Structure of Paxisterol, a Sterol from *Penicillium paxilli*

Tohru Yasuzawa, Mayumi Yoshida and Hiroshi Sano*
Tokyo Research Laboratories, Kyowa Hakko Kogyo Co. Ltd., 3-6-6 Asahimachi, Machida-shi, Tokyo 194, Japan

The complete structure of paxisterol **1**, produced by a *Penicillium* species, was determined by means of spectral and chemical studies of the parent compound and several of its derivatives. Paxisterol is a unique sterol, having an acetal structure at C-18. Application of 2D-INADEQUATE technique in ^{13}C NMR spectral analysis was useful in this structure determination, where all carbon–carbon bonds in the molecule were demonstrated. Absolute stereochemistry was confirmed by the CD spectrum of 3-keto derivative **10** of compound **1** to be the same as that of cholesterol. An interesting acetal migration reaction was observed during the chemical conversions.

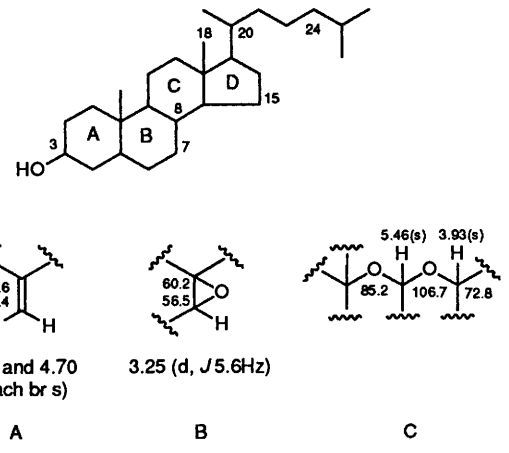
Paxisterol **1**, isolated from the culture broth of *Penicillium paxilli*, has analgesic activity in the mouse acetic acid writhing assay.[1] We describe here the structure determination of paxisterol, a unique sterol having an acetal structure at C-18.

Results and Discussion

Compound **1** was obtained as needles, m.p. 198–199 °C (from EtOAc); $[\alpha]_D^{25}$ −60.8° (*c* 1.0, $CHCl_3$); λ_{max}(MeOH) end-absorption. The IR spectrum exhibited absorption bands $\nu_{max}(CHCl_3)$ 3600 and 3450 (OH), 1640 (C=C) and 1035 cm^{-1} (C–O–C). The electron-impact mass spectrum, *m/z* 442 (M^+, 6%), 424, 396, 353, 345, 313 and 93 (100) (Found: M^+, 442.3068. $C_{28}H_{42}O_4$ requires *M*, 442.3081), and elementary analysis (Found: C, 76.2; H, 9.2. $C_{28}H_{42}O_4$ requires C, 76.33; H, 9.15%) indicated that paxisterol **1** had the molecular formula $C_{28}H_{42}O_4$. The proton signal pattern in the ^1H NMR spectrum and the similarity of the ^{13}C NMR spectrum of compound **1** with that of 5α-cholestan-3β-ol indicated that paxisterol **1** possessed a 5α-cholestan-3β-ol skeleton. The characteristic signals in the ^1H NMR spectrum of compound **1** arose from four methyl groups [δ 0.86 (3 H, s, 19-H_3), 1.02 (6 H, d, 26- and 27-H_3) and 1.36 (3 H, s, 21-H_3)], three oxygen-bearing methine protons [δ 3.93 (br s, 15-H), 3.54 (m, 3-H) and 3.25 (br d, 7-H)], one acetal proton [δ 5.46 (s, 18-H)], and one exomethylene group [δ 4.74 and 4.70 (each br s, 28-H_a and 28-H_b)]. Among these, the methine proton signal at δ 3.54 was assigned to 3-H and the β-configuration of the 3-hydroxy group was suggested from its coupling pattern. Another methine [δ_H 3.25; δ_C 56.5] appeared to form an epoxide ring together with the quaternary carbon at δ_C 60.2 (from their ^{13}C NMR chemical shifts).

The oxygen bonding to another methine (δ_H 3.93; δ_C 72.8) was found to be involved in the acetal group. Another oxygen of the acetal group was bonded to the quaternary carbon at δ_C 85.2. From this preliminary examination of ^1H NMR ^{13}C NMR and ^1H–^{13}C COSY spectra it was found that paxisterol **1** contained a 5α-cholestan-3β-ol skeleton and the three moieties shown in Scheme 1: exomethylene group (A), trisubstituted epoxide group (B) and acetal group (C).

The Location of the Exomethylene Group.—The exomethylene group in paxisterol **1** was characterized by two broad singlets at δ 4.74 and 4.70 in the ^1H NMR spectrum and by ^{13}C NMR signals at δ 155.6 and 106.4 (Table 1). Vicinal coupling between terminal methyl protons (26-H_3 and 27-H_3) and 25-H, long-range coupling between one of the exomethylene proton (28-H_a) and 25-H, and an NOE between 28-H_a and 26-H_3 were



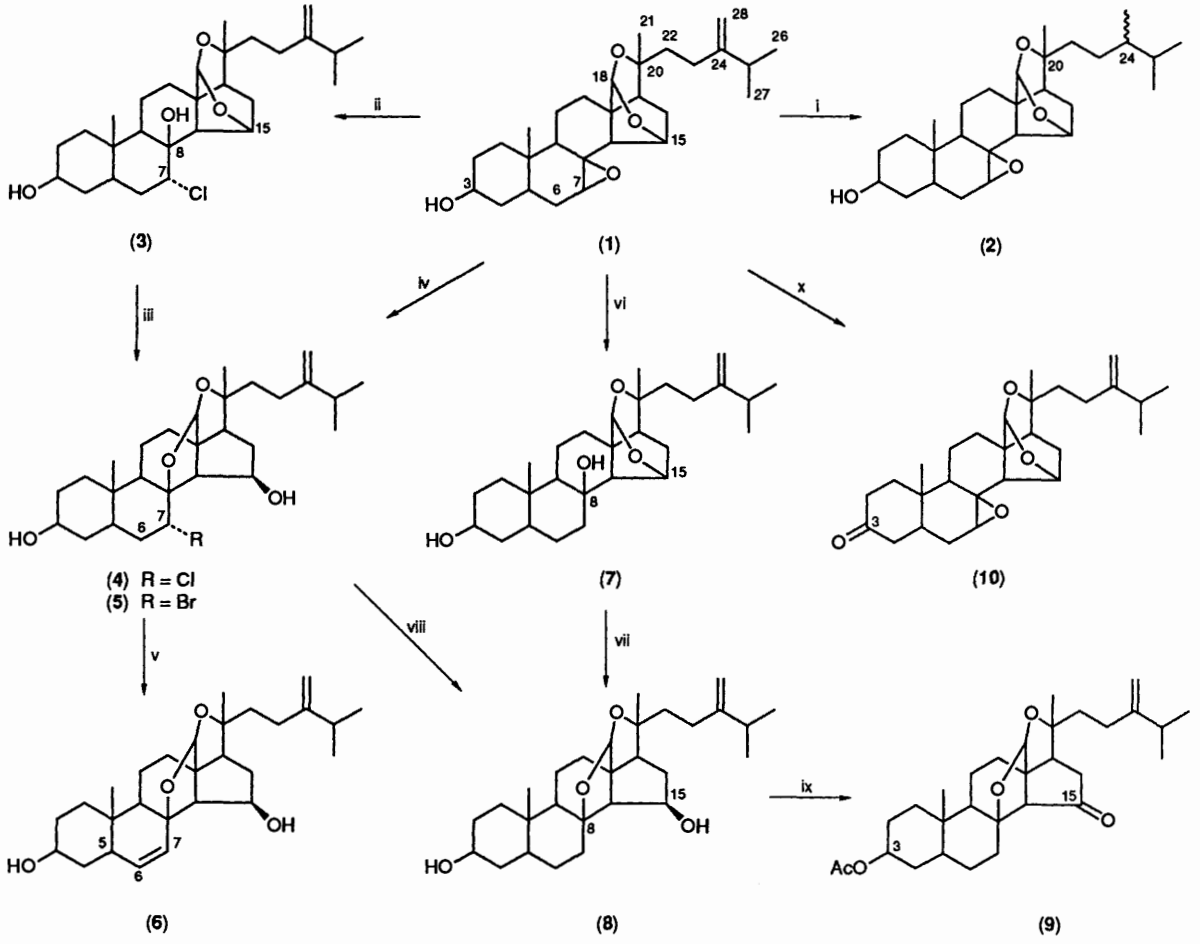
Scheme 1. Partial structures of paxisterol **1**.

Table 1. ^{13}C Chemical shifts (δ_C: $CDCl_3$; 50.28 MHz) of the side-chain moieties of paxisterol **1** and 24-methylenecholest-5-en-3β-ol.

Carbon	**1**	24-Methylenecholest-5-en-3β-ol
20	85.2	35.6
21	27.3	18.7
22	39.6	34.5
23	29.5	30.9
24	155.6	156.6
25	33.9	33.7
26	21.9	21.8
27	21.9	22.0
28	106.4	106.0

observed. The EIMS showed characteristic fragment ions severed at C-20, C-22, and C-24. These results indicated that the exomethylene group was located at C-24. The ^{13}C chemical shifts of the C-23-through-C-28 atoms of compound **1** were very similar to those of 24-methylenecholest-5-en-3β-ol,[2] indicating that both compounds had the same side-chain at C-20, involving the 24-exomethylene group (Table 1).

Additional proof was obtained as follows. Hydrogenation of paxisterol **1** with palladium/carbon under hydrogen led to a mixture of 24*R*-and 24*S*-dihydro derivative (**2**) (Scheme 2) whose ^{13}C NMR signals of the C-20 side-chain resembled those

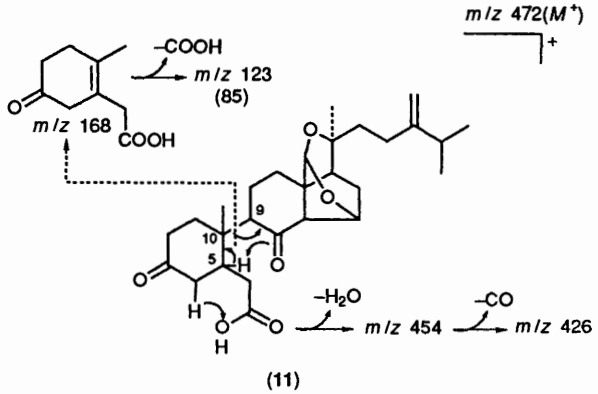


Scheme 2. *Reagents and conditions:* i, H_2/Pd–C; ii, HCl–THF, 15 min, room temperature; iii, PTSA, $CHCl_3$; iv, HCl–THF, 1 h, 50 °C for **4**; HBr–THF, 4 h, 50 °C for **5**; v, KOH–MeOH; vi, $LiAlH_4$, THF; vii, HCl–THF; viii, Bu_3SnH, PhMe; ix, Ac_2O–Py, Jones' reagent; x, PCC, CH_2Cl_2.

of 24-methylcholest-5-en-3β-ol.[3] The side-chain structure of compound **1** was thus deduced to be that of a 24-methylene-5α-cholestan-3β-ol type.

The Location of the Epoxide Group.—The ^1H–^1H COSY spectrum of compound **1** displayed vicinal coupling between the epoxide methine proton (δ 3.25) and a methylene resonating at δ *ca.* 1.6 and 1.8 (each 1 H, br, 6-H_2). Proof that the methylene was attached to the epoxide methine was also established by the ^1H NMR spectrum of compound **4** which was thought to be formed by addition of HCl to the epoxide group of compound **1**, followed by migration of the acetal from 8-OH to 15-OH (Scheme 2). A decoupling experiment on compound **4** showed the connectivity of a chlorine-bearing methine [δ 5.16 (1 H, t, *J* 2.9 Hz, 7-H)] and an adjacent methylene [δ 1.54 (1 H, dt, *J* 14.5 and 2.9 Hz, 6-H_a and 2.03 (1 H, ddd, *J* 14.5, 12.7 and 2.9 Hz, 6-H_b)]. The acetal-migration reaction was confirmed by the isolation of intermediary product **3**, which was converted into isomer **4** by treatment with toluene-*p*-sulphonic acid (PTSA) in chloroform.

Treatment of 7-chloride **4** with base failed to produce vinyl compound **6**; however, smooth reaction proceeded when bromo-compound **5** was treated with potassium hydroxide in methanol under reflux conditions. The existence of another methine adjacent to the methylene which was bonded to the epoxide methine in paxisterol **1** was deduced from the couplings observed between two vinylic protons at δ 5.74 (1 H, dd, *J* 2.9 and 1.4 Hz, 6-H) and 6.40 (1 H, dd, *J* 1.5 and 1.4 Hz, 7-H) and an adjacent methine proton in the ^1H NMR spectrum of compound **6**. Such a situation for a trisubstituted epoxide group is apparently present only in ring B or ring D of the cholestane skeleton.



Scheme 3. Diagnostic mass fragmentation of keto carboxylic acid **11**.

Paxisterol readily underwent oxidation with chromic anhydride in sulphuric acid to give the keto carboxylic acid **11**, formed by oxidative cleavage of the epoxide group.[4] As shown in Scheme 3, in the mass spectrum of compound **11** there are peaks corresponding to the loss of water and a CO group (*m/z* 454 and 426), indicating the presence of an active hydrogen on the γ-position to the carboxy group. The fragment ion peak of ring A arising from the cleavage between C-9 and C-10 including

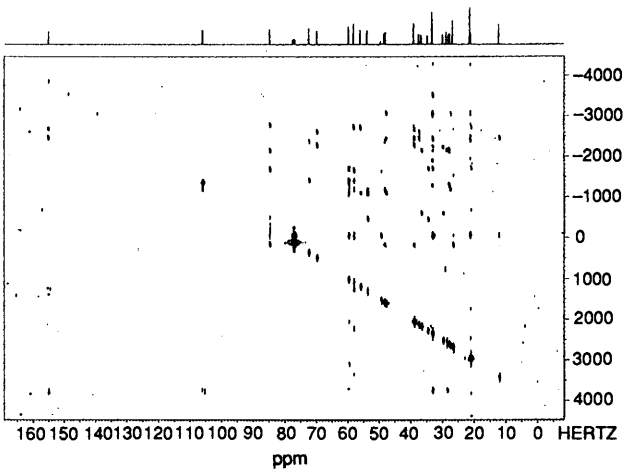
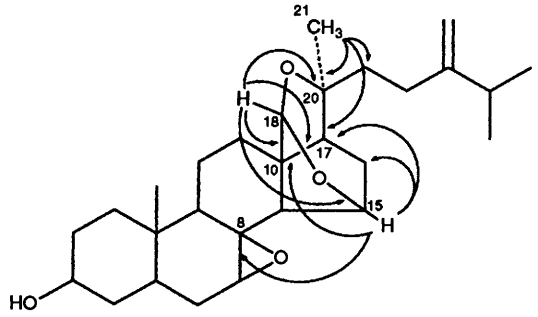


Fig. 1. 2D-INADEQUATE spectrum of paxisterol **1**.

the carboxy moiety (*m/z* 168) was also observed. From these findings the carboxymethyl group was determined to be present on C-5 in structure **11**, thus confirming the location of the epoxide group to be on C-7, -8 in compound **1**.

The Location of the Acetal Moiety.—Since the signal corresponding to the C-18 methyl group, which is usually found in sterols, was missing in the ^1H and ^{13}C NMR spectra of paxisterol **1**, the location of the acetal group was estimated to be at C-18. A COLOC experiment of compound **1** showed that the acetal proton [δ 5.46 (1 H, s, 18-H)] was long-range-coupled both with C-20 at δ 85.2; this was attributed to the long-range coupling with C-21 methyl protons (δ 1.36), and with C-15 at δ_C 72.8; this was assigned by the observation of long-range coupling between 15-H and C-8, C-10, and C-17, confirming the connection between C-18 and C-20, and C-18 and C-15, both through an ether linkage (Scheme 4).



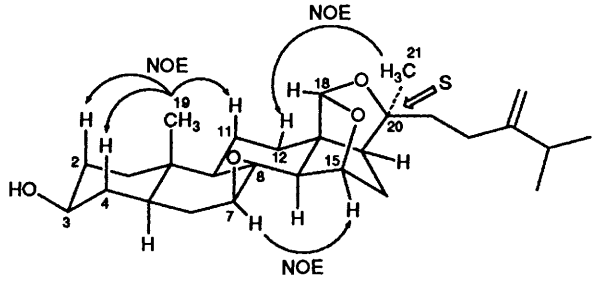
(1)

Scheme 4. Long-range couplings around acetal group in paxisterol **1**.

The location of the acetal moiety was also strongly supported by the following chemical transformations. Lithium aluminium hydride reduction of the C-7, -8 epoxide group of paxisterol **1** yielded the 8-hydroxy derivative **7**. Acetal migration from 15-O to 8-OH in compound **7** readily proceeded under acidic conditions [HCl tetrahydrofuran (THF)] to form compound **8** (Scheme 2). Compound **8** was also obtained upon treatment of the chloride **4** with tributyltin hydride in hot toluene. As expected, the ^{13}C NMR signal at δ_C 71.9 (C-8) of compound **7** had shifted downfield to δ_C 91.8, and a new proton signal of a secondary hydroxy group [δ 4.25 (br d, 15-OH)] was observed for compound **8**, showing the exchange of the C-18 acetal moiety to the 8-OH group from the 15-O(H), which are close in space. The typical IR absorption for a five-membered-ring ketone at 1750 cm^{-1} of compound **9**, which was prepared by selective acetylation of 3-OH in compound **8**, followed by Jones oxidation (Scheme 2), indicated that the newly formed OH group in compound **8**, in other words the attached position of the original acetal oxygen in paxisterol **1**, was on ring D. Furthermore, as the NOE between epoxide methine on C-7 and the acetal oxygen-bonded methine (δ 3.25) was observed in the ^1H NMR spectrum of paxisterol **1**, the acetal oxygen was deduced to be bonded at C-15. Therefore the location of the acetal moiety was finally decided to be present at C-15/C-18/C-20.

Additional skeletal confirmation, including the exomethylene, epoxide and acetal groups, was obtained by 2D-INADEQUATE studies of paxisterol **1** as shown in Fig. 1. All bonds between the carbon atoms were observed in this spectrum.

Absolute Stereochemistry.—All-β-configuration for C-18, C-20, 15-OH and 7,8-epoxide was evident from the preceding migration reaction between the acetal present at C-15/C-18/C-20 and 8-OH in compound **7**. The β-configuration of the C-19 angular methyl and the *S*-configuration of C-20 were determined by the NOE observed from the C-19 methyl protons (δ 0.86) to 2-H^β, 4-H^β and 11-H^β (δ 1.41, *ca.* 1.2, and *ca* 1.7, respectively) and from the C-21 methyl protons (δ 1.36) to 12-H^β (δ 2.37), respectively, as shown in Scheme 5.



(1)

Scheme 5. Absolute stereochemistry of paxisterol **1**.

A CD study was finally carried out to ascertain the absolute stereochemistry of paxisterol **1**. 3-Keto derivative **10** showed a positive Cotton effect in its CD spectrum, similar to that of cholest-5-en-3-one, thus indicating that paxisterol possessed the same absolute stereochemistry as cholesterol.

Experimental

^1H and ^{13}C NMR spectra were recorded on a Bruker AM400 spectrometer. Chemical shifts are given from $SiMe_4$ as internal standard, and the coupling constants were reported in hertz. ^{13}C Spectral data are reported in Tables 1 and 2. IR spectra were measured on a Shimadzu IR-27G IR spectrometer. Mass spectra and high-resolution data were recorded on a Hitachi M-80B spectrometer at 70 eV. Optical rotations were measured on a Perkin-Elmer 141 polarimeter, and CD spectra were obtained from a JASCO J-500A spectropolarimeter. M.p.s were taken on a Yanagimoto micro melting point apparatus and are uncorrected. Wakogel C-200 was used for column chromatography, and analytical TLC separations were performed using precoated Silica Gel 60 F_{254} (E. Merck) visualized by a spray of 1% $Ce(SO_4)_2$–10% H_2SO_4 followed by heating.

Catalytic Reduction of Paxisterol **1**.—To a solution of paxisterol **1** (620 mg) in methanol (50 ml) was added 10% palladium–carbon catalyst (450 mg) and the mixture was stirred

Table 2. ^{13}C NMR data [$\delta_C(CDCl_3$; 50.28 MHz)] for paxisterol **1** and its derivatives.

Carbon	1	2	3	4	5	6	7	8	9	10	11
1	38.1	38.1	37.6	37.5	37.4	36.2	37.8	37.8	37.5	39.4	37.2
2	30.7	30.8	30.8	30.5	30.2	30.7	31.0	30.8	26.7	37.3	34.0
3	70.8	71.0	71.0	70.8	70.6	71.4	71.4	71.1	73.3	210.6	210.5
4	37.4	37.4	36.8	36.9	36.8	35.1	37.8	38.0	33.7	43.9	42.6
5	39.8	39.8	36.8	36.7	37.9	45.2	45.4	44.8	44.5	42.0	39.1
6	28.6	28.6	36.9	33.7	34.5	126.4	24.9	25.5	25.1	28.9	30.3
7	56.5	56.5	33.0	60.6	54.7	135.0	40.3	31.8	31.7	56.1	176.7
8	60.2	60.2	65.3	93.9	93.7	89.2	71.9	91.8	90.7	60.3	205.5
9	48.7	48.7	75.0	48.2	48.0	55.1	53.8	54.7	54.5	48.0	55.2
10	33.8	33.9	47.3	36.5	36.6	34.5	35.8	36.3	36.3	34.0	36.5
11	21.7	21.7	35.9	20.6	20.5	21.1	18.9	21.0	20.9	21.9	25.4
12	28.4	28.40 28.42	18.9	38.1	37.9	38.5	28.8	38.6	41.4	28.2	27.5
13	58.7	58.6	28.2	63.9	63.8	65.5	57.3	64.1	60.2	58.6	63.5
14	54.5	54.6	57.6	63.5	64.4	64.4	61.6	64.4	62.8	54.3	64.3
15	72.8	72.8	56.3	73.5	73.5	72.9	74.4	73.1	210.2	72.8	72.4
16	35.3	35.5 35.4	74.2	32.4	32.3	32.6	35.2	32.7	32.8	35.3	35.0
17	49.1	48.9 49.0	34.9	52.6	52.5	52.3	49.2	52.5	45.4	49.0	49.2
18	106.7	106.1	49.2	109.2	109.3	107.7	107.1	108.3	108.1	106.6	105.1
19	12.8	12.8	106.9	12.1	12.2	11.1	12.5	12.1	12.0	12.0	16.7
20	85.2	85.6 85.5	12.6	88.7	88.5	88.2	85.1	88.1	87.5	85.2	86.1
21	27.3	27.43 27.41	85.3	24.9	24.8	24.7	27.2	24.9	24.2	27.4	27.1
22	39.6	39.10 39.22	27.2	37.0	36.8	36.9	39.6	36.9	36.5	39.6	39.5
23	29.5	28.9 29.2	39.5	28.3	28.2	28.3	29.5	28.4	28.3	29.4	30.3
24	155.6	39.4 39.15	29.4	155.3	155.1	155.4	155.5	155.4	155.2	155.6	155.2
25	33.9	31.8 31.9	155.4	33.9	33.8	33.9	34.0	33.9	34.0	33.9	33.8
26	21.9	18.2 17.7	34.0	21.9	21.8	21.9	21.9	21.9	21.94	21.9	21.8
27	21.9	20.2 20.4	21.9	21.9	21.8	21.9	21.9	21.9	21.89	21.9	21.8
28	106.4	15.5 15.3	21.9	106.8	106.8	106.7	106.5	106.7	106.6	106.4	106.5
CO*Me*									21.4		
COMe									170.7		

for 2 h under hydrogen at room temperature. After the catalyst had been filtered off the solvent was evaporated off under reduced pressure to afford a powder, which was subjected to silica gel column chromatography and eluted with $CHCl_3$–MeOH (10:1) to give a mixture of (24R)- and (24S)-24,28-*dihydropaxisterol* **2** (440 mg, 71%) as needles, m.p. 234–235 °C (from EtOAc); $[\alpha]_D^{22}$ −55.3° (*c* 0.3, $CHCl_3$); $\nu_{max}(CHCl_3)$ 3600 and 3450 cm^{-1} (OH); *m/z* 445 (MH^+, 52%), 398 (100), 300, 273, and 243 (Found: M^+, 444.3272. $C_{28}H_{44}O_4$ requires *M*, 444.3237).

HCl Addition to Paxisterol **1**.—To a solution of paxisterol **1** (50 mg) in THF (5 ml) was added 2M-hydrochloric acid (1 ml), and the mixture was stirred at room temperature for 15 min. The reaction mixture was neutralized with aq. $NaHCO_3$ and evaporated to give a residue, which was dissolved in EtOAc (50 ml) and the solution was washed with water (50 ml). The water layer was extracted twice with EtOAc (100 ml). The combined EtOAc extracts and mother liquor were washed with saturated aq. NaCl and dried with $MgSO_4$. The EtOAc solution was evaporated to afford a residue, which was subjected to silica gel column chromatography and eluted with hexane–EtOAc (3:1) to give 7-*chloro*-8-*hydroxy derivative* **3** (52 mg, 96%) as needles, m.p. 171–172 °C (from EtOAc); $[\alpha]_D^{22}$ −50.8° (*c* 0.3, $CHCl_3$); $\nu_{max}(CHCl_3)$ 3610 and 3480 cm^{-1} (OH); *m/z* 478 (M^+, 3%), 461, 436, 381, 337, 257, 123, 107 and 81 (100) (Found: M^+, 478.2849. $C_{28}H_{43}ClO_4$ requires *M*, 478.2848).

HCl Addition followed by Acetal Migration of Paxisterol **1**.—To a solution of paxisterol **1** (1.005 g) in THF (30 ml) was added 2M-hydrochloric acid (1.2 ml), and the mixture was stirred at 50 °C for 1 h, neutralized with aq. $NaHCO_3$, and evaporated to give a residue, which was dissolved in $CHCl_3$ (150 ml) and washed with water (150 ml). The water layer was extracted twice with $CHCl_3$ (200 ml). The combined $CHCl_3$ extracts and mother liquor were washed with saturated aq. NaCl and dried with $MgSO_4$. The $CHCl_3$ solution was evaporated to afford a residue, which was subjected to silica gel column chromatography and eluted with hexane–EtOAc (2:1) to give 7-*chloro*-15-*hydroxy derivative* **4** (745 mg, 69%) as needles, m.p. 169–170 °C (from EtOAc); $[\alpha]_D^{22}$ −43.7° (*c* 0.2, $CHCl_3$); $\nu_{max}(CHCl_3)$ 3620 and 3450 cm^{-1} (OH); *m/z* 478 (M^+, 4%), 460 (100), 435, 381, 363, 337, 291, 257, 123, 107 and 81 (Found: M^+, 478.2866. $C_{28}H_{43}ClO_4$ requires *M*, 478.2848).

Acetal Migration from 15-O *to* 8-OH *in Compound* **3**.—To a solution of compound **3** (630 mg) in $CHCl_3$ was added PTSA (100 mg) and the mixture was stirred at room temperature for 4 h, diluted with $CHCl_3$ (100 ml), and neutralized with aq. $NaHCO_3$ checking by universal test paper (Togo Roshi Kaisha, Japan), and washed with saturated aq. NaCl. The $CHCl_3$ solution was dried with $MgSO_4$ and evaporated to give a residue, which was chromatographed on a silica gel column and eluted with hexane–EtOAc (2:1) to give 15-hydroxy derivative **4** (521 mg, 83%).

HBr Addition followed by Acetal Migration of Paxisterol **1**.—To a solution of paxisterol **1** (750 mg) in THF (25 ml) was added 2M-hydrobromic acid (1.5 ml) and the mixture was stirred at 50 °C for 4 h. The reaction mixture was neutralized with aq. $NaHCO_3$ checking by universal test paper (Togo Roshi Kaisha, Japan) and evaporated to give a residue, which was dissolved in EtOAc (100 ml) and the solution was washed with water (100 ml). The water layer was extracted twice with EtOAc (200 ml). The combined EtOAc extracts and mother liquor were washed with saturated aq. NaCl and dried with $MgSO_4$. The EtOAc solution was evaporated to afford a residue, which was subjected to silica gel column chromatography and eluted with hexane–EtOAc (2:1) to give 7-*bromo*-15-*hydroxy derivative* **5** (620 mg, 7·0%) as needles, m.p. 211–212 °C (from EtOAc)

(Found: C, 64.0; H, 8.5. $C_{28}H_{43}BrO_4$ requires C, 64.24; H, 8.28%); $[\alpha]_D^{22}$ −82.0° (*c* 0.3, $CHCl_3$); $\nu_{max}(CHCl_3)$; 3620 and 3450 cm^{-1} (OH); *m/z* 523 (MH^+, 0.2%), 506, 504, 481, 479, 425 and 81 (100).

Elimination of HBr from Bromide **5**.—To a solution of the bromide **5** (275 mg) in MeOH (30 ml) was added KOH (5 g) and the mixture was heated to reflux for 5 h. The solution was concentrated under reduced pressure to give a residue, which was dissolved in EtOAc (50 ml), and the solution was washed with water (50 ml). The water layer was extracted twice with EtOAc (60 ml). The combined EtOAc extracts and mother liquor were washed with saturated aq. NaCl, dried with $MgSO_4$, and evaporated to afford a residue, which subjected to silica gel column chromatography and eluted with hexane–EtOAc (2:1) to give 6-*ene derivative* **6** (215 mg, 93%) as needles, m.p. 177–178 °C (from EtOAc) (Found: C, 75.7; H, 9.85. $C_{28}H_{42}O_4$ requires C, 75.98; H, 9.56%); $[\alpha]_D^{22}$ −56.7° (*c* 0.5, $CHCl_3$); $\nu_{max}(CHCl_3)$ 3620 and 3430 cm^{-1} (OH); *m/z* 442 (M^+, 3%), 424, 406, 328, 312, 300, 283 and 81 (100) (Found: M^+, 442.3107. $C_{28}H_{42}O_4$ requires *M*, 442.3081).

$LiAlH_4$ Reduction of Paxisterol **1**.—To a stirred solution of paxisterol **1** (1.23 g) in THF (50 ml) at room temperature under nitrogen was added $LiAlH_4$ (250 mg) and the mixture was refluxed for 30 min. After the solution had cooled, MeOH (5 ml) was added followed by water (1 ml). Dil. HCl (100 ml) was added, and the mixture was extracted twice with EtOAc (200 ml). The combined extracts were washed successively with saturated aq. $NaHCO_3$ and saturated aq. NaCl, dried with $MgSO_4$, and evaporated to yield 8-*hydroxy derivative* **7** (1.21 g, 99%) as needles, m.p. 206–207 °C (from EtOAc); $[\alpha]_D^{22}$ −25.6° (*c* 0.4, $CHCl_3$); $\nu_{max}(CHCl_3)$ 3630 and 3550 cm^{-1} (OH); *m/z* 445 (MH^+, 60%), 426 (100), 401, 329, 301, 257, 123, 107 and 81 (Found: M^+, 444.3249. $C_{28}H_{44}O_4$ requires *M*, 444.3237).

Acetal Migration from 15-O *to* 8-OH *in Compound* **7**.—To a solution of compound **7** (613 mg) in THF (50 ml) was added 2M-hydrochloric acid (2 ml) and the mixture was refluxed for 2 h. After neutralization with aq. $NaHCO_3$ checking by universal pH test paper (Togo Roshi Kaisha, Japan), the solvent was evaporated off under reduced pressure. The residue was dissolved in $CHCl_3$ (50 ml) and the solution was washed with water (50 ml). The water layer was extracted twice with $CHCl_3$ (100 ml). The combined $CHCl_3$ extracts and mother liquor were washed successively with saturated aq. $NaHCO_3$ and saturated aq. NaCl and dried with $MgSO_4$. The $CHCl_3$ solution was evaporated to afford a residue, which was subjected to silica gel column chromatography and eluted with hexane–EtOAc (1:1) to give 15-*hydroxy derivative* **8** (415 mg, 71%) as needles, m.p. 171–172 °C (from EtOAc) (Found: C, 75.4; H, 10.0. $C_{28}H_{44}O_4$ requires C, 75.63; H, 9.97%); $[\alpha]_D^{22}$ −9.3° (*c* 0.3, $CHCl_3$); $\nu_{max}(CHCl_3)$ 3600 and 3440 cm^{-1} (OH); *m/z* 444 (M^+, 8%), 426 (100), 408, 401, 329, 301 and 257.

Tributyltin Hydride Reduction of Chloride **4**.—To a solution of compound **4** (14 mg) in toluene (0.5 ml) were added tributyltin hydride (20 μl) and α,α′-azoisobutyronitrile (9 mg) and the mixture was stirred at 80 °C for 21 h. To the reaction mixture was added $CHCl_3$ (1 ml) and the mixture was stirred for 30 min, then evaporated to afford a residue, which was subjected to silica gel column chromatography and eluted with hexane–EtOAc (2:1) to give compound **8** (2.2 mg).

Acetylation followed by Jones Oxidation of Compound **8**.—A solution of compound **8** (50 mg) in a mixture of acetic anhydride (0.5 ml) and pyridine (1 ml) under nitrogen was kept for 1 h at room temperature. Water (0.5 ml) was added, and after 30 min the solvent was evaporated off. To a stirred solution of the residue in acetone (2 ml) was added Jones' reagent (0.2 ml) and the mixture was stirred for 30 min at room temperature, then neutralized with aq. $NaHCO_3$ checking by universal pH test paper (Togo Roshi Kaisha, Japan). Water (50 ml) was added and the mixture was extracted three times with EtOAc (150 ml). The combined extracts were washed with saturated aq. NaCl, dried with $MgSO_4$, and evaporated to afford a yellowish powder, which was subjected to silica gel column chromatography and eluted with hexane–EtOAc (3:1) to give 3-O-*acetyl*-15-*keto derivative* **9** (45 mg, 83%) as needles, m.p. 210–211 °C (from EtOAc); $[\alpha]_D^{22}$ +18.9° (*c* 0.2, $CHCl_3$); $\nu_{max}(CHCl_3)$ 1745 (ester) and 1725 cm^{-1} (CO); *m/z* 484 (M^+, 54%), 466, 441, 387, 341 (100), 289, 139, 123 and 107 (Found: M^+, 484.3181. $C_{30}H_{44}O_5$ requires *M*, 484.3186).

Pyridinium Chlorochromate (PCC) Oxidation of Paxisterol **1**.—To a solution of compound **1** (802 mg) in CH_2Cl_2 (20 ml) at room temperature was added pyridinium chlorochromate (1.61 g). After 2 h, the reaction mixture was dissolved in EtOAc (100 ml) and washed with saturated aq. $NaHCO_3$. The water layer was extracted twice with EtOAc (200 ml). The combined EtOAc extracts and mother liquor were washed with saturated aq. NaCl, dried with $MgSO_4$, and evaporated to afford a yellowish residue, which was subjected to silica gel column chromatography and eluted with hexane–EtOAc (1:2) to give 3-*keto derivative* **10** (399 mg, 50%) as needles, m.p. 226–227 °C (from EtOAc) (Found: C, 76.2; H, 9.2. $C_{28}H_{40}O_4$ requires C, 76.33; H, 9.15%); $[\alpha]_D^{22}$ −29.3° (*c* 0.3, $CHCl_3$); $\nu_{max}(CHCl_3)$ 1705 cm^{-1} (CO); *m/z* 440 (M^+, 18%), 422, 394, 351, 343 (100), 325, 311 and 298 (Found: M^+, 440.2935. $C_{28}H_{40}O_4$ requires *M*, 440.2925).

Jones Oxidation of Paxisterol **1**.—To a solution of paxisterol **1** (60 mg) in acetone (5 ml) was added Jones' reagent (0.2 ml) and the mixture was stirred for 1 h at room temperature, then evaporated to give a residue, which was dissolved in EtOAc (30 ml), and the solution was washed with water (30 ml). The water layer was extracted twice with EtOAc (90 ml). The combined EtOAc extracts and mother liquor were washed with saturated aq. NaCl, dried with $MgSO_4$, and evaporated to afford a yellowish residue, which was subjected to silica gel column chromatography and eluted with $CHCl_3$–MeOH–AcOH (30:1:0.1) to give *keto carboxylic acid* **11** (33 mg, 52%) as a powder, $[\alpha]_D^{22}$ −5.0° (*c* 0.2, $CHCl_3$); $\nu_{max}(CHCl_3)$ 3200 and 1710 cm^{-1} (CO_2H and CO); *m/z* 472 (M^+, 9%), 454, 426, 168, 123 (85) and 81 (100) (Found: M^+, 472.2835. $C_{28}H_{40}O_6$ requires *M*, 472.2822).

Acknowledgements

We thank Dr. Y. Saitoh and Mr. T. Iida for helpful discussions, Mr. A. Nakamura and Mr. M. Okubo for 400 MHz NMR spectra, Mrs. Y. Yasuzawa for mass spectra and Miss K. Yoshikawa for technical assistance.

References

1 H. Nakano, M. Hara, Y. Yamashita, K. Ando and K. Shuto, *J. Antibiot.*, 1988, **41**, 409.
2 B. M. Jagodzinska, J. S. Trimmer, W. Fenical and C. Djerassi, *J. Org. Chem.*, 1985, **50**, 1453.
3 N. Koizumi, Y. Fujimoto, T. Takesita and N. Ikekawa, *Chem. Pharm. Bull.*, 1979, **27**, 38.
4 P. D. Bartlett, G. L. Fraser and R. B. Woodward, *J. Am. Chem. Soc.*, 1941, **63**, 495.

Paper 0/01805H
Received 23rd *April* 1990
Accepted 9th *July* 1990