Penasterol, a Novel Antileukemic Sterol from the Okinawan Marine Sponge *Penares* sp.

Jie-fei Cheng, Jun'ichi Kobayashi,* Hideshi Nakamura, and Yasushi Ohizumi
Mitsubishi-Kasei Institute of Life Sciences, Minamiooya, Machida, Tokyo 194, Japan
Yoshimasa Hirata
Faculty of Pharmacy, Meijo University, Nagoya 468, Japan
Takuma Sasaki
Cancer Research Institute, Kanazawa University, Kanazawa 920, Japan

A novel lanosterol-derived metabolite, penasterol (**1**), with potent antileukemic activity has been isolated from the Okinawan marine sponge *Penares* sp. and the structure elucidated on the basis of evidence provided by spectroscopy and chemical transformations. This is the first isolation of a marine sterol with a 14-carboxy group potentially important within the framework of sterol biosynthesis.

Although the biosynthetic pathway from lanosterol to cholesterol has been well investigated,[1] the lanosterol-related natural sterol with a 14-carboxy group is very rare and the only example is lyofolic acid,[2] a triterpenoid from a terrestrial plant. During our survey of bioactive compounds from marine organisms,[3] a novel lanosterol-derived metabolite, penasterol (**1**), with potent antileukemic activity has been isolated from the Okinawan marine sponge *Penares* sp. Here we describe the isolation and structure determination of (**1**), which is the first marine sterol with a 14-carboxy group[4] potentially important within the framework of sterol biosynthesis.

Results and Discussion

The sponge *Penares* sp. collected at Okinawa Island was kept frozen until used. The methanol extract of the sponge was partitioned between toluene and water. The toluene-soluble material exhibiting antileukemic activity was chromatographed on a silica gel column ($CHCl_3$–CH_3OH, 97.5:2.5) and a Sephadex LH-20 column ($CHCl_3$–CH_3OH, 1:1) to furnish penasterol (1) (0.02% wet weight) as an antileukemic compound.

The molecular formula, $C_{30}H_{48}O_3$, of (**1**) was deduced from the electron impact mass spectrum (*m/z* 456, M^+) and the ^{13}C n.m.r. data (see Table). The similarity of the ^{13}C resonances of (**1**) to those of lanosterol (**7**)[5] (Table) suggested that compound (**1**) is a lanosterol-like sterol. The presence of a carboxy group in (**1**) was implied by the i.r. bands at 3 600—2 500 and 1 695 cm^{-1}, and confirmed by the mass fragment peak at *m/z* 411 ($M^+ - CO_2H$), and the ^1H and ^{13}C chemical shifts at δ_H 11.7 and δ_C 176.4. A deuterium-exchangeable proton at δ_H 3.99 (br s) indicated the presence of a hydroxy group. The ^1H and ^{13}C chemical shifts and the ^1H coupling constants of C*H*OH (δ_H 2.99, dd, *J* 5.0, 10.3 Hz; δ_C 76.9) were consistent with those of sterols with a 3β-OH group.[6]

The C-20 to C-27 side chain of (**1**) was defined as the same as that of lanosterol (**7**) by analyzing the ^1H and ^{13}C n.m.r. data. The ^1H signals at δ_H 1.63 (3 H, s) and 1.55 (3 H, s) were assigned to the vinyl methyl protons at C-26 and C-27, while those at δ_H 5.06 (1 H, t, *J* 7.0 Hz) and 0.89 (3 H, d, *J* 6.5 Hz) were assignable to the olefinic proton at C-24 and the methyl protons at C-20, respectively. The ^{13}C chemical shifts of C-20 to C-27 of (**1**) were almost equal to those of lanosterol (**7**)[5] (see Table) or desmosterol.[7]

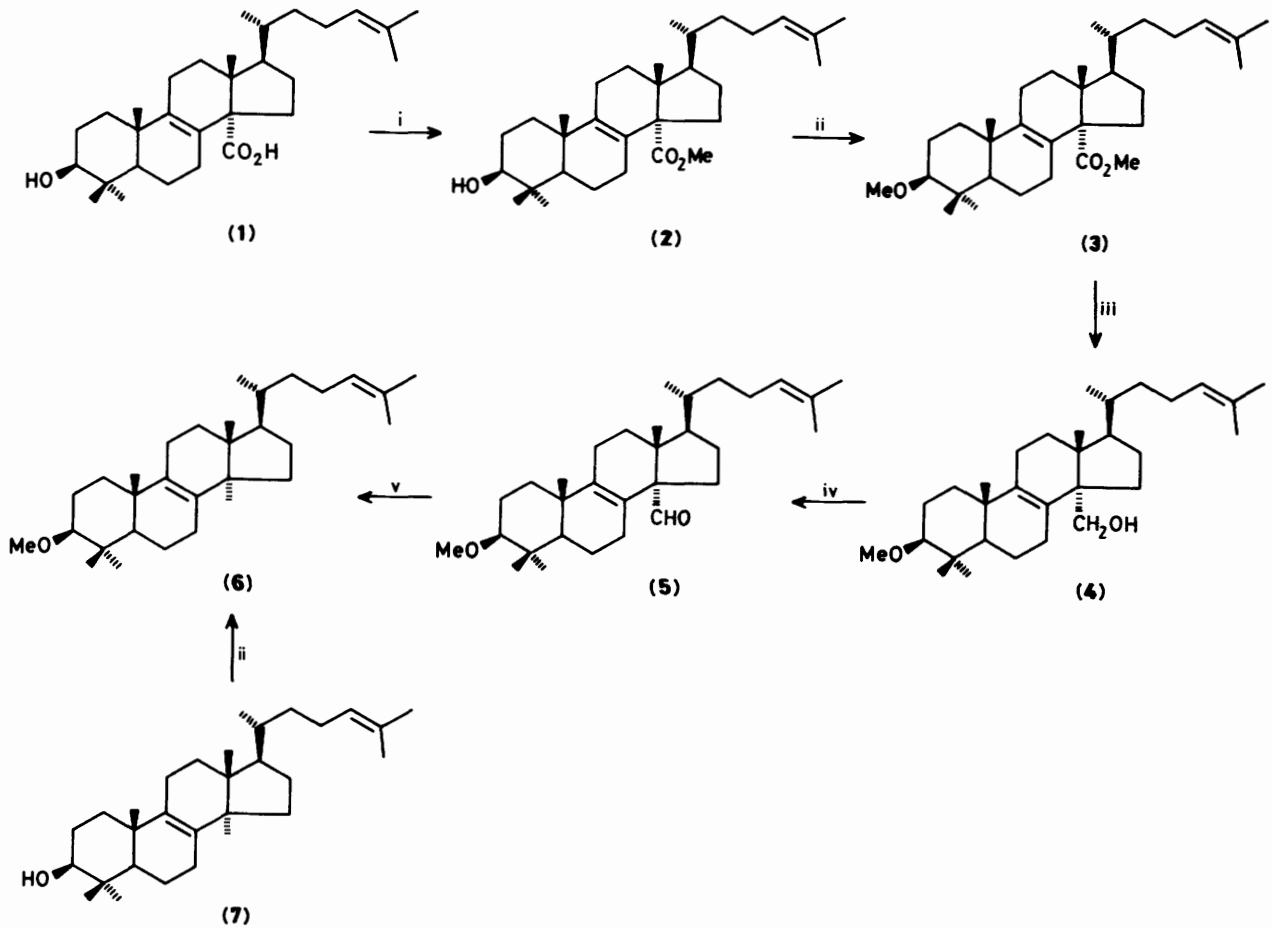
The ^1H n.m.r. spectrum of (1) showed the four tertiary methyl signals at δ_H 0.69, 0.70, 0.89, and 0.95 in addition to the three methyl signals of the side chain. Since the distortionless enhancement by polarization transfer (DEPT) experiment also confirmed the presence of the seven methyl groups in (**1**) instead of eight as usually found in C_{30} sterols, the remaining methyl group was considered to have been oxidized to the carboxy group. The carbon (δ_H 61.7) bearing a carboxy group in (**1**) was assigned to C-14 by comparison with the ^{13}C chemical shifts of the analogous sterols with a C-4,[8] C-10,[9] or C-14[10] carboxy group. This assignment was supported by resistance of (**1**) to its lactonization, excluding the possibility of a CO_2H substitution at C-4 or C-10. The presence of a 13-methyl group in (**1**) was indicated by its characteristic higher-field resonances (δ_H 0.69; δ_C 17.1). Thus the structure of penasterol was assigned as (**1**).

The skeleton and stereochemistry of penasterol (**1**) was confirmed by the following chemical transformations of (**1**) into 3-*O*-methyl-lanosterol (**6**) (Scheme 1). Methylation of (**1**) with

Table. ^{13}C N.m.r. data for (δ_C/p.p.m.; 22.5 Hz) lanosterol (**7**), penasterol (**1**), and 3-*O*-methyl-lanosterol (**6**)

Position	(7)[a]	(1)[b]	(6)[c]
1	35.7	34.9	35.5
2	27.9	27.3	28.0
3	79.0	76.9	88.7
4	39.0	38.3	38.8
5	50.5	50.0	50.9
6	18.3	17.8	18.1
7	28.3	28.4	28.1
8	134.4	130.0	134.3
9	134.4	140.1	134.4
10	37.1	36.9	37.0
11	21.1	21.5	21.0
12	26.6	27.3	26.5
13	44.6	46.0	44.5
14	49.4	61.7	49.8
15	30.9	30.8	30.8
16	31.1	27.3	31.1
17	50.7	49.6	50.4
18	15.8	17.1	16.1
19	19.1	18.9	19.1
20	36.6	35.0	36.3
21	18.8	18.1	18.6
22	36.3	35.6	36.3
23	25.0	24.1	24.9
24	125.3	124.6	125.2
25	130.8	129.8	129.4
26	17.6	17.3	17.6
27	25.7	25.3	25.6
28	24.3	176.4	24.2
29	28.1	27.7	28.1
30	15.4	15.3	15.7
OMe			57.4

[a] Assignments according to ref. 5. [b] Recorded in $(CD_3)_2SO$. [c] Recorded in $CDCl_3$.



Scheme 1. *Reagents:* i, CH_2N_2, MeOH; ii, NaH, MeI, THF, reflux; iii, $LiAlH_4$, THF, reflux; iv, PCC, CH_2Cl_2; v, NH_2NH_2 (80%), KOH, $HOCH_2CH_2OH$, 180 °C

CH_2N_2 furnished the methyl ester (**2**) (CO_2CH_3, δ_C 176.6 and δ_H 3.62; $\nu_{max.}$ 1 700 cm^{-1}). Treatment of (**2**) or (**1**) with NaH–MeI afforded (**3**) (3-OCH_3, δ_H 3.37). Reduction of the ester part of (**3**) followed by oxidation of the hydroxymethyl group of (**4**) with pyridinium chlorochromate (PCC) in CH_2Cl_2 gave the aldehyde (**5**) (CHO, δ_C 198.7 and δ_H 9.40). Finally the aldehyde (**5**) was reduced to (**6**) ($[\alpha]_D^{24}$ +59.1°), the physicochemical properties of which were identical with an authentic sample of 3-*O*-methyl-lanosterol ($[\alpha]_D^{24}$ +53.0°) derived from lanosterol (**7**) with NaH–MeI. The ^1H n.m.r. spectra of the two samples of 3-*O*-methyl-lanosterol (**6**) derived from the compound (**5**) or lanosterol (**7**) are shown in the Figure (a) or (b), respectively.

The C-14 demethylation of lanosterol (**7**) or the $\Delta^{7,24}$-isomer (**8**) *via* oxidation of the 14-methyl group to the aldehyde (**9**) and subsequent elimination of formic acid [(**10**)→(**11**)] is the first step in lanosterol metabolism of yeast or mammals (Scheme 2).[11,12] A C-14 demethylated sterol derivative has been isolated from marine sponges,[13] but no lanosterol-derived metabolite with a formyl or a carboxy group at C-14. Penasterol (**1**) may be an important intermediate in considering sterol biosynthesis of marine sponges. It was tested for antitumor activity against L1210 murine leukemia cells *in vitro*. Penasterol (**1**) was cytotoxic, exhibiting an IC_{50} value of 3.6 μg/ml.

Experimental

M.p.s were determined with a Yanagimoto micro melting point apparatus and are uncorrected. I.r. spectra were recorded on a Hitachi 260-50 i.r. spectrophotometer as KBr pellets and u.v. spectra on a JASCO 660 UV/VIS spectrophotometer for solutions in methanol. Optical rotations were measured on a JASCO DIP-360 polarimeter. ^1H and ^{13}C N.m.r. spectra were recorded on a Bruker AM-500 (500 MHz for ^1H n.m.r.) and JEOL FX-90Q (22.5 MHz for ^{13}C n.m.r.) in $CDCl_3$ (δ_H 7.27 and δ_C 76.9 as standard) or [$^2H_6$]dimethyl sulphoxide (δ_H 2.50 and δ_C 39.5 as standard) solution. Mass spectra (e.i.m.s.) were obtained with a Shimadzu GC-MS QP-1000A spectrometer at 70 eV. Wako C-300 silica gel was used for glass column chromatography. T.l.c. was carried out on Merck silica gel GF_{254}. Lanosterol was purchased from Sigma Chemical Co.

Isolation of Penasterol (**1**).—The sponge (1.55 kg, wet weight), collected by netting at Unten Bay (−70 m), Okinawa Island, in June 1987, was kept frozen until used. The methanol (2 × 1 500 ml) extract was dissolved in methanol–toluene (3:1; 200 ml) and then partitioned between toluene (2 × 1 000 ml) and 1M aqueous NaCl (1 000 ml). The toluene-soluble material (10.2 g) was partially subjected to a silica gel column ($CHCl_3$–CH_3OH, 97.5:2.5) and Sephadex LH-20 ($CHCl_3$–CH_3OH, 1:1) to furnish *penasterol* (**1**) (90 mg from 2.5 g of toluene extract, 0.02% wet weight of the sponge), as needles from hexane–ethyl acetate (1:1), m.p. 197—201 °C; $[\alpha]_D^{26}$ −54.5° (*c* 0.1 in MeOH); $\lambda_{max.}$(MeOH) 205 nm (ε 8 000); $\nu_{max.}$ 3 600—2 500, 2 950, 2 850, 1 695, 1 460, 1 370, 1 250, 1 200, 830, and 760 cm^{-1}; δ_H[$(CD_3)_2SO$] 11.7 (1 H, br, CO_2H), 5.06 (1 H, t, *J* 7.0 Hz, 24-H), 3.39 (1 H, br s, 3-OH), 2.99 (1 H, dd, *J* 5.7, 10.3 Hz, 3-H),

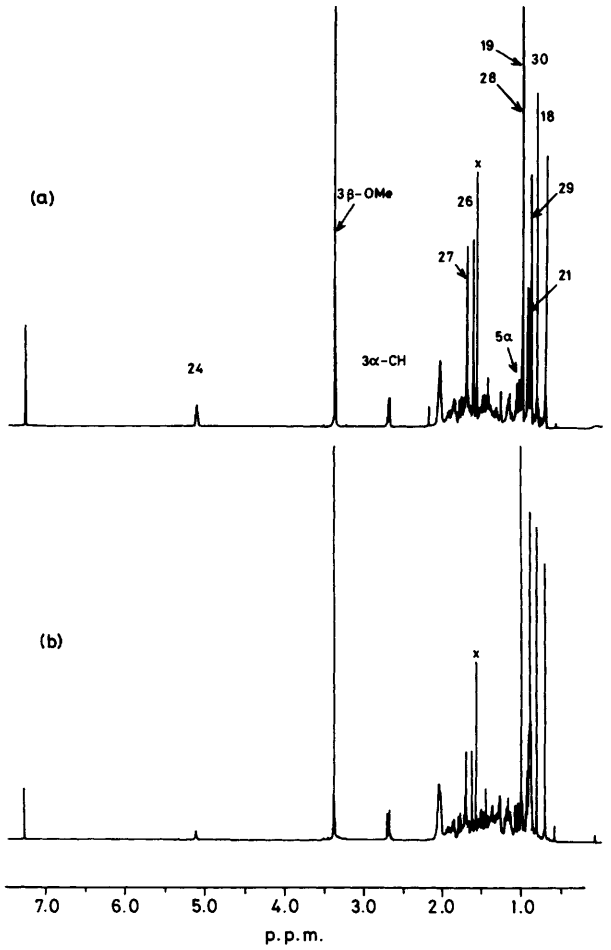
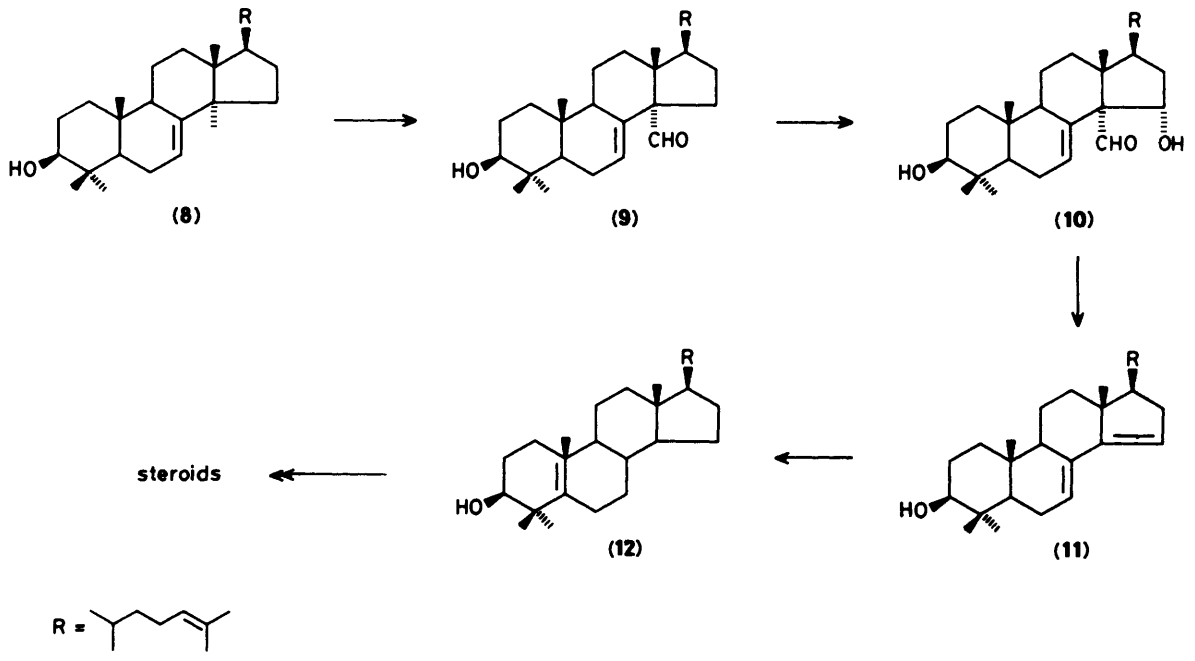


Figure. 500 MHz ^{1}H N.m.r. spectra of 3-*O*-methyl-lanosterol (**6**) in $CDCl_3$. (a) The sample derived from penasterol (**1**) and (b) that prepared from lanosterol as shown in Scheme 1. The mark x is due to water

1.63 (3 H, s, 27-Me), 1.55 (3 H, s, 26-Me), 0.95 (3 H, s, 19-Me), 0.89 (3 H, s, 29-Me), 0.88 (3 H, d, *J* 6.8 Hz, 21-Me), 0.70 (3 H, s, 30-Me), 0.69 (3 H, s, 18-Me), and 0.96—2.28 (23 H, m); δ_C (see Table); *m/z* 456 (M^+), 411 ($M^+ - CO_2H$), and 393 ($M^+ - CO_2H - H_2O$).

Esterification of Penasterol (**1**).—An excess of ethereal diazomethane was added to (**1**) (90 mg) in MeOH at room temperature during 2 h. Evaporation of the solvent yielded quantitatively the *ester* (**2**), as a colourless solid, m.p. 126—127 °C; $[\alpha]_D^{26}$ −36.4° (*c* 0.22 in MeOH); $\lambda_{max.}$(MeOH) 206 nm (ε 6 700); $\nu_{max.}$ 3 500, 2 925, 2 850, 1 700, 1 450, 1 370, 1 240, 1 200, and 1 030 cm^{-1}; $\delta_H(CDCl_3)$ 5.09 (1 H, t, *J* 7.1 Hz, 24-H), 3.62 (3 H, s, 14-CO_2Me), 3.25 (1 H, dd, *J* 4.5, 11.7 Hz, 3-H), 1.67 (3 H, s, 27-Me), 1.61 (3 H, s, 26-Me), 1.08 (1 H, dd, *J* 2.1, 12.6 Hz, 5-H), 1.02 (3 H, s, 19-Me), 0.99 (3 H, s, 29-Me), 0.92 (3 H, d, *J* 5.7 Hz, 21-Me), 0.82 (3 H, s, 30-Me), 0.76 (3 H, s, 18-Me), and 1.26—2.11 (22 H, m); *m/z* 470 (M^+), 411 ($M^+ - CO_2Me$), and 393 ($M^+ - CO_2Me - H_2O$).

Methylation of Compound (**2**).—Compound (**2**) (90 mg) was added to a suspension of NaH (50%; 80 mg) in THF (4 ml) and the mixture was stirred under reflux for 1 h. An excess of methyl iodide was added and stirring was continued for a further 1 h. The reaction mixture was neutralized with ice water–hydrochloric acid and extracted with chloroform (2 × 50 ml). The extract was dried (Na_2SO_4), evaporated under reduced pressure, and the residue purified on a silica gel column using ethyl acetate–hexane (2:98) as eluant to give *compound* (**3**) (70 mg, 76%) as a colourless solid, $[\alpha]_D^{25}$ −25° (*c* 0.4 in MeOH); $\lambda_{max.}$(MeOH) 206 nm (ε 7 000); $\nu_{max.}$ 2 925, 2 850, 1 710, 1 450, 1 380, 1 190, and 1 105 cm^{-1}; $\delta_H(CDCl_3)$ 5.09 (1 H, t, *J* 7.1 Hz, 24-H), 3.62 (3 H, s, 14-CO_2Me), 3.37 (3 H, s, 3-OMe), 2.68 (1 H, dd, *J* 4.2, 11.7 Hz, 3-H), 1.67 (3 H, s, 27-Me), 1.60 (3 H, s, 26-Me), 1.06 (1 H, dd, *J* 2.2, 12.6 Hz, 5-H), 1.02 (3 H, s, 19-Me), 0.98 (3 H, s, 29-Me), 0.92 (3 H, d, *J* 5.7 Hz, 21-Me), 0.80 (3 H, s, 30-Me), 0.76 (3 H, s, 18-Me), and 1.38—2.18 (22 H, m); *m/z* 484 (M^+), 425 ($M^+ - CO_2Me$), and 393 ($M^+ - CO_2Me -$ MeOH).



Scheme 2.

Reduction of Compound (**3**).—A THF solution of compound (**3**) (70 mg) was added to an excess of lithium aluminium hydride suspension in THF (4 ml) and the mixture was stirred under reflux for 1 h. After work-up, the residue was passed through a short silica gel column (6% ethyl acetate in hexane) to furnish *compound* (**4**) (50 mg, 75%) as colourless crystals, m.p. 90 °C; $[\alpha]_D^{25}$ +53.3° (*c* 0.75 in $CHCl_3$); $\nu_{max.}$ 3 500, 2 925, 2 850, 1 450, 1 370, 1 100, and 1 020, cm^{-1}; $\delta_H(CDCl_3)$ 5.09 (1 H, t, *J* 7.1 Hz, 24-H), 3.60 (1 H, d, *J* 11.8 Hz, 28-H), 3.38 (3 H, s, 3-OMe), 3.20 (1 H, d, *J* 11.8 Hz, 28-H), 2.68 (1 H, dd, *J* 4.2, 11.7 Hz, 3-H), 1.85—2.10 (1 H, br s, OH), 1.69 (3 H, s, 27-Me), 1.61 (3 H, s, 26-Me), 1.05 (3 H, s, 19-Me), 0.99 (3 H, s, 29-Me), 0.92 (3 H, d, *J* 6.5 Hz, 21-Me), 0.82 (3 H, s, 30-Me), 0.71 (3 H, s, 18-Me), and 1.06—2.10 (22 H, m); $\delta_C(CDCl_3)$ 140.3 (s, C-9), 130.9 (s, C-8), 129.6 (s, C-25), 125.1 (d, C-24), 88.4 (d, C-3), 66.6 (s, C-14), 57.5 (q, OMe), 55.9 (t, C-28), 51.4 (d, C-5), 50.4 (d, C-17), 45.1 (s, C-13), 38.9 (s, C-4), 37.7 (t, C-10), 36.1 (3 × C, C-20, C-22, C-1), 31.1 (t, C-16), 30.1 (t, C-15), 28.1 (t, C-7), 28.0 (t, C-2), 27.9 (t, C-12), 25.6 (q, C-2), 24.8 (2 × C, C-23, C-28), 21.9 (t, C-11), 19.1 (q, C-19), 18.5 (q, C-21), 18.1 (t, C-6), 17.4 (q, C-26), and 16.1 (2 × C, C-18, C-30); *m/z* 439 (M^+ − OH), 425 (M^+ − CH_2OH), and 393 (M^+ − CH_2OH − MeOH).

Oxidation of Compound (**4**).—Compound (**4**) (25 mg) in CH_2Cl_2 (5 ml) was oxidized with pyridinium chlorochromate (PCC) at room temperature for 2 h. The reaction mixture was filtered through a short silica gel column (ethyl acetate), and purified by a silica gel column (1% ethyl acetate in hexane) to yield the *aldehyde* (**5**) (20 mg, 80%), m.p. 133—136 °C; $[\alpha]_D^{25}$ −256° (*c* 0.35 in $CHCl_3$) and −244° (*c* 0.35 in cyclohexane); $\lambda_{max.}$(MeOH) 212 nm (ε 1 900), 234 (1 300), and 306 nm (100); $\nu_{max.}$ 2 925, 2 850, 1 700, 1 450, 1 360, 1 100, and 840 cm^{-1}; $\delta_H(CDCl_3)$ 9.40 (1 H, s, 14-CHO), 5.06 (1 H, t, *J* 6.9 Hz, 24-H), 3.37 (3 H, s, 3-OMe), 2.66 (1 H, dd, *J* 4.2, 11.5 Hz, 3-H), 1.66 (3 H, s, 27-Me), 1.59 (3 H, s, 26-Me), 1.06 (3 H, s, 19-Me), 0.96 (3 H, s, 29-Me), 0.91 (3 H, d, *J* 6.5 Hz, 21-Me), 0.80 (3 H, s, 30-Me), 0.75 (3 H, s, 18-Me), and 1.00—2.28 (23 H, m); $\delta_C(CDCl_3)$ 198.7 (d, C-28), 143.9 (s, C-9), 129.6 (s, C-25), 124.8 (d, C-24), 122.8 (s, C-8), 88.3 (d, C-3), 66.9 (s, C-14), 57.5 (q, O-Me), 53.0 (t, C-15), 51.3 (d, C-17), 50.5 (d, C-5), 46.0 (s, C-13), 38.9 (s, C-4), 38.0 (t, C-10), 35.8 (2 × C, C-20, C-22), 35.5 (t, C-1), 30.7 (t, C-16), 29.6 (t, C-29), 27.9 (t, C-2), 27.6 (t, C-7), 25.6 (q, C-27), 24.7 (t, C-12), 22.6 (t, C-23), 19.4 (q, C-19), 18.5 (q, C-21), 18.0 (t, C-6), 17.6 (q, C-26), 16.8 (q, C-18), and 16.1 (q, C-30); *m/z* 454 (M^+), 425 (M^+ − CHO), and 393 (M^+ − CHO − MeOH).

3-O-*Methyl-lanosterol* (**6**).—The aldehyde (**5**) (20 mg) was mixed with 80% NH_2NH_2 (2 ml), ethylene glycol (4 ml), and KOH (1 g) and the mixture heated under reflux at 180 °C for 2 h; the temperature was then raised to 200 °C to remove the solvent. The residue was diluted with water and extracted with $CHCl_3$ (2 × 50 ml), and the chloroform extract was dried (Na_2SO_4). Evaporation of the solvent under reduced pressure and the purification of the residue on a silica gel column (1% ethyl acetate in hexane) afforded *compound* (**6**) (13 mg, 69%), m.p. 118—120 °C; $[\alpha]_D^{24}$ +59.1° (*c* 0.22 in $CHCl_3$); $\lambda_{max.}$(MeOH) 210 nm (ε 5 000); $\nu_{max.}$ 2 950, 2 825, 1 470, 1 380, 1 110, 910, and 800 cm^{-1}; $\delta_H(CDCl_3)$ 5.11 (1 H, t, *J* 7.0 Hz, 24-H), 3.37 (3 H, s, 3-OMe), 2.68 (1 H, dd, *J* 4.3, 11.8 Hz, 3-H), 1.69 (3 H, s, 27-Me), 1.61 (3 H, s, 26-Me), 1.04 (1 H, dd, *J* 2.1, 12.7 Hz, 5-H), 0.99 (6 H, s, 28-Me, 19-Me), 0.92 (3 H, d, *J* 6.4 Hz, 21-Me), 0.88 (3 H, s, 29-Me), 0.80 (3 H, s, 30-Me), 0.70 (3 H, s, 18-Me), and 1.10—2.10 (22 H, m); δ_C (see Table); *m/z* 440 (M^+), 425 (M^+ − Me), and 393 (M^+ − Me − MeOH).

Compound (**6**) was also prepared by methylation of lanosterol (**7**). Thus, lanosterol (**7**) (20 mg) was added to the suspension of NaH (20 mg) in THF (2 ml) and the mixture stirred under reflux for 1 h. An excess of MeI was added and stirring continued for a further 1 h. After neutralization with water–hydrochloric acid, the reaction mixture was extracted with chloroform (2 × 25 ml), and the combined extracts were dried (Na_2SO_4) and evaporated under reduced pressure. The residue was chromatographed on a silica gel column using chloroform and then ethyl acetate–hexane (2:98) as eluants to yield *compound* (**6**) (10 mg, 50%), the mixed [with (**6**) derived from (**5**)] m.p. 118—120 °C; $[\alpha]_D^{24}$ +53.0° (*c* 0.22 in $CHCl_3$); $\lambda_{max.}$(MeOH) 210 nm (ε 4 700); $\nu_{max.}$ identical with values given above; δ_H (see Figure); $\delta_C(CDCl_3)$ 134.5 (s, C-9), 134.3 (s, C-8), 130.6 (s, C-25), 125.2 (d, C-24), 88.7 (d, C-3), 57.4 (q, OMe), 51.0 (d, C-5), 50.4 (d, C-17), 49.8 (s, C-14), 44.5 (s, C-13), 38.8 (s, C-4), 37.0 (s, C-10), 36.3 (d, C-20), 36.2 (t, C-22), 35.5 (t, C-17), 31.1 (t, C-16), 30.8 (t, C-15), 28.1 (2 × C, C-7, C-29), 28.0 (t, C-2), 26.4 (t, C-12), 25.6 (t, C-27), 24.9 (t, C-23), 24.2 (t, C-28), 21.0 (t, C-11), 19.1 (q, C-19), 18.6 (t, C-27), 18.1 (t, C-6), 17.6 (q, C-26), 16.1 (1, C-18), and 15.7 (q, C-30); *m/z* 440 (M^+), 425 (M^+ − Me), and 393 (M^+ − Me − MeOH).

Acknowledgements

We thank Dr. T. Hoshino (Mukaishima Marine Biological Station, Hiroshima University) for his kind identification of the marine sponge, Mr. Z. Nagahama for his assistance in collecting the marine sponge, and Miss M. Hamashima of this Institute for her technical assistance.

References

1 M. Lindberg, F. Gautschi, and K. Bloch, *J. Biol. Chem.*, 1963, **238**, 1661; W. L. Miller and J. L. Gaylor, *J. Biol. Chem.*, 1970, **245**, 5369; G. J. Schroepfer, Jr., *Ann. Rev. Biochem.*, 1982, **51**, 555.
2 J. Sakakibara, Y. Hotta, and M. Yasue, *Yakugaku Zasshi*, 1971, **91**, 1318; J. Sakakibara, Y. Hotta, M. Yasue, Y. Iitaka, and K. Yamazaki, *J. Chem. Soc., Chem. Commun.*, 1974, 839.
3 J. Kobayashi, H. Nakamura, Y. Ohizumi, and Y. Hirata, *Tetrahedron Lett.*, 1986, **27**, 1191; J. Kobayashi, M. Ishibashi, H. Nakamura, Y. Ohizumi, Y. Yamasu, T. Sasaki, and Y. Hirata, *ibid.*, p. 5755; M. Ishibashi, Y. Ohizumi, T. Sasaki, H. Nakamura, Y. Hirata, and J. Kobayashi, *J. Org. Chem.*, 1987, **52**, 450; M. Ishibashi, Y. Ohizumi, M. Hamashima, H. Nakamura, Y. Hirata, T. Sasaki, and J. Kobayashi, *J. Chem. Soc., Chem. Commun.*, 1987, 1127.
4 L. J. Goad, in 'Marine Natural Products. Chemical and Biological Perspectives,' ed. P. J. Scheuer, Academic Press, New York, 1978, vol. II, p. 75.
5 S. A. Knight, *Org. Magn. Reson.*, 1974, **6**, 603.
6 M. Hirotani, T. Furuya, and M. Shiro, *Phytochemistry*, 1985, **24**, 2055.
7 J. L. C. Wright, A. G. McInnes, S. Shimizu, D. G. Smith, J. A. Walter, D. Idler, and W. Khalil, *Can. J. Chem.*, 1978, **56**, 1898.
8 K. H. Pegel and C. B. Rogers, *J. Chem. Soc., Perkin Trans. 1*, 1985, 1711.
9 T. Nakatsu, R. P. Walker, J. E. Thompson, and D. J. Faulkner, *Experientia*, 1983, **39**, 759.
10 J. Sakakibara, Y. Hotta, and M. Yasue, *Yakugaku Zasshi*, 1975, **95**, 911.
11 K. Alexander, M. Akhtar, R. B. Boar, J. F. McGhie, and D. H. R. Barton, *J. Chem. Soc., Chem. Commun.*, 1972, 383.
12 Y. Aoyama and Y. Yoshida, *Biochem. Biophys. Res. Commun.*, 1986, **134**, 659.
13 I. Kitagawa, M. Kobayashi, Y. Okamoto, M. Yoshikawa, Y. Hamamoto, Abstracts, 20th Symposium on the Chemistry of Natural Products, Sapporo, Japan, 1987, p. 568.

Received 16*th November* 1987; *Paper* 7/2032