[Chem. Pharm. Bull. 21(2) 323—328 (1973)] UDC 547.92.02 : 591.05

Marine Sterols. I. Sterols of Annelida, *Pseudopotamilla occelata* Moore

Masaru Kobayashi, Motohito Nishizawa, Kagemi Todo, and Hiroshi Mitsuhashi

Faculty of Pharmaceutical Sciences, Hokkaido University[1]

(Received July 29, 1972)

Sterols of annelida of the class Polychaeta, *Pseudopotamilla occelata* Moore, were identified as 22-*trans*-24-norcholesta-5,22-dien-3β-ol (4.4%), 22-*trans*-cholesta-5,22-dien-3β-ol (6%), cholesterol (50.1%), brassicasterol (5.5%), desmosterol (17.9%), 24-methylcholest-5-en-3β-ol (3.4%), 24-methylenecholest-5-en-3β-ol (9.3%), 24-ethylcholesta-5,22-dien-3β-ol (0.5%), and 24-ethyl- and -ethylidene-chloest-5-en-3β-ol (total 2%). The major six sterols were isolated and their structure characterized. Gas–liquid chromatography showed the probable presence of 22-*cis*- isomer of cholesta-5,22-dien-3β-ol.

Marine invertebrates contain a variety of sterols which have been studied from comparative and phylogenetic point of view for a long time.[2] During the last few years some workers reported the occurrence of biogenetically interesting C_{22}, C_{26}, and C_{30} sterols in marine invertebrates.[3] Among these, the C_{26} sterols seem to occur rather widely. At the present time, there are no explanations about the biogenesis of these sterols with unusual side chain.

Although the sterol distribution in marine intertebrates has been studied extensively, there are little informations concerning that of annelids. Only cholesterol has been identified in marine and terrestrial species of this phylum.[4] The present paper will show that annelida of the class Polychaeta, *Pseudopotamilla occelata*, collected in Hokkaido, contains at least eleven Δ^5-sterols including C_{26} sterol and desmosterol.

The sterol fraction (0.31% of the dried material) was separated by saponification of the ether extract followed by silica gel chromatography and crystallization from methanol. Preliminary identification of the components was made with free sterol, methyl ether, and trimethylsilyl (TMS) ether derivatives by combined gas–liquid chromatography–mass spectrometry (GLC/MS) using 3% OV-1 column. Each chromatogram was found to consist of 7 peaks (Fig. 1). Every peak showed prominent fragments, *i.e.*, molecular ion (M), M-Me, M-ROH, M-Me-ROH, M-side chain-ROH (*m/e* 255), along with strong ions at *m/e* 129 and M-129 in each TMS ether. This cracking pattern fully confirms that these peaks contain 3β-hydroxy-Δ^5-sterol derivatives.[5a] Other ions such as M–side chain (*m/e* 273, 287, 345),[6] M-side chain-42 (*m/e* 231, 245, 303), M-side chain-42-ROH (*m/e* 213), and M-side chain-27-RO (*m/e* 229) appeared in lower intensity than those of Δ^7-analogs. The ions, M-side chain-27 (*m/e* 246, 260, 318) was very weak. It was the major ion in Δ^7-sterols of starfish, parti-

1) Location: *Kita-12-jo, Nishi-5-chome, Sapporo, Hokkaido.*
2) J. Austin, "The Sterols of Marine Invertebrates and Plants," in "Advances in Steroid Biochemistry and Pharmacology," Vol. 1, ed. by M.H. Briggs, Academic Press, Inc., New York, 1970, p. 73—96.
3) *a*) D.R. Idler, P.M. Wiseman, and L.M. Safe, *Steroids*, **16**, 451 (1970); *b*) A. Kanazawa and S. Teshima, *Bull. Jap. Soc. Sci. Fish.*, **37**, 675 (1971); *c*) M. Kobayashi, R. Tsuru, K. Todo, and H. Mitsuhashi, *Tetrahedron Letters*, **1972**, 2935; *d*) A. Alcaide, J. Viala, F. Pinte, M. Itoh, T. Nomura, and M. Barbier, *C.R.*, **273**, 1386 (1971); *e*) Y.M. Sheikh, C. Djerassi, and B.M. Tursch, *Chem. Commun.*, **1971**, 217; *f*) D.R. Idler, L.M. Safe, and E.F. MacDonald, *Steroids*, **18**, 545 (1971).
4) A.de Waele, *Bull. Acad. Belg. Cl. Sci.*, **16**, 592 (1930); C.G. Wilber and W.M. Bayors, *Biol. Bull. Woods Hole*, **93**, 99 (1947).
5) *a*) B.A. Knights, *J. Gas Chromatogr.*, **5**, 273 (1967); *b*) S.G. Wyllie and C. Djerassi, *J. Org. Chem.*, **33**, 305 (1968).
6) The mass numbers are arranged in the order of free sterol, methyl ether, and TMS ether in this paper.

cularly when the side chain is unsaturated.[3c] The ions due to M-side chain-2H (*m/e* 271, 285, 343) were observed in every peak except in peak 3, suggesting that these peaks contain sterols with unsaturated side chain.[5b]

Peak 1——The molecular ions (*m/e* 370, 384, 442) and other fragments, M-Me (*m/e* 355, 369, 427), M-ROH (*m/e* 352), and M-Me-ROH (*m/e* 337), show that peak 1 is C_{26} sterol and diunsaturated. The prominent ions at *m/e* 300, 314, and 372 derived from the cleavage of C-20 and C-22 with one hydrogen transfer, are the main feature of C-22 unsaturated Δ^5- and Δ^7-sterols.[5b] The *m/e* 97 ion due to unsaturated C_7H_{13} side chain was the base peak in three mass spectra.[3a] The sterol was isolated as its acetate.

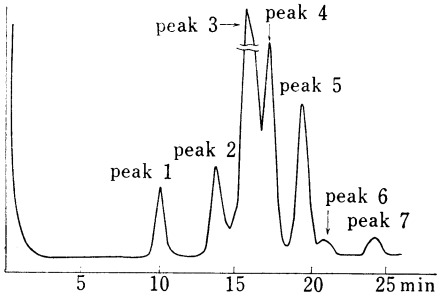

Fig. 1. Gas Chromatogram of Sterol Methyl Ether Mixture from *P. occelata* by a Column of 3% OV-1 at 260°

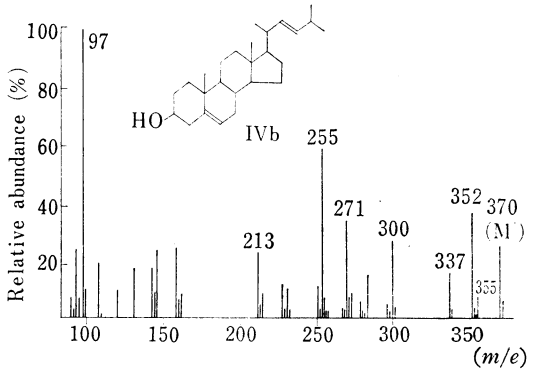

Fig. 2. Mass Spectrum of the Peak 1 of Free Sterol

Peak 2——The ions, M (*m/e* 384, 398, 456), M-Me (*m/e* 369, 383, 441), M-ROH (*m/e* 366), M-Me-ROH (*m/e* 351), and at *m/e* 300, 314, 372, show that peak 2 is cholesta-5,22-dien-3β-ol.

Peak 3——The cracking pattern and retention time agreed with those of cholesterol and its methyl and TMS ether derivatives.

Peak 4——The main fragments are the same as those of peak 2 except that ions at *m/e* 300, 314, and 372 were accompanied by ions with one mass unit smaller. The ions at *m/e* 271, 285, and 343 are far more intense than those of peak 2. In each of the three spectra, the ion at *m/e* 253 (M-side chain-ROH-2H) is more intense than the ion at *m/e* 255. These, along with its retention time, indicate that peak 4 is desmosterol.[7] The mass spectra revealed the simultaneous presence of another sterol with molecular ions at *m/e* 398, 412, and 470 as a minor component. The absence of the ions due to McLafferty-type cleavage[5] suggests it is 24-methylcholesta-5,22-dien-3β-ol. Both components were isolated as acetate and their structure characterized.

Peak 5——The ions at *m/e* 314, 328, and 386 due to the cleavage between C-22 and C-23 by McLafferty type of rearrangement and the molecular ions (*m/e* 398, 412, 470, and 400, 414, 472) suggest that peak 5 is composed of 24-methyl- and 24-methylene-cholest-5-en-3β-ol. The latter sterol was isolated as acetate.

Peak 6[8]——The ions, M (*m/e* 426, 484), M-Me (*m/e* 411, 469), M-ROH (*m/e* 394), M-Me-ROH (*m/e* 379), and the prominent ions at *m/e* 314 and 372 due to the allylic cleavage with one hydrogen transfer[5b] suggest that peak 6 is 24-ethyl-cholesta-5,22-dien-3β-ol.

Peak 7——The ions, M (*m/e* 414, 428, 486), M-Me (*m/e* 399, 413, 471), M-ROH (*m/e* 396), and M-Me-ROH (*m/e* 381) show that peak 7 is 24-ethylcholest-5-en-3β-ol. Other weak molecular ions at *m/e* 412, 426, and 484, and the strong ions at *m/e* 314, 328, and 386, suggest the co-existence of 24-ethylidene-cholest-5-en-3β-ol.

7) C.J.W. Brooks, E.C. Horning, and J.C. Young, *Lipids*, 3, 391 (1968).
8) The mass spectrum of free sterol is not available.

The GLC of the sterol acetate by a column of 1.5% SE-30 showed 7 peaks with the same pattern as by 3% OV-1. Chromatography of the sterol acetate on silver nitrate-impregnated silicic acid[9] gave 6 major sterols which corresponded to peaks 1 to 5, found to be pure or above 97% pure by GLC. The isolated sterols amounted to 93% of the original mixture and are discussed in the order of their elution.

The first eluate (I), mp 115—116.5° was identified with cholesterol acetate by mixed melting point and spectral data.

The second eluate (IIa), mp 149—150.5°, corresponded to peak 4. The nuclear magnetic resonance (NMR) spectrum showed absorptions of two secondary methyls at δ 0.80 (28-Me) and 0.96 (21-Me), terminal dimethyl at δ 0.82, and olefinic protons at δ 5.34 (1H, 6-H) 5.04—5.24 (2H, multiplet, 22,23-H). Hydrolysis of IIa gave a free sterol (IIb), mp 142.5—145°. Although the melting points and specific rotations (free sterol, $[\alpha]_D$ −50°; acetate, $[\alpha]_D$ −55.3° are intermediate values reported for brassicasterol (mp 146—151,° $[\alpha]_D$ −62°; acetate, mp 152—159°, $[\alpha]_D$ −64°), and its C-24R epimer (mp 141—147°, $[\alpha]_D$ −41°; acetate, mp 135—138°, $[\alpha]_D$ −46°),[10] it was rather regarded as slightly impure brassicasterol and not a mixture of epimers, from the melting point of its acetate and its NMR spectrum which showed no splitting of secondary and angular methyl groups. The inseparability of these C-24 alkylated epimers by GLC was noted by some workers.

The third eluate (IIIa), mp 121—129°, corresponded to peak 2, cholesta-5,22-dien-3β-ol acetate (reported,[10] mp 126—128°). The structure was confirmed by NMR signals of secondary methyl at δ 0.995 (21-Me), terminal dimethyl at δ 0.851, angular methyl at δ 0.685 (18-Me) and 1.015 (19-Me), and olefinic protons at δ 5.0—5.25 (2H, multiplet, 22,23-H) and 5.30 (1H, multiplet, 6-H). Strong infrared (IR) absorptions at 957 and 968 cm^{-1} supported the *trans* configuration at C-22.[11] Hydrolysis of IIIa gave a free sterol (IIIb), mp 131—133° (reported,[10] mp 133—136°).

The fourth eluate (IVa), mp 141—142°, which corresponded to peak 1, showed NMR signals at δ 0.677 (18-Me), 0.926 (6H, doublet, terminal dimethyl), 1.003 (19-Me), 2.013 (OAc), 4.35—4.8 (1H, broad, 3α-H), 5.1—5.25 (2H, multiplet, 22,23-H), and 5.32 (1H, multiplet, 6-H). These data add complementary proof to mass spectral data for $\Delta^{5,22}$-diunsaturated C_{26} sterol which has a terminal isopropyl group. The free sterol (IVb), mp 134—138°, showed NMR signals at δ 0.681 (18-Me), 0.930 (6H, doublet, terminal dimethyl), 1.00 (19-Me), 0.984 (3H, doublet, 21-Me. In acetate, it was enveloped by another signals), 3.3—3.8 (1H, broad, 3α-H), 5.14—5.28 (2H, multiplet, 22,23-H), and 5.32 (1H, multiplet, 6-H). The *trans* configuration at C-22 was supported by IR absorptions at 958 and 970 cm^{-1}. These spectral data indicate that peak 1 is 22-*trans*-24-norcholesta-5,22-dien-3β-ol (reported,[12] mp 138—140°), first isolated by Idler, *et al.*[3a] from scallop and not the 27-nor structure. Asteroids generally convert the Δ^5-sterol into Δ^7-sterols[13] and, as a hypothesis, it is suggested that the $\Delta^{5,22}$-C_{26} sterol may be the precursor of asterosterol, 24-norcholesta-7,22-dien-3β-ol, found in asteroids collected in Hokkaido.[3c]

The fifth eluate (Va), mp 96.5—97.5°, which corresponded to peak 4, was identified with desmosterol acetate (reported,[14] mp 99°). Its NMR spectrum showed a terminal vinylic dimethyl at δ 1.59 and 1.67, and C-24 olefinic proton at δ 5.05 as a slightly broad triplet. Hydrolysis of Va gave a free sterol (Vb), mp 117.5—119° (repoted,[14] mp 121—122°).

---

9) H.E. Vroman and C.F. Cohen, *J. Lipid Res.*, **8**, 150 (1962).
10) J. Jacques, H. Kagan, and G. Ourisson, "Tables of Selected Constants," Vol. 14, 1a, ed. by S. Allar, Pergamon Press, Inc., New York, 1965.
11) L.F. Fieser and M. Fieser, "Steroids," Reinhold, Inc., New York, 1959, p. 172.
12) M. Fryberg, A.C. Oehlschlager, and A.M. Unrau, *Chem. Commun.*, **1971**, 1194.
13) U.H.M. Fagerlund and D.R. Idler, *Can. J. Biochem. Physiol.*, **38**, 997 (1960).
14) D.R. Idler, A. Saito, and P. Wiseman, *Steroids*, **11**, 465 (1968).

The sixth and most polar component (VIa), mp 132—133.5,° which corresponded to peak 5, amounted to 9.3% of the original mixture and recovery seemed quantitative. It was identified with 24-methylencholest-5-en-3β-ol acetate (reported,[10] mp 134—136°) by strong IR absorption of terminal methylene at 895 cm^{-1} and by NMR signals at δ 0.66 (18-Me), 0.985 (19-Me), 0.986 (doublet, terminal dimethyl), 4.64 and 4.69 (each 1H, multiplet, terminal methylene), and 5.35 (1H, multiplet, 6-H). Hydrolysis of VIa gave a free sterol (VIb), mp 142—143.5° (reported,[10] mp 145—146°).

The GLC of sterol methyl ether on 1.5% OV—17 showed a better separation as shown in Fig. 3. Each of peak 2 and 4 separated into two components. In this condition, brassicasterol is eluted faster than desmosterol[15] and, in fact, the isolated brassicasterol and desmonsterol amounted to 4% and 17% of the mixture in accordance with the result of GLC. From the GLC retention time, the shoulder of peak 2 seems to be 22-*cis* isomer of cholesta-5,22-dien-3β-ol (IIIb) recently isolated by Idler, *et al.*[16] from scallop. Based on the gas-liquid chromatography (GLC) and spectral data, the sterols of *Pseudopotamilla occelata* are summarized in Fig. 3.

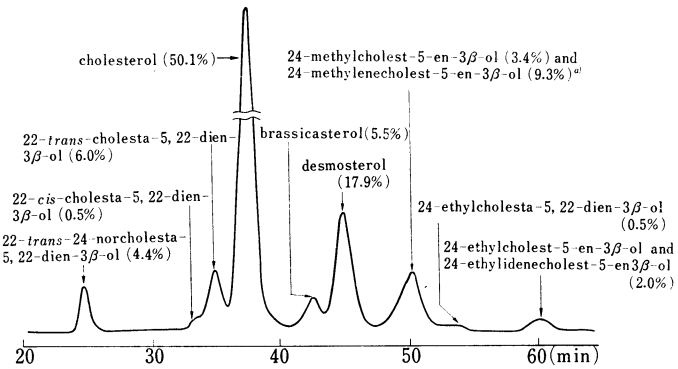

Fig. 3. Gas Chromatogram and Composition of Sterol Methyl Ether Mixture from *P. occelata* by a Column of 1.5% OV-17 at 250°

a) Calculated from GLC and the amount isolated.

The observed sterol distribution showed a close resemblance to that of the clam, *Tapes philippinarum*, except for the absence of C_{22} sterol.[15] The stanols or sterols with conjugated diene moiety were not detected. Although the sterol biosynthesis was demonstrated to occur in some annelida of the class Polychaeta,[17] there remains some doubt as to the extent of endogenous synthesis. These animals are plankton feeders, and since the sterols described occur in variable marine plants, at least part of the sterols may be derived from phytoplankton. Recent reports, as exemplified by echinoderms,[18] invariably pointed out the greater importance of dietary preference and mode of internal modification of digested sterols in marine invertebrates. Moreover, Barvier, *et al.*[19] reported the occurrence of Δ^5-unsaturated C_{26} sterol in marine plankton. Some rhodophytae contain a high amount of desmosterol[14] and other sterols are reported in many rhodophytae and phaeophytae.[2]

Experimental

Melting points were determined on a Kofler hot-stage and are not corrected. NMR spectra were recorded in $CDCl_3$ solution at 100 MHz, with Me_4Si as internal standard. The GLC was carried out using a

15) S. Teshima, A. Kanazawa, and T. Ando, *Kagoshima Daigaku Suisan Gakubu Kiyo*, **20**, 131 (1971).
16) D.R. Idler and P. Wiseman, *Comp. Biochem. Physiol.*, **38**, 581 (1971).
17) J.A.M. Wootton and L.D. Wright, *Comp. Biochem. Physiol.*, **5**, 253 (1962).
18) L.J. Goad, I. Rubinstein, and A.G. Smith, *Proc. Roy. Soc.* (London), Ser. B, **180**, 223 (1972).
19) J.L. Boutry, A. Alcaide, and M. Barbier, *C. R.*, **272**, 1022 (1971).

1.8 m×3 mm i.d. glass column packed with 1.5% SE-30 on 60—80 mesh Chromosorb W at 250° or 3 m×3 mm i.d. glass column packed with 1.5% OV-17 on 80—100 mesh Shimalite W at 250°, with N_2 carrier gas flow-rate of 60 ml/min. The GLC/MS was carried out using a 3 m×3 mm i.d. column packed with 3% OV-1 on 100—120 mesh gas Chrome Q at 260°, with He carrier gas flow-rate of 2.0 kg/cm². Hydrolysis of steroi acetate was carried out by refluxing in 5% KOH-MeOH for 20 min, followed by usual work-up throughout.

Isolation of Sterols——The dried and ground material of *P. occelata* (1.08 kg) was extracted with ether in a Soxhlet apparatus. The extract (32 g) was saponified with 10% KOH–MeOH and the non-saponifiable material extracted with ether in a liquid-liquid extraction apparatus. The extract (4.7 g) was chromatographed over silica gel and the fractions with the same polarity of cholesterol were combined and recrystallized from MeOH to a crystalline sterol mixture (3.3 g). Sterol acetate was obtained in a usual manner, with Ac_2O in pyridine.

The GLC/MS of Eree Sterol——*m/e*: Peak 1: 370, 355, 352, 337, 300, 273, 271, 255, 231, 229, 213, 97; Peak 2: 384, 369, 366, 351, 341, 300, 273, 271, 255, 111; Peak 3: 386, 371, 368, 353, 301, 275, 273, 260, 255, 247; Peak 4: 398, 384, 369, 366, 351, 300, 299, 273, 271, 269, 255, 253, 245, 229, 213; Peak 5: 400, 398, 383, 380, 365, 314, 271, 255, 253, 231, 229, 213; Peak 7: 414, 412, 399, 396, 381, 341, 329, 314, 303, 296, 282, 275, 273, 271, 255, 231, 229.

The GLC/MS of Sterol Me Ether——A solution of 50 mg of crude sterol in 1 ml of MeI was treated with 50 mg of Ag_2O at intervals over 1 hr under reflux. After refluxing further 3 hrs, the solution was filtered and the solvent was evaporated. The residue was dried and applied to GLC/MS directly. *m/e*: Peak 1: 384, 369, 352, 337, 314, 287, 285, 259, 255, 253, 213, 97; Peak 2: 398, 383, 366, 351, 314, 287, 285, 255, 213, 111; Peak 3: 400, 385, 368, 353, 329, 326, 301, 275, 255, 247; Peak 4: 412, 398, 383, 366, 351, 327, 314, 313, 299, 285, 255, 253, 245, 213; Peak 5: 414, 412, 397, 380, 365, 328, 313, 296, 285, 253, 229, 213; Peak 6: 426, 411, 394, 351, 314, 287, 285, 255; Peak 7: 428, 426, 413, 396, 381, 328, 287, 285, 255, 213.

The GLC/MS of Sterol TMS Ether——A solution of 50 mg of crude sterol in 1 ml of dry pyridine was treated with 0.2 ml of hexamethyl disilazane and 0.1 ml of trimethylchlorosilane for 5 min. After addition of 5 ml of $CHCl_3$ and 3 ml of H_2O, the organic layer was washed with H_2O untill free from pyridine. The mixture was evaporated, dried, and applied to GLC/MS directly. *m/e*: Peak 1: 442, 427, 352, 337, 313, 255, 231, 129, 97; Peak 2: 456, 441, 372, 366; 351, 327, 255, 129; 111; Peak 3: 458, 443, 368, 353, 329, 301, 275, 255, 247, 229, 213; Peak 4: 470, 456, 441, 372, 366, 351, 343, 327, 281, 253, 245, 129; Peak 5: 472, 470, 455, 386, 382, 380, 367, 365, 343, 341, 296, 257, 129; Peak 6: 484, 469, 394, 386, 379, 372, 351, 345, 343, 318, 296, 255, 253, 129; Peak 7: 486, 484, 471, 396, 386, 381, 357, 345, 329, 303, 296, 275, 255, 129.

Column Chromatography of Sterol Acetate——A solution of crude sterol acetate (220 mg) in hexane was applied on a column of 125 g of silver nitrate-impregnated silicic acid (1: 4) and eluted with a mixture of hexane–benzene (5:1). The fractions (10 ml) were collected automatically and monitored by GLC on 1.5% SE-30 column at 250°.

Fraction 1——This was found to be composed of cholesterol acetate (I) and a trace of its C-24 alkylated derivatives. The evaporation residue (116 mg) was recrystallized from MeOH to afford pure chlolesterol acetate (I), mp 115—116.5°. $[\alpha]_D$ −34.5° (c=2, $CHCl_3$). The identity was confirmed by mixed mp 115—116°, TLC, GLC, and IR and mass spectra.

Fraction 2——This was found to be composed of an equal mixture of I and 24-methylcholesta-5,22-dien-3β-ol acetate (IIa) (2.4 mg).

Fraction 3——This was found to consist of IIa (98%) and 2% of I. The evaporation residue (8.8 mg) was recrystallized from MeOH to IIa, as colorless plates, mp 149—150.5° $[\alpha]_D$ −55.3° (c=0.9, $CHCl_3$). NMR δ: 0.67 (18-Me), 0.80 (3H, d, J=7 Hz, 28-Me), 0.82 (6H, d, J=7 Hz, 26,27-Me), 0.96 (3H, d, J=7 Hz, 21-Me), 1.00 (19-Me), 4.58 (1H, m, 3α-H), 5.04—5.24 (2H, m, 22,23-H), 5.34 (1H, m, 6-H). Hydrolysis of IIa gave the free sterol (IIb), mp 142.5—145°, $[\alpha]_D$ −50° (c=2.0, $CHCl_3$).

Fraction 4——This fraction (14.7 mg) consisted of cholesta-5,22-dien-3β-ol acetate (IIIa, 97.5%), I (2%), and IIa (0.5%). The evaporation residue was recrystallized from MeOH to IIIa, as colorless plates, mp 121—129°, $[\alpha]_D$ −51.7° (c=1.5, $CHCl_3$). NMR δ: 0.685 (18-Me), 0.851 (6H, d, J=7 Hz, 26,27-Me), 1.015 (19-Me), 0.995 (3H, d, J=7 Hz, 21-Me), 4.54 (1H, m, 3α-H), 5.0—5.25 (2H, m, 22,23-H), 5.30 (1H, m, 6-H). IR ν_{max}^{Nujol} cm^{-1}: 3100—3500, 957, 968. Hydrolysis of IIIa gave the free sterol (IIIb), mp 131—133°, $[\alpha]_D$ −50° (c=1.14, $CHCl_3$).

Fraction 5——This fraction (3.1 mg) consisted of an equal amount of IIIa and 24-norcholesta-5,22-dien-3β-ol acetate (IVa).

Fraction 6——This was found to consist of IVa. The evaporation residue (8 mg) was recrystallized from MeOH to IVa as colorless plates, mp 141—142°, $[\alpha]_D$ −43.5 (c=0.8, $CHCl_3$). NMR δ: 0.677 (18-Me), 0.926 (6H, d, J=7 Hz, 26,27-Me), 1.003 (19-Me), 2.013 (OAc), 4.35—4.8 (1H, m, 3α-H), 5.1—5.25 (2H, m, 22,23-H), 5.32 (1H, m, 6-H). Hydrolysis of IVa gave free sterol (IVb) as needles, mp 134—138°, $[\alpha]_D$ −44° (c=0.5, $CHCl_3$). NMR δ: 0.681 (18-Me), 0.930 (6H, d, J=6.5 Hz, 26,27-Me), 0.984 (3H, d, J=7 Hz, 21-Me), 1.00 (19-Me), 3.2—3.8 (1H, m, 3α-H), 5.10—5.28 (2H, m, 22,23-H), 5.28—5.4 (1H, m, 6-H). IR ν_{max}^{Nujol} cm^{-1}: 958, 970.

Fraction 7——The evaporation residue (37.6 mg) was recrystallized from MeOH to desmosterol acetate (Va) as colorless powdery crystals, mp 96.5—97.5°, $[\alpha]_D$ −37.8° (c=3.7, $CHCl_3$). NMR δ: 0.69 (18-Me), 0.93 (3H, d, J=7 Hz, 21-Me), 1.00 (19-Me), 1.59 (3H, s, 26- or 27-Me), 1.67 (3H, s, 26- or 27-Me), 2.00 (OAc), 4.4—4.8 (1H, m, 3α-H), 5.05 (1H, broad triplet, J=7 Hz, 24-H), 5.36 (1H, m, 6-H). The acetate (Va) was hydrolyzed to give the free sterol (Vb) as colorless plates, mp 117.5—119°, $[\alpha]_D$ −42.3° (c=2.6, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3200, 835, 796.

Fraction 8——Elution with hexane–benzene (2:1) gave 2.3 mg of a complex mixture of fractions 1 to 7.

Fraction 9——This fraction (20.4 mg) consisted of 24-methylenecholest-5-en-3β-ol acetate (VIa). Recrystallization from MeOH gave colorless plates, mp 132—133.5°. $[\alpha]_D$ −41.4 (c=2.0, $CHCl_3$). NMR δ: 0.66 (18-Me), 0.985 (19-Me), 0.986 (6H, d, J=7 Hz, 26,27-Me), 4.64 and 4.69 (each 1H, m, 28-H), 5.35 (1H, m, 6-H). Hydrolysis of VIa gave the free sterol (VIb) as plates, mp 142.5—143°, $[\alpha]_D$ −39.1° (c=1.1, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1728, 1250, 885.

Acknowledgement We are grateful to Japan Electron Optics Lab. Ltd. for the measurement of GLC/MS spectra.