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Studies on Marine Natural Products. II.[1] New Polyhydroxylated Sterols from the Soft Coral *Lobophytum pauciflorum* (Ehrenberg)

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Three new polyhydroxylated sterols (II), (IV) and (VI), along with the known sterol (I), were isolated from the Japanese soft coral *Lobophytum pauciflorum* (Ehrenberg). The structures of II, IV and VI were established to be 24ξ-methylcholestane-3β,5α,6β,25-tetrol, 24ξ-methylcholestane-1β,3β,5α,6β-tetrol and 24-methylenecholestane-1β,3β,5α,6β-tetrol, respectively.

Keywords——soft coral; *Lobophytum pauciflorum* (Ehrenberg); tetrahydroxy 24ξ-methylcholesterols; tetrahydroxy 24-methylenecholesterol; ^{13}C NMR

Extensive studies on sterols from marine sources have recently been reported.[3,4] Some of these sterols are of interest in connection with the biosynthesis of their characteristic side chain moieties.[3b] Marine sterols are often found in highly oxygenated forms, and such sterols sometimes show pharmacological activities. We have therefore investigated the chemical constituents of several Japanese soft corals. In the present paper we describe the isolation and structural studies of three new polyhydroxylated sterols (II), (IV) and (VI) from the soft coral *Lobophytum pauciflorum* (Ehrenberg). We have already isolated 13-membered carbocyclic cembranolide diterpenes from this soft coral.[1]

The ether extract, obtained from *Lobophytum pauciflorum* (Ehrenberg) according to the procedure shown in Fig. 1, was chromatographed on a silica gel column, eluting with *n*-hexane-acetone mixtures of increasing polarity and then with methanol, to give fractions 1—9 in order of elution. From fractions 5 and 6, 13-membered carbocyclic cembranolides were isolated.[1] Fractions eluted with a more polar solvent system appeared to contain sterols. Fraction 8, which was eluted with *n*-hexane–acetone (1:1), was subjected to high-performance liquid chromatography using a silica gel pre-packed column eluted with chloroform–methanol mixtures, to give two crystalline substances, I (mp 226°) and II (mp 255—256°), and an amorphous substance (mp 234—240°) in order of elution.

The amorphous substance was shown to be a mixture of a sterol and its dehydro derivative by analyses of the proton nuclear magnetic resonance (^1H-NMR) spectrum and the mass spectrum (MS) [*m/e* 450 (M^+) and *m/e* 448 (M^+)], although only a single spot was seen on thin-layer chromatography with various solvent systems. Since the infrared (IR) spectrum of the mixture showed hydroxyl absorption but no acetoxyl absorption, separation

1) Part I: Y. Yamada, S. Suzuki, K. Iguchi, K. Hosaka, H. Kikuchi, Y. Tsukitani, H. Horiai, and F. Shibayama, *Chem. Pharm. Bull.*, **27**, 2394 (1979).
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3) *a)* L. Minale and G. Sodano, "Marine Natural Products Chemistry," Plenum Press, New York and London, 1977, p. 87; *b)* C. Djerassi, R.M.K. Carlson, S. Popov, and T.H. Varkony, *ibid.*, 1977, p. 111. *c)* F.J. Schmitz, "Marine Natural Products," Vol. 1, Academic Press, New York, 1978, p. 241.
4) Some recent studies include: N. Theobald, J.N. Shoolery, C. Djerassi, T.R. Erdman, and P.J. Scheuer, *J. Am. Chem. Soc.*, **100**, 5574 (1978); N. Theobald, R.J. Wells, and C. Djerassi, *J. Am. Chem. Soc.*, **100**, 7677 (1978); A. Maquestiau, Y. van Haverbeke, R. Flammang, H. Mispreuve, M. Kaisin, J.C. Braekman, D. Daloze, and B. Tursch, *Steroid*, **31**, 31 (1978).

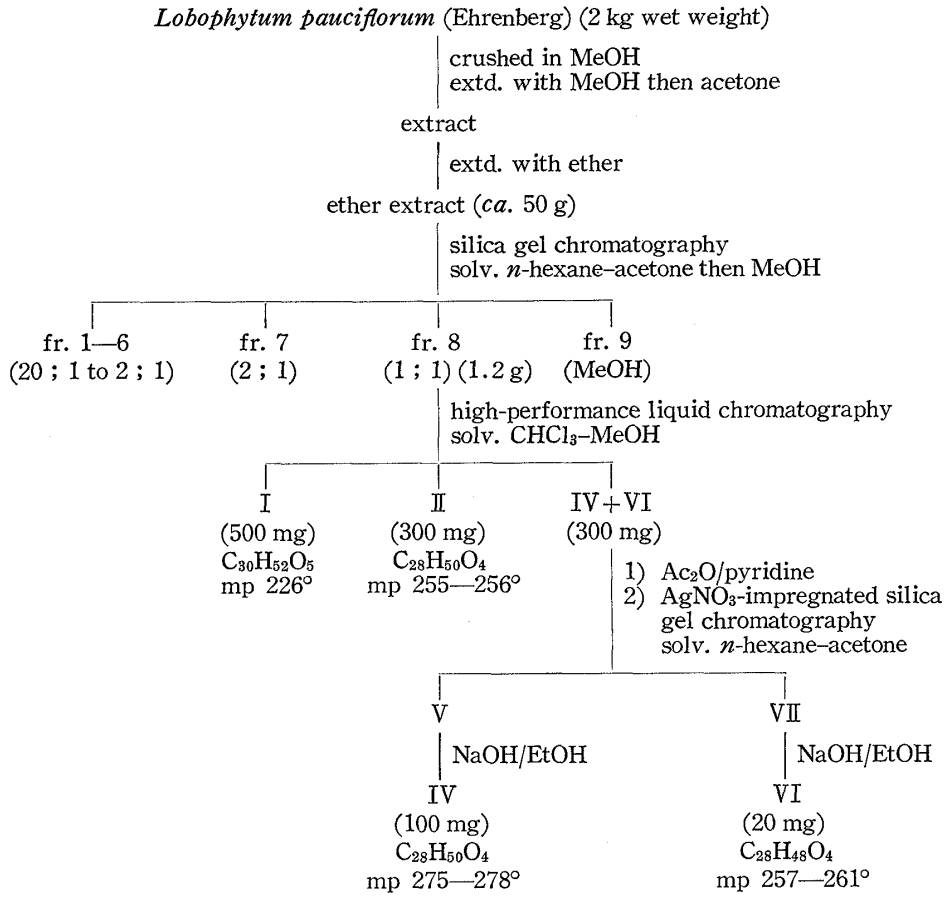


Fig. 1. Isolation of Polyhydroxylated Sterols from *Lobophytum pauciflorum* (Ehrenberg)

of this mixture was attempted by the following procedure; 1) acetylation of the mixture, 2) separation by silver nitrate-impregnated silica gel chromatography, and 3) alkaline hydrolysis of each acetate. Compound (IV) (mp 275—278°) and (VI) (mp 257—261°) were thus separated, though each substance still remained amorphous. The ^{1}H-NMR spectrum of the original mixture was shown to be an overlap of the spectra of IV and VI, and this was also the case with their mass spectra.

The spectral data for the crystalline compound (I)[$C_{30}H_{52}O_5$, $[\alpha]_D^{20}$ −17° (*c* 0.3, EtOH)] suggested that I must be 24ξ-methylcholestane-3β,5α,6β,25-tetrol 25-monoacetate, previously isolated from the soft coral *Sarcophyton elegans* by Djerassi *et al.*[5] The physical properties of I coincided in every respect with those of an authentic specimen.

The physical data for II, IV and VI are shown in Table I. These data indicate that the compounds are new polyhydroxylated sterols.

The spectral data for compound (II) clearly show the presence of four tertiary methyl groups, two secondary methyl groups and four hydroxy groups. Acetylation of II gave a diacetate (III) [mp 217—220°, ^{1}H-NMR δ 2.03 (3H, s) and 2.07 (3H, s)]. Comparison of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of II with that of the known compound (I) led to the assignment of the structure shown for II. As summarized in Table II,[6] the signals of both compounds are closely related, except for the absence of signals due

5) J.M. Moldowan, B.M. Tursch, and C. Djerassi, *Steroid*, **24**, 387 (1974).
6) Assignments of the ^{13}C signals were made on the basis of the chemical shift rules[7] and by comparison with the spectra of related sterols.[8]
7) J.B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, 1972.
8) J.W. Blunt, *Aust. J. Chem.*, **28**, 1017 (1975).

to an acetyl group in II and the signals underlined. The remarkable high field shift (−13.3 ppm) of the C-25 signal together with the low field shifts (+3.6—+4.7 ppm) of the C-24, C-26 and C-27 signals suggests that the acetoxyl group at the C-25 position in I is replaced by a hydroxyl group in II. This was confirmed by alkaline hydrolysis of I to II. Thus the structure of II was established as 24ξ-methylcholestane-3β,5α,6β,25-tetrol.[9]

Table I. Physical Data for II, IV and VI

	II	IV	VI
Molecular formula	$C_{28}H_{50}O_4$	$C_{28}H_{50}O_4$	$C_{28}H_{48}O_4$
$[\alpha]_D^{20}$ (EtOH)	−21° (*c* 0.3)	−10° (*c* 0.28)	−3° (*c* 0.14)
ν_{max}^{KBr} cm^{-1}	3400, 1040	3370, 1040, 1005	3350, 1638, 1040, 1005, 885
^{1}H-NMR (CD_3OD) δ	0.72(3H, s)	0.72(3H, s)	0.72(3H, s)
	0.90(3H, d, J=7.0 Hz)	0.81(3H, d, J=6.0 Hz)	0.96(3H, d, J=6.0 Hz)
	0.97(3H, d, J=6.5 Hz)	0.86(6H, d, J=6.5 Hz)	1.03(6H, d, J=7.0 Hz)
	1.13(6H, s)	0.95(3H, d, J=6.0 Hz)	1.13(3H, s)
	1.17(3H, s)	1.13(3H, s)	3.43(1H, br)
	3.46(1H, br)	3.43(1H, br)	3.88(2H, m)
	4.00(1H, m)	3.95(2H, m)	4.66(1H, brs)
			4.72(1H, br)

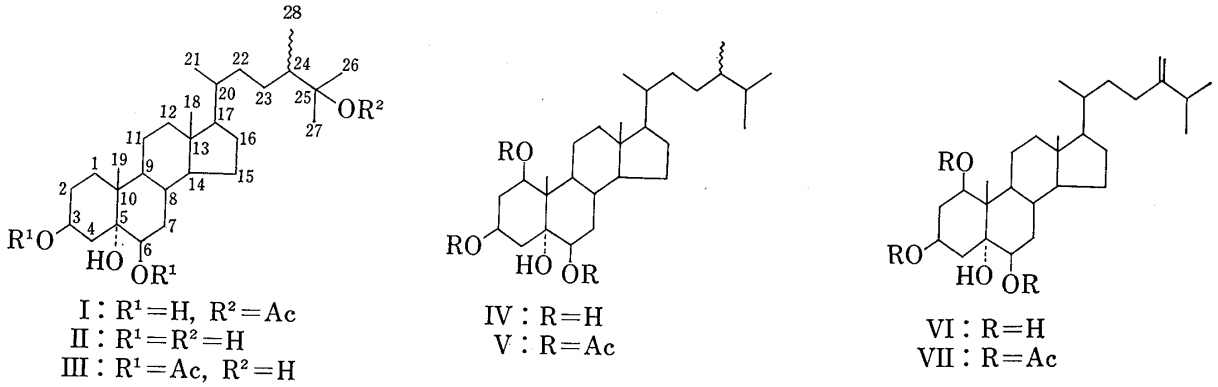
Table II. ^{13}C-NMR Chemical Shifts[a] of the Sterols (I), (II) and (IV)

Carbon	I	II	IV
C-1	32.4	32.5	73.7(72.5)
C-2	33.2	33.3	44.1[b] (44.3)
C-3	67.3	67.4	65.3(65.2)
C-4	42.7	42.8	43.2[b]
C-5	75.8	75.9	76.9
C-6	76.2	76.3	77.0
C-7	35.6	35.5[b]	35.7
C-8	31.3	31.2	31.7[c]
C-9	45.8	46.0	47.1
C-10	39.0	39.1	44.9(45.3)
C-11	21.7	21.8	25.0[d] (25.6)
C-12	40.5	40.6	41.3
C-13	43.0	43.0	42.6
C-14	56.3[b]	56.4[c]	56.7
C-15	24.6	24.6	24.9[d]
C-16	28.0[c]	28.4[d]	28.5
C-17	56.4[b]	56.5[c]	56.7
C-18	12.4	12.4	12.5
C-19	17.2	17.3	10.7(11.8)
C-20	35.0	36.8	34.0
C-21	19.1	19.3	19.0
C-22	36.5	35.7[b]	36.6
C-23	28.5[c]	28.6[d]	31.0
C-24	42.3	46.0	39.3
C-25	85.5	72.2	31.9[c]
C-26	23.0[d]	26.6[e]	17.8[e]
C-27	23.4[d]	28.1[e]	20.7[e]
C-28	14.6	15.1	15.7
$OCO\underline{C}H_3$	22.3[d]		
$O\underline{C}OCH_3$	169.9		

a) The spectra were taken with a JEOL FX-100 spectrometer (25.0 MHz) in C_5D_5N with TMS as an internal reference.
b)—*e*) Assignments may be reversed in each column.

9) This is the first isolation of II[5] from a natural source.

The third compound (IV) showed the same molecular formula ($C_{28}H_{50}O_4$) as II. Comparison of the ^{1}H–NMR spectra of IV and II showed that the hydroxyl group at C-25 must be absent in IV; instead of a singlet [1.13(6H)] due to the methyl protons at C-26 and C-27 in II, a doublet at δ 0.86 (6H, J=6.5 Hz) due to an isopropyl group was observed in IV. In addition to the changes on the side chain moiety, a new signal due to a proton on a carbon atom of a secondary alcohol appeared at δ 3.95 (1H, m) (overlapping with the multiplet of the C-3 proton), which was shifted to lower field at δ 5.20 (1H, dd, J=6.0, 11 Hz) in the triacetate (V). The coupling constants of this signal suggested that the secondary hydroxyl group was located at either C-1 or C-12. Analysis of the ^{13}C–NMR spectrum of IV suggested its location at C-1 with a β-configuration, on the basis of the following findings. By applying the hydroxyl substituent effects on the ^{13}C signals of sterols reported by Djerassi *et al.*,[10] the ^{13}C chemical shifts for C_1-β-OH, C_1-α-OH, C_{12}-β-OH and C_{12}-α-OH were calculated. The calculated values in the case of C_1-β-OH(values parenthesized in Table II) are in good agreement with the observed chemical shifts. Other carbon signals of IV appeared at almost the same positions as those of II. Thus the structure of IV was elucidated as 24ξ-methylcholestane-1β,3β,5α,6β-tetrol.



I : R^1=H, R^2=Ac
II : R^1=R^2=H
III : R^1=Ac, R^2=H

IV : R=H
V : R=Ac

VI : R=H
VII : R=Ac

Fig. 2

In the ^{1}H–NMR spectrum of VI, a doublet due to the secondary methyl group at C-24[11] disappeared, while signals due to a terminal methylene group were observed at δ 4.66 and 4.72. This change in the side chain moiety was confirmed by analysis of the mass spectrum, which showed strong peaks at *m/e* 364 ($M^+-C_6H_{12}$), 349 ($M^+-C_6H_{12}-CH_3$), 346 ($M^+-C_6H_{12}-H_2O$) and 328 ($M^+-C_6H_{12}-2H_2O$). These peaks are apparently derived by McLafferty rearrangement, typical of the mass spectra of 24-methylenecholesterol derivatives.[12] The ^{1}H–NMR spectra of VI and its acetate (VII) were very similar to those of IV and its acetate (V) (Table I and "Experimental"), respectively, except for the changes in the side chain moiety. The ^{13}C–NMR spectrum of a mixture of IV and VI indicated the presence of the same steroidal moiety in both compounds; corresponding signals due to the steroidal moiety of IV and VI completely overlapped with each other.[13] Thus, the structure of VI was shown to be 24-methylenecholestane-1β,3β,5α,6β-tetrol.

10) H. Eggert, C.L. VanAntwerp, N.S. Bhacca, and C. Djerassi, *J. Org. Chem.*, **41**, 71 (1976).
11) The signals of the secondary methyl group at C-24 appear at δ 0.87 ppm in I, 0.90 ppm in II and 0.81 ppm in IV.
12) S.G. Wyllie and C. Djerassi, *J. Org. Chem.*, **33**, 305 (1968); M. Bortolotto, J.C. Braekman, D. Dalose, and B. Tursch, *Bull. Soc. Chim. Belg.*, **85**, 27 (1976).
13) The ^{13}C-NMR spectrum of the mixture clearly showed signals due to the side chain carbons of VI at δ 155.6 (C-24), 106.5 (C-28), 43.9, 36.1 (C-22 and C-23) and 28.2 (C-25), in addition to the signals due to the steroidal moiety and the side chain moiety of IV, as listed in Table II.

Experimental

All melting points are uncorrected. Optical rotations were taken with a Jasco DIP-4 digital polarimeter. IR spectra were obtained with a Hitachi 215 spectrometer. ^{1}H-NMR spectra were measured at 100 MHz on a JEOL PS-100 spectrometer and ^{13}C-NMR spectra were measured at 25.0 MHz on a JEOL FX-100 spectrometer. Chemical shifts are given on the δ (ppm) scale with tetramethylsilane as an internal standard. Mass spectra were recorded on a Hitachi RMU-7L spectrometer.

Isolation of the Sterols (I), (II), (IV) and (VI)——The wet soft corals of *Lobophytum pauciflorum* (Ehrenberg) (2 kg), collected at coral reefs of Ishigaki Island in Okinawa Prefecture, were crushed in MeOH (20 l) and extracted with MeOH (20 l) then with acetone (20 l) at room temperature overnight. The combined residue was suspended in water (3 l) and extracted twice with ether (2 l). The ether residue (*ca.* 50 g) was chromatographed on a silica gel column (800 g, 60 mm×700 mm), eluting with *n*-hexane–acetone mixtures of increasing polarity (20: 1 to 1: 1) and then with MeOH; fr. 1 (20: 1, 4 l), fr. 2 (10: 1, 2 l), fr. 3 (10: 1, 2 l), fr. 4 (5: 1, 2 l), fr. 5 (5: 1, 2 l), fr. 6 (2: 1, 2 l), fr. 7 (2: 1, 2 l), fr. 8 (1: 1, 4 l) and fr. 9 (MeOH). Fraction 8 (1.2 g) was subjected to high-performance liquid chromatography using a silica gel pre-packed column [Merck (B)]. Elution with $CHCl_3$–MeOH (20: 1, then 10: 1) gave three fractions [(1) (500 mg), (2) (300 mg) and (3) (300 mg)]; fractions (1) and (2) were crystalline, but fraction (3) was amorphous.

Recrystallization (from MeOH) of the crystals obtained from fraction (1) gave 24ξ-methylcholestane-3β,5α,6β,25-tetrol 25-monoacetate (I) as colorless needles. mp 226°. $[\alpha]_D^{20}$ −17° (*c* 0.3, EtOH). IR ν_{max}^{KBr} 3400, 1730, 1260, 1045 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.68 (3H, s), 0.87 (3H, d, J=6.4 Hz), 0.93 (3H, d, J=5.2 Hz), 1.17 (3H, s), 1.38 (6H, s), 1.97 (3H, s), 3.54 (1H, brm), 4.09 (1H, m). The ^{13}C-NMR data are listed in Table II. These physical properties coincided in every respect with those of an authentic specimen.

Recrystallization (from MeOH) of the crystals obtained from fraction (2) gave 24ξ-methylcholestane-3β,5α,6β,25-tetrol (II) as colorless needles. MS *m/e*: 432 (M^+-H_2O). Other physical properties of II are listed in Table I.

The amorphous substance obtained from fraction (3) was shown to be a mixture of two closely related compounds by analyses of the ^{1}H-NMR and the mass spectra [*m/e*: 450 (M^+) and *m/e*: 448 (M^+)]. The mixture (42 mg) was treated with acetic anhydride (0.5 ml) in pyridine (1 ml) at room temperature for 23 hr, giving an acetate mixture (60 mg). This mixture was then chromatographed on a 10% $AgNO_3$-impregnated silica gel column (10 g, 12 mm×160 mm) and the column was eluted with *n*-hexane–acetone mixtures (97: 3 and then 96: 4), giving the triacetate (V) from fractions 101—136 and the triacetate (VII) from fractions 171—230. V; mp 111—113°. $[\alpha]_D^{20}$ −33° (*c* 0.28, EtOH). ^{1}H-NMR ($CDCl_3$) δ: 0.68 (3H, s), 0.78 (3H, d, J=6.0 Hz), 0.84 (6H, d, J=7.0 Hz), 0.91 (3H, d, J=4.0 Hz), 1.24 (3H, s), 2.00 (6H, s), 2.08 (3H, s), 4.62 (1H, brs), 5.20 (1H, dd, J=6.0, 11 Hz), 5.20 (1H, m). ^{13}C-NMR (C_5D_5N) δ: 10.7 (q), 12.2 (q), 15.7 (q), 17.8 (q), 19.0 (q), 20.7 (q), 21.1 (q), 21.2 (q), 21.8 (q), 23.6 (t), 24.7 (t), 28.3 (t), 31.0 (t), 31.7 (t), 31.8 (d), 32.0 (d), 33.9 (2d), 36.5 (t), 37.2 (t), 39.4 (d), 40.6 (t), 42.2 (s), 43.8 (s), 45.3 (t), 55.8 (d), 56.4 (d), 68.3 (d), 75.2 (s), 75.9 (d), 76.7 (d), 170.0 (s), 170.1 (2s). VII; mp 91—95°. $[\alpha]_D^{20}$ −29° (*c* 0.22, EtOH). ^{1}H-NMR ($CDCl_3$) δ: 0.68 (3H, s), 0.95 (3H, d, J=4.0 Hz), 1.02 (6H, d, J=6.5 Hz), 1.24 (3H, s), 2.00 (6H, s), 2.08 (3H, s), 4.63 (1H, brs), 4.70 (2H, brs), 5.18 (1H, dd, J=5.0, 11 Hz), 5.18 (1H, brm).

The triacetate (V) was subjected to alkaline hydrolysis. A solution of V (28 mg) in EtOH (2 ml) was treated with 5% aqueous NaOH solution (1 ml) and the mixture was stirred at room temperature for 23 hr. Water (10 ml) was added and the mixture was extracted twice with AcOEt (40 ml). Usual work-up gave 24ξ-methylcholestane-1β,3β,5α,6β-tetrol (IV) as a colorless amorphous solid (17 mg). *Anal.* Calcd for $C_{28}H_{50}O_4 \cdot 2/3H_2O$: C, 72.68; H, 11.18. Found: C, 72.33; H, 10.93. MS *m/e*: (relative intensity) 450 (M^+, 2), 432 (31), 414 (65), 396 (29), 359 (29), 345 (53), 305 (18), 287 (19), 95 (100). Other physical properties are listed in Table I.

Similar alkaline hydrolysis of the triacetate (VII) (17 mg) gave 24-methylenecholestane-1β,3β,5α,6β-tetrol (VI) as a colorless amorphous solid (3 mg). *Anal.* Calcd for $C_{28}H_{48}O_4 \cdot 3/2H_2O$: C, 70.69; H, 10.81. Found: C, 70.68; H, 10.53. MS *m/e*: (relative intensity) 448 (M^+, 12), 433 (16), 430 (12), 412 (23), 394 (8), 364 (45), 349 (33), 346 (28), 328 (37), 321 (99), 303 (47), 285 (37), 249 (31), 95 (100). Other physical properties are listed in Table I.

Acetylation of II——A solution of II (60 mg) in pyridine (1 ml) was treated with acetic anhydride (0.5 ml) and the mixture was allowed to stand at room temperature for 24 hr. Usual work-up gave the diacetate (III) as colorless plates. mp 217—220°. *Anal.* Calcd for $C_{32}H_{54}O_6$: C, 71.86; H, 10.19. Found: C, 71.99; H, 10.30. IR ν_{max}^{KBr}: 3460, 3420, 1723, 1703 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.70 (3H, s), 0.88 (3H, d, J=6.0 Hz), 0.92 (3H, d, J=4.0 Hz), 1.15 (9H, s), 2.03 (3H, s), 2.07 (3H, s), 4.70 (1H, brs), 5.10 (1H, brm). ^{13}C-NMR (C_5D_5N) δ: 12.4 (q), 15.5 (q), 16.6 (q), 19.3 (q), 21.3 (q), 21.4 (q), 21.5 (t), 24.4 (t), 26.6 (q), 27.5 (t), 28.2 (q), 28.3 (t), 28.5 (t), 31.4 (d), 32.0 (t), 32.5 (t), 35.5 (d), 36.7 (t), 37.5 (t), 39.0 (s), 40.4 (t), 43.0 (s), 45.2 (d), 46.0 (d), 56.1 (d), 56.2 (d), 71.5 (d), 72.2 (s), 74.3 (s), 76.7 (d), 170.2 (s), 170.3 (s).

Alkaline Hydrolysis of I——A solution of I (49 mg) in diethylene glycol (1 ml) was treated with KOH (200 mg), and the mixture was heated at 200° for 30 min. The mixture was extracted twice with $CHCl_3$–

MeOH (6: 4, 10 ml) and twice with $CHCl_3$ (10 ml). The organic layer was washed with water and dried. Removal of the solvent gave a crystalline substance (49 mg), which was recrystallized from EtOH–H_2O to give II (29 mg).

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