 (5), 220 (14), and 205 (100).

Synthesis of **1**.—To a solution of gammaceran-3β,21α-diol [**4**] (28 mg) in pyridine (2 ml) was added a solution of CrO_3 (50 mg) in pyridine (2 ml) under stirring at 5° and the mixture was kept at room temperature for 5 h. The usual workup yielded a solid (25 mg), which was purified by prep. tlc (solvent=$CHCl_3$) to give gammaceran-3,21-dione as prisms, mp 307–310° (MeOH/$CHCl_3$), $[\alpha]^{23}D$ +90.9° (*c*=0.45, $CHCl_3$), eims $[M]^+$ *m/z* 440, which was identified by direct comparison (mmp, co-tlc, ir, ^1H and ^{13}C nmr and eims) with compound **1**.

COMPOUND **2**.—Prisms, mp 310–312° (MeOH-$CHCl_3$), $[\alpha]^{23}D$ +3.6°, (*c*=0.69, $CHCl_3$); ir ν max 3465 (OH), 2945, 2872, 1457, 1386, 1365, 1244, 1142, 1067, and 994 cm^{-1}; ^1H and ^{13}C nmr, see Tables 1 and 2; hreims $[M]^+$ *m/z* 444.3976 ($C_{30}H_{52}O_2$ requires 444.3965); eims *m/z* $[M]^+$ 444 (7%), 426 (9), 411 (3), 408 (1), 393 (1), 220 (14), 207 (71), 203 (14), and 189 (100).

Acetylation of **2**.—Compound **2** (40 mg) was dissolved in pyridine and Ac_2O (each 1 ml) and the mixture was kept at room temperature overnight. The usual workup afforded diacetate [**2a**] (41 mg), mp 335–338° (MeOH/$CHCl_3$), $[\alpha]^{23}D$ −38.1° (*c*=0.59, $CHCl_3$); ir ν max 2845, 2872, 1724 (OAc), 1465, 1451, 1393, 1373, 1250 (OAc), 1183, 1059, 1043, 1033, 1018, and 990 cm^{-1}; ^1H and ^{13}C nmr, see Tables 1 and 2; eims *m/z* $[M]^+$ 528 ($C_{34}H_{56}O_4$, 4%), 468 (10), 453 (2), 408 (11), 393 (2), 262 (14), 249 (8), 203 (13), and 189 (100).

Oxidation of **2**.—A solution of compound **2** (26 mg) in pyridine (2 ml) was oxidized with a mixture of CrO_3 (50 mg) and pyridine (2 ml) under the same conditions described above to give the dione, mp 307–310° (MeOH/$CHCl_3$), $[\alpha]^{23}D$ +92° (*c*=0.58, $CHCl_3$), identical in all respects (mmp, co-tlc, ir, ^1H and ^{13}C nmr and eims) with **1**.

COMPOUND **3**.—Needles, mp 324–327° (MeOH/$CHCl_3$), $[\alpha]^{23}D$ +37.1° (*c*=0.65, $CHCl_3$); ir ν max 3542 (OH), 2941, 2871, 1701 (six-membered-ring ketone), 1461, 1386, 1364, 1312, 1233, 1067, and 998 cm^{-1}; ^1H and ^{13}C nmr, see Tables 1 and 2; hreims $[M]^+$ *m/z* 442.3817 ($C_{30}H_{50}O_2$ requires 442.3808); eims *m/z* $[M]^+$ 442 (11%), 424 (15), 409 (12), 232 (3), 222 (4), 218 (4), 207 (56), 205 (31), 203 (12), and 189 (100).

Acetylation of **3**.—Compound **3** (15 mg) was acetylated as usual (pyridine and Ac_2O, each 1 ml) to give the keto-acetate [**3a**] (15 mg), mp 275–277° (MeOH/$CHCl_3$), $[\alpha]^{23}D$ +17.2° (*c*=0.43, $CHCl_3$); ir ν max 2945, 2872, 1724 (OAc), 1451, 1393, 1373, 1250 (OAc), 1043, 1033, 1018, and 990 cm^{-1}; ^1H and ^{13}C nmr, see Tables 1 and 2; eims *m/z* $[M]^+$ 484 (3%), 424 (18), 409 (10), 249 (5), 232 (4), 218 (26), 205 (28), 203 (12), and 189 (100).

Oxidation of **3**.—A mixture of **3** (10 mg) in pyridine (1.5 ml) was treated with a solution of CrO_3 (19 mg) in pyridine (1.5 ml) under the same conditions described above. The resulting product was purified by prep. tlc (plate=20×20 cm, solvent=$CHCl_3$) to give the dione (8 mg), mp 307–309° (MeOH-$CHCl_3$), $[\alpha]^{23}D$ +91° (*c*=0.25, $CHCl_3$), identical in all respects (mmp, co-tlc, ir, ^1H and ^{13}C nmr and eims) with gammaceran-3,21-dione [**1**].

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LITERATURE CITED

1. T. Makino, in: "New Illustrated Flora of Japan." Ed. by M. Ono, H. Ohba, and M. Nishida, Hokuryu-kan, Tokyo, 1989, p. 8.

2. S. Uyeo, J. Okada, S. Matsunaga, and J.W. Rowe, *Tetrahedron*, **24**, 2859 (1968).
3. F.H. Allen, N.W. Isaacs, O. Kennard, and W.D.S. Motherwell, *J. Chem. Soc., Perkin Trans. II*, 498 (1973).
4. R. Tanaka and S. Matsunaga, *Phytochemistry*, **29**, 3267 (1990).
5. R. Tanaka and S. Matsunaga, *J. Nat. Prod.*, **54**, 1337 (1991).
6. R. Tanaka, A. Inoshiri, M. Yoneda, T. Ishida, A. Numata, and S. Matsunaga, *Phytochemistry*, **29**, 3263 (1990).
7. R. Tanaka and S. Matsunaga, *Phytochemistry*, **30**, 1983 (1991).
8. R. Tanaka, Y. Usami, Y. In, T. Ishida, T. Shingu, and S. Matsunaga, *J. Chem. Soc., Chem. Commun.*, 1351 (1992).
9. R. Tanaka and S. Matsunaga, *Phytochemistry*, **31**, 3535 (1992).
10. J. Takayasu, R. Tanaka, S. Matsunaga, H. Ueyama, H. Tokuda, T. Hasegawa, A. Nishino, H. Nishino, and A. Iwashima, *Cancer Lett.*, **53**, 141 (1990).
11. J. Cerny, A. Vystrcil and S. Huneck, *Chem. Ber.*, **96**, 3021 (1963).
12. W.-H. Hui and M.-M. Li, *J. Chem. Soc., Perkin Trans. I*, 897 (1977).

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