MARINE STEROLS. VI(*1*). STEROLS OF THE SCALLOP, *PATINOPECTEN YESSOENSIS*

Masaru Kobayashi and Hiroshi Mitsuhashi

Faculty of Pharmaceutical Sciences,
Hokkaido University, Sapporo 060, Japan

Received: 8/15/75

ABSTRACT

The sterols of the scallop, *Patinopecten yessoensis* Jay, was found to contain over 20 components. The major components were Δ^5-sterols, and lesser amount of ring-saturated sterols were also present. Biogenetically unusual C_{26} sterols (24-norcholesta-5,22-dien-3β-ol and 24-norcholest-22-en-3β-ol) and 24(28)-*cis*-24-propylidenecholest-5-en-3β-ol (29-methylisofucosterol), 22-*trans*-27-*nor*-(24S)-24-methylcholesta-5,22-dien-3β-ol (occelasterol), and a new sterol, 22-*trans*-27-*nor*-(24S)-24-methylcholest-22-en-3β-ol (patinosterol), were isolated and their structures were confirmed. Occurrence of 22-*trans*-(24S)-24-methylcholesta-5,22-dien-3β-ol (24-epibrassicasterol) was confirmed. 22-*cis*-Cholesta-5,22-dien-3β-ol was not found.

INTRODUCTION

Within the classes of phylum Mollusca, the class Pelecypoda (bivalves) is unique in its great diversity of sterols (*2*). The sterols of scallops had been repeatedly studied (*2,3*) and as many as 13 sterols were reported in the recent examination (*4*).

Three novel sterols, 22-*trans*-24-norcholesta-5,22-dien-3β-ol [15a] (*4a*), 24(28)-*cis*-24-propylidenecholest-5-en-3β-ol (29-methylisofucosterol) [18a] (*4b*), and 22-*cis*-cholesta-5,22-dien-3β-ol (22-*cis*-dehydrocholesterol) (*4c*), were reported to occur in the Canadian scallop, *Placopecten magellanicus* by Idler *et al.* and were subsequently reported in many marine invertebrates (*5,6*). However, the original identification of 22-*cis*-dehydrocholesterol was insufficient and there was a significant discrepancy in the IR spectrum and chromatographic mobilities between natural and synthetic compounds (*1*).

Recently we found two new sterols, 22-*trans*-27-*nor*-(24S)-24-methylcholesta-5,22-dien-3β-ol (occelasterol) [13a] and its Δ^7-isomer, amuresterol [23], both having a novel 27-*nor*-24-methylcholestane-type side chain, from a marine annelida, *Pseudopotamilla occelata* (*1*), and a starfish, *Asterias amurensis* (*7*), and suggested that "22-*cis*-dehydrocholesterol", reported from many marine invertebrates (*4c*,*6*), is in fact occelasterol (*1*).

Since the Japanese scallop, *Patinopecten yessoensis* Jay, the closely related species, contained a high proportion of the component which corresponded to occelasterol (or to 22-*cis*-dehydrocholesterol) in GLC (peak 2, Fig. 1), we investigated its sterol composition with the purpose of confirming the identity with occelasterol as well as looking for the presence of new sterols. The sterols of *P. yessoensis* had been studied by Kita *et al.* (*8*) and they reported the presence of clionasterol and 24-methylenecholesterol but, these reports before the introduction of GLC and mass spectrometry should be reexamined.

RESULTS

The sterol mixture was obtained in 0.1% yield from the fresh materia. Its gas chromatogram (Fig. 1) was largely identical in peak numbers and their relative intensities with those reported for *P. magellanicus* (*4c*).

The sterol acetate mixture was separated into 36 fractions by chromatography over silver nitrate-impregnated silicic acid column (Fig. 2) and some of the enriched fractions were further divided into subfractions by silver nitrate-impregnated TLC. Examination of each fraction by combination of GLC and TLC analyses revealed that each of the GLC peaks were not homogeneous and they were mainly composed of Δ^5-sterols and minor amounts of ring-saturated sterols and also

associated with a number of unidentified minor components.

Fraction 1 was a mixture of Δ^5-sterols and lesser amount of fully saturated sterols. The less polar subfraction was composed of cholestanol acetate [1b], 24ξ-methylcholestanol acetate [2b], 24ξ-ethylcholestanol acetate [3b], and a saturated C_{30} sterol.

The structure of the C_{30} sterol was deduced as 24ξ-ethyl-4ξ-methylcholestanol acetate [4b] from the mass spectrum which showed molecular ion (M^+) at *m/e* 472, M^+-Me at 457, and other ions at *m/e* 412 (M^+-AcOH), 397 (M^+-AcOH and Me), 290 (M^+-side chain and 41), 289 (M^+-side chain and 42), 271 (M^+-side chain and AcOH), 244 (M^+-side chain and C-16 to C-17 and AcOH), 344 (M^+- C-1 to C-4), 230 (M^+-side chain and AcOH and 41), and 229 (M^+-side chain and AcOH and 42). This cracking pattern was common in the mass spectra of other saturated sterols except for the

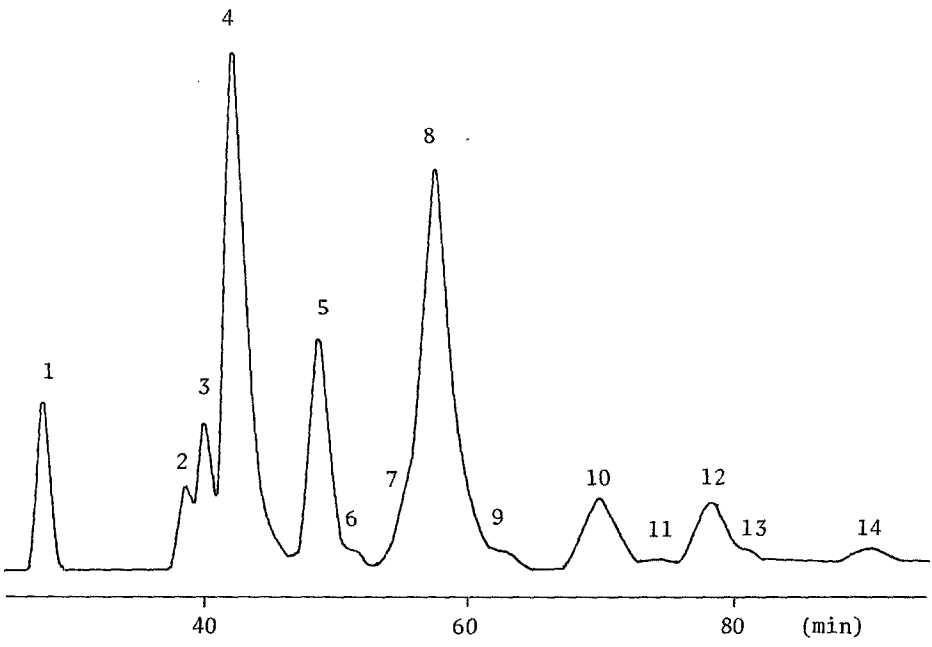


Figure 1. Gas chromatogram of the sterol mixture from *P. yessoensis*

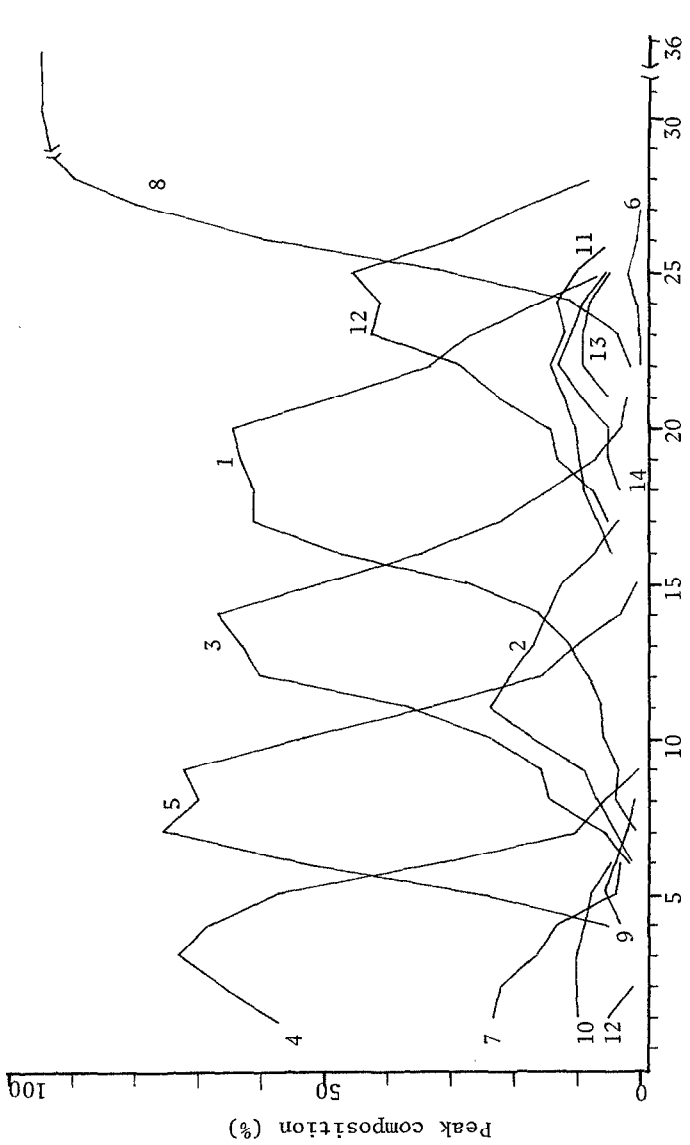


Figure 2. Column chromatography of the steryl acetate mixture from *P. yessoensis*

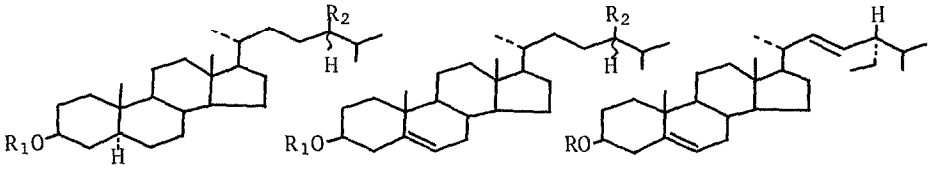
presence of a extra nuclear methyl group in 4b. From the biogenetic grounds, this is 4ξ- or 14α-methyl but the latter is excluded from the ions derived by ring D cleavage. The more polar subfraction of the fraction 1 was found to be composed of cholesterol acetate [5b], 24ξ-

methylcholesterol acetate [6b], and 24ξ-ethylcholesterol acetate [7b] from the mass spectra (M^+-AcOH, *m/e* 368, 382, and 396).

Fractions 6 to 11 yielded seven mono- and di-unsaturated sterols by several TLC separations. The subfraction, slightly more polar than Δ^5-sterols, was separated into three bands by repeated development with a mixture of benzene and hexane. The upper band (peak 9) gave 22-*trans*-(24R)-24-ethylcholesta-5,22-dien-3β-ol acetate (poriferasterol acetate) [8b], mp 140-140.5° (lit.(*9*), mp 140-141°), $[\alpha]_D$ -52±3°. NMR δ 0.70 (18-Me), 1.03 (19-Me), 1.025 (3H, d, *J*=7 Hz, 21-Me), 2.05 (OAc), 4.4-4.8 (1H, m, 3α-H), 5.0-5.2 (2H, m, 22,23-H), 5.36 (1H, m, 6-H). Hydrolysis of the acetate gave poriferasterol [8a], mp 154-157.5° (lit.(*10*), mp 157-158°), $[\alpha]_D$ -47±3°. IR (chloroform), 958, 972 cm^{-1} (*trans*-disubstituted double bond) (*11*). Mass spectrum, *m/e* 412 (M^+), 369 (M^+-C_3H_7), 351 (M^+-C_3H_7 and H_2O), 300 (M^+- C-22 to C-29 and 1H), 271 (M^+-side chain and 2H), 255 (M^+-side chain and H_2O).

The melting point of the free sterol was slightly lower than that reported for poriferasterol but clearly distinguishable from that of stigmasterol (lit.(*10*), mp 169-170°), which is isomeric at C-24.

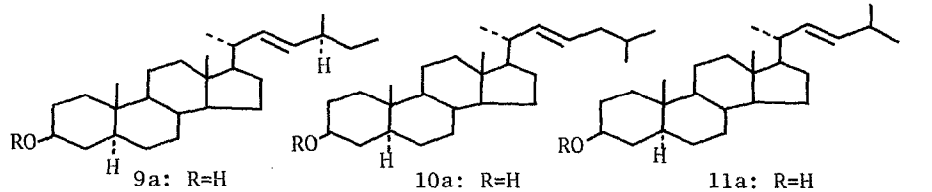
The lower band (peak 3) gave 22-*trans*-cholest-22-en-3β-ol acetate [10b], mp 106° (lit.(*5e*), mp 104-105°), $[\alpha]_D$ 0±3°. IR (chlorform), 960, 970 cm^{-1}, NMR δ 0.66 (18-Me), 0.82 (19-Me), 0.854 (6H, d, *J*=6.5 Hz, isopropyl), 0.99 (3H, d, *J*=6.2 Hz, 21-Me), 4.4-4.8 (1H, m, 3α-H), 5.14-5.32 (2H, m, 22,23-H). Hydrolysis of the acetate gave the free sterol [10a], mp 121° (lit.(*5e*), mp 117-117.5°), $[\alpha]_D$ +12±3°. Mass spectrum, *m/e* 386 (M^+), 302 (M^+- C-22 to C-27 and 1H), 287 (302-Me), 273 (M^+-side chain and 2H), 257 (M^+-side chain and H_2O). This sterol was recently found in the sponge, *Hymeniacidon perleve* (*5e*).



1a: R_1=H, R_2=H
b: R_1=Ac, R_2=H
2a: R_1=H, R_2=Me
b: R_1=Ac, R_2=Me
3a: R_1=H, R_2=Et
b: R_1=Ac, R_2=Et
4a: R_1=H, R_2=Et (4ξ-Me)
b: R_1=Ac, R_2=Et (4ξ-Me)

5a: R_1=H, R_2=H
b: R_1=Ac, R_2=H
6a: R_1=H, R_2=Me
b: R_1=Ac, R_2=Me
7a: R_1=H, R_2=Et
b: R_1=Ac, R_2=Et

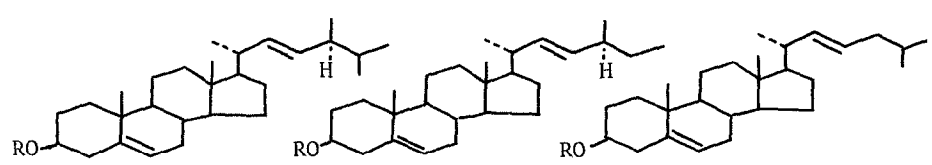
8a: R=H
b: R=Ac



9a: R=H
b: R=Ac

10a: R=H
b: R=Ac

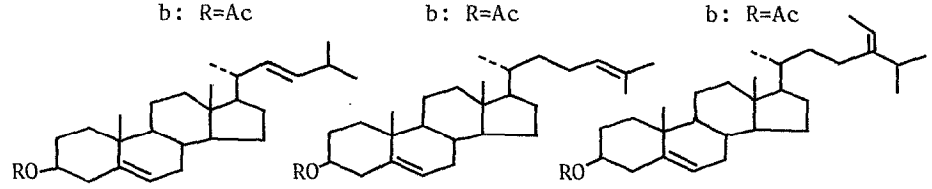
11a: R=H
b: R=Ac



12a: R=H
b: R=Ac

13a: R=H
b: R=Ac

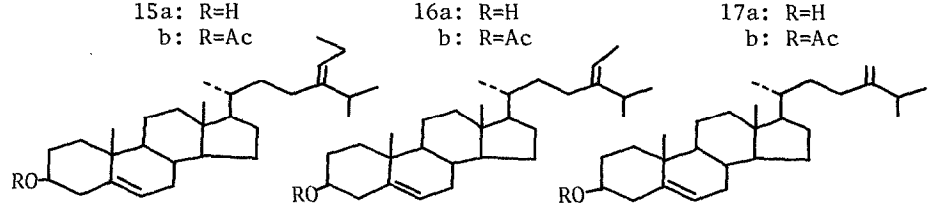
14a: R=H
b: R=Ac



15a: R=H
b: R=Ac

16a: R=H
b: R=Ac

17a: R=H
b: R=Ac



18a: R=H
b: R=Ac

19a: R=H
b: R=Ac

20a: R=H
b: R=Ac

The middle band corresponded to peak 2 and also yielded a C_{27} sterol acetate, mp 129-130°, $[\alpha]_D$ 0±3°. Hydrolysis of the acetate gave the free sterol, mp 132-133°, $[\alpha]_D$ 0±3°. It was a new compound and designated patinosterol. Patinosterol showed close resemblance to 22-*trans*-cholest-22-en-3β-ol [10a] in IR and mass spectral properties. However, it showed a slightly shorter retention time than 10a in GLC and its acetate was slightly less polar than 10b on argentation chromatography. This relation was observed between occelasterol [13a] and 22-*trans*-cholesta-5,22-dien-3β-ol [14a], and also between amuresterol [23] and 22-*trans*-cholesta-7,22-dien-3β-ol (*1,7*).

The mass spectrum of patinosterol (Fig. 3) showed molecular ion at *m/e* 386 and other ions at 371 (M^+-Me), 353 (M^+-Me and H_2O), 275 (M^+-side chain), and 257 (M^+-side chain and H_2O), indicating that it is a monounsaturated C_{27} sterol ($C_{27}H_{46}O$) having an unsaturated C_8H_{15} side chain. The ions at *m/e* 273 (M^+-side chain and 2H) and 302 (allylic cleavage of C-20 and C-22 with one hydrogen transfer) suggest that the double bond is located at C-22 (*12*) and, from the IR absorptions at 958 and 972 cm^{-1}, it is *trans*-disubstituted (*11*).

The NMR spectrum of patinosterol acetate showed signals of 18-Me (δ 0.66), 19-Me (0.822), acetoxy-methine (4.5-4.9), acetoxy-Me (2.04), and two olefinic protons at 5.1-5.24 (unresolved m, 22,23-H) as in cholest-22-en-3β-ol acetate [10b] but the terminal dimethyl signal of 10b (δ 0.854) was replaced by a secondary methyl doublet at δ 0.925 (J=6.7 Hz) which was partially enveloped by 21-Me doublet at 0.995. This difference indicates that patinosterol bears a secondary methyl at C-24 and lacks 27-methyl and indeed the NMR pattern of the side chain signals was identical with that of occelasterol acetate (*1*) and amure-

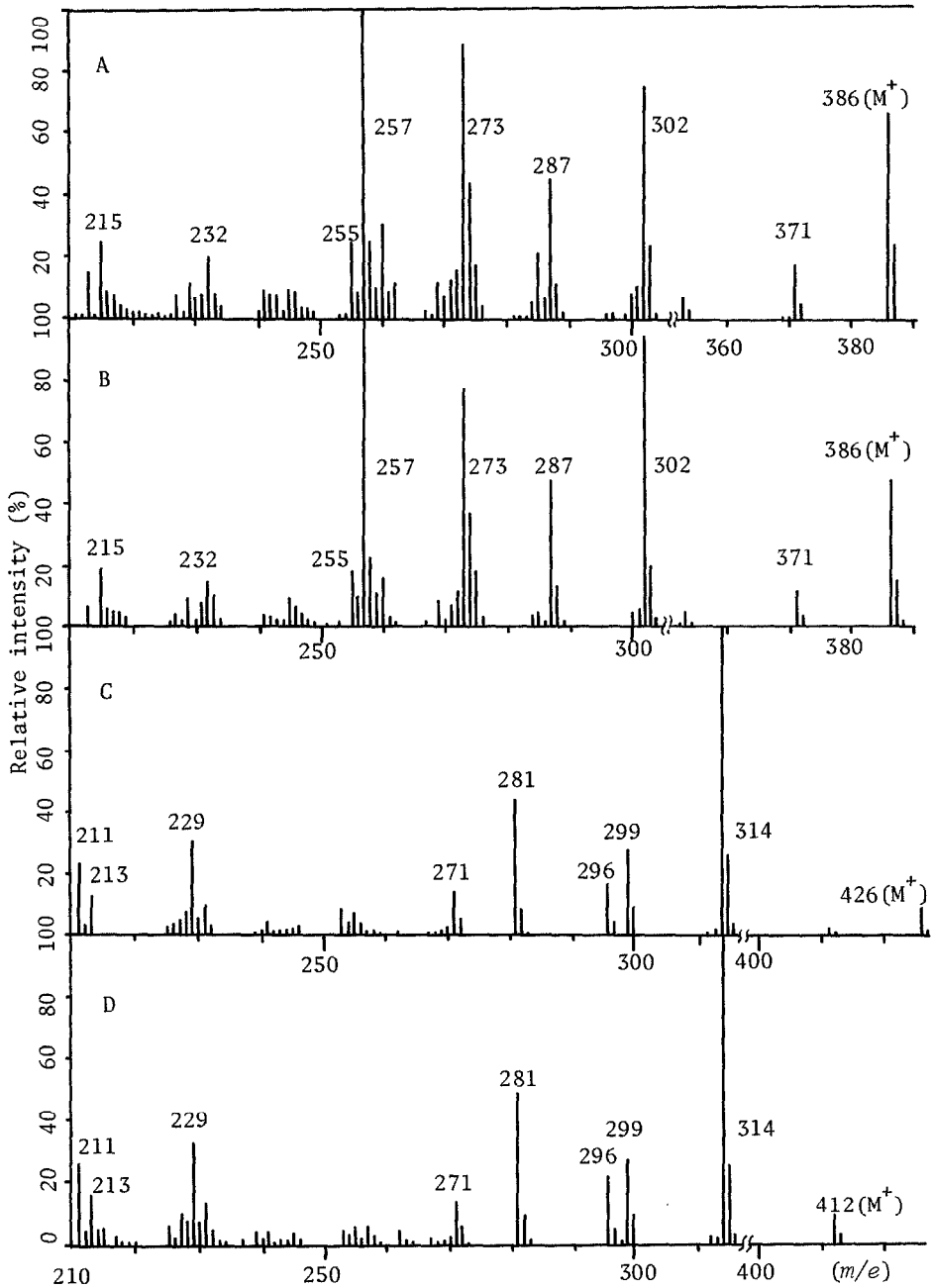


Figure 3. Mass spectra of patinosterol (A), cholest-22-en-3β-ol (B), 29-methylisofucosterol (C), and isofucosterol (D)

sterol acetate (*7*). The primary methyl triplet signal falls exactly on that of 19-methyl (δ 0.82) but a part of the signal was present at δ 0.75. These evidences establish that this new sterol, patinosterol, is 22-*trans*-27-*nor*-24-methylcholest-22-en-3β-ol [9a], the third member of a class of sterols having an "amurestane-type" side chain.
The chemical shift of 18- and 19-methyl and the broad nature of hydroxymethine in NMR fall well within the boundary of 5α-H, 3β-hydroxy steroid system (*13,14*). The configuration at C-24 was tentatively assigned as 24S (24α), the same as occelasterol and amuresterol from the biogenetic grounds.

The next polar subfraction was a mixture of peaks 1 and 5, and purified by several separations by TLC. The less polar band (peak 1) gave 24-norcholest-22-en-3β-ol acetate [11b], mp 113-113.5° (lit.(*5c*), mp 117-121°), $[\alpha]_D$ +1±2°. IR (chloroform), 960, 970 cm^{-1}. NMR δ 0.64 (18-Me), 0.81 (19-Me), 0.92 (6H, d, *J*=6.7 Hz, isopropyl), 0.97 (3H, d, *J*=7 Hz, 21-Me), 4.4-4.8 (1H, m, 3α-H), 5.1-5.25 (2H, m, 22,23-H). Hydrolysis of the acetate gave the free sterol [11a], mp 124.5-128° (lit.(*15*), mp 121-123°), $[\alpha]_D$ +5±2°. Mass spectrum, *m/e* 372 (M^+), 357 (M^+-Me), 339 (M^+-Me and H_2O), 302 (M^+- C-22 to C-26 and 1H), 287 (302-Me), 273 (M^+-side chain and 2H), 257 (M^+-side chain and H_2O). It was also reported in the sterol mixture of the sponge, *H. perleve* (*5c*), and a tunicate, *Halocynthia roretzi* (*5a*). The more polar band (peak 5) gave 24-methylcholesta-5,22-dien-3β-ol acetate [12b], mp 154-155°, $[\alpha]_D$ -46°, and it was the third major component in the scallop sterol mixture. The IR and mass spectra and melting point of free sterol (151-152°) were identical with those of brassicasterol(mp151°)(*16*). However, there was a slight deviation in the NMR pattern of C-21 and

C-28 secondary methyl signals (*10,17*) and its specific rotation (-40.9 was clearly distinguishable from that of brassicasterol (-66°) (*10*). Partial hydrogenation of the acetate over 10% palladium-charcoal followed by hydrolysis gave 22,23-dihydro derivative, identical with campesterol, mp 159-160°, $[\alpha]_D$ -33.7° (lit.(*10*), mp 160-161°, $[\alpha]_D$ -33°). 22,23-Dihydrobrassicasterol was reported mp 158-159°, $[\alpha]_D$ -44° (*10*). The identity with campesterol was also supported by the NMR which showed two doublets at δ 0.805 and 0.853 due to the characteristic non-equivalent isopropyl group of campesterol (*10*). From these results it is concluded that $\Delta^{5,22}$-C_{28} sterol from the scallop is 24-epibrassicasterol (22-*trans*-(24S)-24-methylcholesta-5,22-dien-3β-ol) [12a] (*18*).

Fractions 10 and 11 were individually separated by TLC with a mixture of chloroform and hexane. The band next to 12b was composed of peaks 2 and 3 and they were occelasterol acetate (peak 2) and 22-*trans*-cholesta-5,22-dien-3β-ol acetate (peak 3). Purification of the upper zone of the band by several TLC separations gave occelasterol acetate [13b] mp 145°, mixed mp 143-145°, $[\alpha]_D$ -48° (lit.(*1*), mp 142-144°, $[\alpha]_D$ -47° NMR δ 0.69 (18-Me), 1.025 (19-Me), 1.00 (3H, d, *J*=6.7 Hz, 21-Me), 0.93 (3H, d, *J*=6.7 Hz, secondary Me at C-24), 4.4-4.8 (1H, m, 3α-H), 5.1-5.4 (2H, m, 22,23-H), 5.36 (1H, m, 6-H). IR (chloroform), 960, 972 cm^{-1}. Hydrolysis of the acetate gave occelasterol [13a], mp 129-131°, $[\alpha]_D$ -43.5° (lit.(*1*), mp 128.5-129.5°, $[\alpha]_D$ -44°). Mass spectrum, m/e 384 (M^+), 369 (M^+-Me), 351 (M^+-Me and H_2O), 300 (cleavage at C-20 a C-22 with 1H transfer), 271 (M^+-side chain and 2H), 255 (M^+-side chain and H_2O). The lower zone gave 22-*trans*-cholesta-5,22-dien-3β-ol acetate [14b], mp 130-131° (lit.(*19*), mp 125-128°), $[\alpha]_D$ -54°.

The next polar subfraction (peak 1) gave 22-*trans*-24-norcholesta-

5,22-dien-3β-ol acetate [15b], mp 143-144.5° (lit.(*20*), mp 142.5°-143°), $[\alpha]_D$ -54°. The subfractions lower than 15b was a complex mixture composed of three major (peaks 6, 11, and 13) and several minor components and were not investigated. The retention time of peaks 6 and 11 corresponded to those of desmosterol acetate [16b] and fucosterol acetate [17b]. Another major component (peak 13, r.rt to cholesterol, 1.94) was not identified.

Fractions 21 to 23 were separated by TLC into two parts, the upper part being a mixture of 15b, 16b, and 17b, and the unidentified sterol, and other less polar minor components. The lower part was composed of peaks 12 and 14. It was separated by TLC with a mixture of hexane and benzene into less polar (peak 14) and more polar band (peak 12). The less polar band gave 29-methylisofucosterol acetate [18b], mp 107°, $[\alpha]_D$ -32±3°, NMR δ 0.69 (18-Me), 1.03 (19-Me), 0.98 (6H, d, *J*=7 Hz, isopropyl), 0.96 (3H, d, *J*=6 Hz, 21-Me), 2.82 (1H, degenerated septet, *J*=7 Hz, 25-H), 4.4-4.8 (1H, m, 3α-H), 5.00 (1H, t, *J*=7 Hz, 28-H), 5.36 (1H, m, 6-H). Hydrolysis of the acetate gave the free sterol [18a], mp 113-114.5° (lit.(*4b*), mp 111-112°), $[\alpha]_D$ -26±3°. Mass spectrum (Fig. 3), *m/e* 426 (M^+), 411 (M^+-Me), 314 (McLafferty-type cleavage at C-22 and C-23), 299 (314-Me), 296 (314-H_2O), 281 (299-H_2O), 271 (M^+-side chain and 2H). The more polar band gave isofucosterol acetate [19b], mp 132-133° (lit.(*21*), mp 132°), $[\alpha]_D$ -32±2°. NMR δ 0.68 (18-Me), 1.01 (19-Me), 0.97 (6H, d, *J*=7 Hz, isopropyl), 0.94 (3H, d, *J*=6 Hz, 21-Me), 1.58 (3H, d, *J*=6.8 Hz, 29-Me), 2.82 (1H, septet, *J*=6.8 Hz, 25-H), 5.10 (1H, q, *J*=7 Hz, 28-H), 5.36 (1H, m, 6-H). The deshielded 25-methine signal (δ 2.82) in compounds 18b and 19b is the most pronounced characteristic of 24(28)-*cis*-sterols (*21*). Hydrolysis of the acetate

gave the free sterol [19a], mp 133.5-135° (lit.(*21*), mp 133.5°), $[\alpha]_D$ -31±2°. Mass spectrum (Fig. 3), *m/e* 412 (M^+), 397 (M^+-Me), 394 (M^+-H_2O), 379 (M^+-Me and H_2O), 314, 299, 296, 281, 271.

The most polar eluent corresponded to peak 8 and was purified from the fraction 36 which gave 24-methylenecholest-5-en-3β-ol acetate [20b], mp 133-135° (lit.(*22*), mp 135-136°), $[\alpha]_D$ -46°.

DISCUSSION

Twenty sterols were identified in the scallop, *P. yessoensis* (Table 1) and when the unidentified minor components were included, the total amounted to more than 30 components.

Two amurestane-type sterols [9a and 13a] were identified in the present study and, since a similar peak was also observed in the sterol mixture of other marine invertebrates in GLC (*1*), the additional isolation would seem to be warranted.

Our results indicate that the peak 2 of the original mixture is composed of occelasterol [13a] and lesser amount of patinosterol [9a], and no trace of 22-*cis*-dehydrocholesterol was detected. The informations gained from recent studies indicate that the sterol components and their relative abundances are largely uniform in the closely related species of marine invertebrates if their feeding habit is similar (*2,23,24*). From this and the reason mentioned before (*1*), the authenticity of 22-*cis*-dehydrocholesterol in *P. magellanicus* must be considered doubtful and it is rather compatible with occelasterol. Other identifications of 22-*cis*-dehydrocholesterol (*6*) are based only on GLC retention time so that they must also be subjected to reinvestigation (*25*).

29-Methylisofucosterol, first isolated from *P. magellanicus* by Idler *et al.* (*4b*), was also confirmed in *P. yessoensis*. Interestingly,

Table 1. Percentage composition of the sterol mixture from *P. yessoensis*

Peak	Relative retention time*	Percentage composition	Component
1	0.665	5.2	22-*trans*-24-Norcholest-22-en-3β-ol [11a]**
			22-*trans*-24-Norcholesta-5,22-dien-3β-ol [15a]
2	0.895	2.6	Patinosterol (22-*trans*-27-*nor*-(24S)-24-methylcholest-22-en-3β-ol) [9a]**
			Occelasterol (22-*trans*-27-*nor*-(24S)-24-methylcholesta-5,22-dien-3β-ol) [13a]
3	0.935	6.2	22-*trans*-Cholest-22-en-3β-ol [10a]**
			22-*trans*-Cholesta-5,22-dien-3β-ol [14a]
4	1.00	27.8	Cholestan-3β-ol [1a]**
			Cholest-5-en-3β-ol [5a]
5	1.14	13.4	24-Epibrassicasterol (22-*trans*-(24S)-24-methylcholesta-5,22-dien-3β-ol) [12a]
6	1.20	1.2	Desmosterol (cholesta-5,24-dien-3β-ol) [16a]***
7	1.31	27.6	24ξ-Methylcholestan-3β-ol [2a]**
			24ξ-Methylcholest-5-en-3β-ol [6a]
8	1.35		24-Methylenecholest-5-en-3β-ol [20a]
9	1.43	1.4	Poriferasterol (22-*trans*-(24R)-24-ethylcholesta-5,22-dien-3β-ol) [8a]
10	1.64	6.4	24ξ-Ethylcholestan-3β-ol [3a]**
			24ξ-Ethylcholest-5-en-3β-ol [7a]
11	1.73	0.6	Fucosterol (24(28)-*trans*-24-ethylidenecholest-5-en-3β-ol) [17a]***
12	1.82	5.6	Isofucosterol (24(28)-*cis*-24-ethylidenecholest-5-en-3β-ol) [19a]
			24ξ-Ethyl-4ξ-methylcholestan-3β-ol [4a]**
13	1.94	0.6	Unidentified
14	2.10	1.3	29-Methylisofucosterol (24(28)-*cis*-24-propylidenecholest-5-en-3β-ol) [18a]

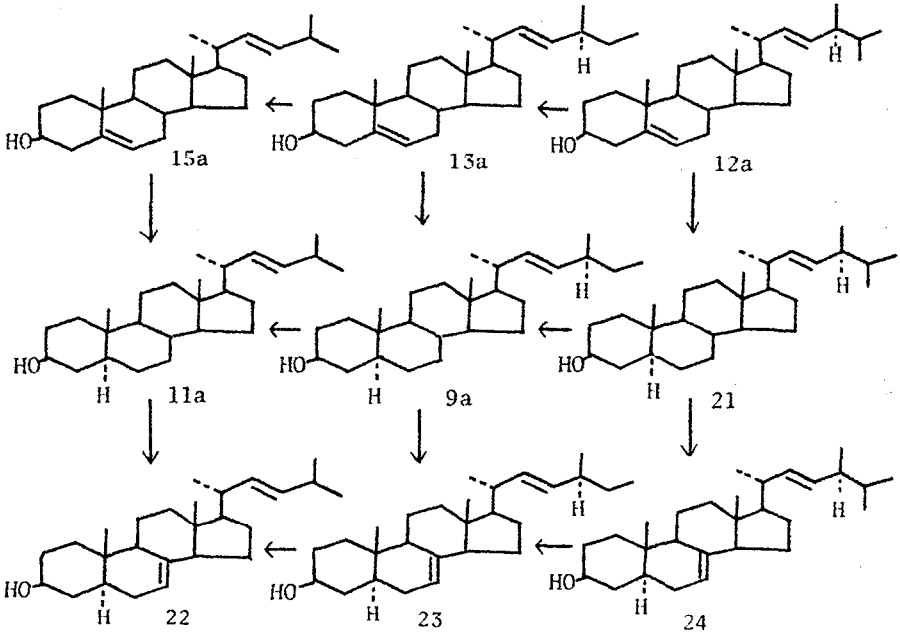
* Relative to cholest-5-en-3β-ol ** Less than 0.5% *** Deduced from the relative retention time

among the marine invertebrates, the scallops contain this unusual sterol and occelasterol most abundantly.

Up to the present, three C_{26} sterols [11a, 15a, and 22] have been found in marine invertebrates (*5,23,27*) but their biogenesis still re-

mains unsettled. Some marine plants are also known to contain 15a in a minor amount (*4a,28,29*). However, Barbier *et al.*(*29*) demonstrated that although the red algae, *Rhodymenia palmata*, contain 15a in 0.5% of the total sterol mixture, it did not incorporate the labeled precursors into 15a. Possibly, the presence of 15a in marine plants is attributed to their absorption or adsorption from marine particulates or solute.

The discovery of three amurestane-type sterols [9a, 13a, and 23] in the present and previous studies (*1,7*) suggests the following biogenetic sequence which is involved in the extremely complex marine food chain. In this case, the primary source might be 24-epibrassicasterol [12a], and recent studies invariably indicate this sterol to be



far more abundant than brassicasterol in marine sources (*30*). In the case of Δ^{22}- and $\Delta^{7,22}$-sterols, the formation process might be duplicated since some marine invertebrates, such as starfish, are capable of

modifying the ingested Δ^5-sterol to Δ^7 through the saturated sterol (*23, 31*). Thus, for example, asterosterol [22] could arise in starfish from 15a and, by the succesive demethylation, from stellasterol [24]. Such a hypothesis is consistent with the fact that in our results, C_{26} and amurestane sterols always occur together, and also associated with a large amount of (24S)-24-methyl sterols [12a or 24] having an identical C-24 configuration (*1,7,27*). Recent discovery of 24-methylcholesta-7,22,25-trien-3β-ol from the starfish, *Leiaster leanchii* by Teshima (*32*) is also suggestive of its intermediacy from stellasterol [24] to amurestерol [23] in starfish.

EXPERIMENTAL

Melting points were determined on a Kofler hot stage and are uncorrected. Optical rotations were measured in $CHCl_3$ soln. NMR spectra were determined on a JEOL PS100 spectrometer operating at 100 MHz in $CDCl_3$ soln. with TMS as internal standard. Mass spectra were determined on a Hitachi RMU7 mass spectrometer. IR spectra were taken on a Hitachi 215 spectrometer in $CHCl_3$ soln. unless specified. GLC was carried out on a Shimadzu GC4BPF gas chromatograph using a glass column (3 m x 3 mm I.D.) packed with 1.5% OV-17 on 80-100 mesh Shimalite W at 252°, with N_2 carrier gas flow-rate of 60 ml/min. Hydrolysis of sterol acetate was carried out by refluxing in 3% KOH-MeOH for 15 min followed by the usual work-up. A mixture of $CHCl_3$ and MeOH was used for all recrystallizations.

Isolation of crude sterol Fresh material of commercial scallop, *P. yessoensis* (5 kg, without shell) was crushed and dried *in vacuo* at 80-90° and extracted with acetone till the extract became colorless. Evaporation of the solvent gave 200 g of a dark brown oil, which was mixed with a solution prepared from KOH (100 g), H_2O (200 ml), and EtOH (800 ml), and refluxed for 7 hr. The mixture was concentrated to 350 ml, diluted with 1.5 liter of H_2O, and extracted with Et_2O and the solvent was evaporated. The solid residue (7.4 g) was dissolved in 25 ml of MeOH, left to stand overnight, and the precipitate formed (5.1 g) was collected. The mother liquor was concentrated to 15 ml, left to stand for 5 days, and the precipitate formed (0.1 g) was collected. The mother liquor was dissolved in 100 ml of Et_2O, washed with H_2O, and evaporated. The residue was dissolved in 5 ml of MeOH and the precipitate (0.2 g) was collected. The combined crude sterol (5.4 g) was acetylated in the usual way with Ac_2O and pyridine at 90° for 3hr and the solvent was evaporated to give the crude sterol acetate.

Fractionation of crude sterol acetate The crude sterol acetate was chromatographed over a column of 20% (w/w) $AgNO_3$-silicic acid (500 g,

100 mesh, Mallinckrodt) with a mixture of benzene and hexane. The composition and the amount of each fraction (1 liter) are given in Table 2 and Figure 2.

Table 2. Column chromatography of crude sterol acetate

Fr.No.	Solvent ratio	Eluent (mg)	Fr.No.	Solvent ratio	Eluent (mg)	Fr.No.	Solvent ratio	Eluent (mg)
1	1:5	216	13		79	25		97
2		301	14		88	26		129
3		801	15		94	27		104
4		547	16		80	28		124
5		113	17		49	29	1:2	130
6		99	18		57	30		64
7		158	19		43	31		80
8		162	20		55	32		75
9		160	21		48	33		132
10		139	22		53	34	1:1	148
11		124	23	3:10	74	35		106
12		87	24		119	36		77

Separation of fraction 1 Fraction 1 was separated over $AgNO_3$-silicic acid column (125 g) into two subfractions. The less polar subfraction was found to be composed of four peaks (r.rt., 1.00, 1.31, 1.64 and 1.82) and corresponded to peaks 4, 7, 10, and 12. GLC/MS: Peak 4 [1b], *m/e* 430 (M^+), 415 (M^+-Me), 370 (M^+-AcOH), 355 (370-Me), 316 (M^+-C-1 to C-4), 275 (M^+-side chain and 42), 276 (M^+-side chain and 41), 215 (M^+-side chain and 42 and AcOH), 230 (M^+-side chain and C-16,17 and AcOH) Peak 7 [2b], *m/e* 444 (M^+), 384 (M^+-AcOH), 330 (M^+- C-1 to C-4), 276, 275 230, 215. Peak 10 [3b], *m/e* 458 (M^+), 398 (M^+-AcOH), 383 (M^+-AcOH and Me), 344 (M^+-C-1 to C-4), 276, 275, 230, 215. Peak 12 [4b], see Results. The more polar subfraction was a mixture of several components. It was purified to one band by TLC which was found, by GLC, to contain three peaks (r.rt., 1.00, 1.31, and 1.64). It was identified as 5b, 6b, and 7b from the mass spectrum of the mixture (M^+-AcOH, *m/e* 368, 382, 396) and GLC comparison with the authentic standards. The contaminants of this subfraction was a trace of monounsaturated C_{27}, C_{28}, C_{29}, and C_{30} sterol acetates from the mass spectrum of unpurified mixture (M^+, *m/e* 428, 442, 456, and 470) assumed to be Δ^{22}-sterols from the biogenetic grounds.

Isolation of poriferasterol [8a], *patinosterol* [9a], *and 22-trans-cholest-22-en-3β-ol* [10a] Fractions 6-9 were separated individually by TLC ($CHCl_3$:hexane=2:3, two stages) to 7 bands (*a* to *h*, Rf: *a*, 0.81, *b*, 0.74, *c*, 0.67, *d*, 0.61, *e*, 0.55, *f*, 0.5, *g*, 0.45, *h*, 0.14). The amount of bands *a* to *e* were less than 5% in total. The bands *f* and *h* were enveloped by the broad band *g*. Band *a* showed one major (r.rt., 0.66) and nine unidentified minor peaks in GLC. The major peak corresponds to peak 1 and it is possibly a saturated C_{26} sterol. Band *b* was also composed of six unidentified peaks. Band *c* was a mixture of 5b, 6b, and 7b. Band *d* was a complex mixture composed of eleven components. The major components were 5b, 8b, 9b, and 10b and two unidentified components (r.rt., 1.15 and 1.31) and one of the unidentified components (r.rt., 1.15) is possibly a 24-methylcholest-22-en-3β-ol acetate from

its GLC retention time and TLC mobility. Band *e* was a mixture of 9b (24%), 10b (75%), and other minute contaminants (less than 1%). Band *f* was a mixture of 11b (21%) and 12b (79%). Band *g* was mainly composed of 12b and a trace amount of 13b. Band *h* was a mixture of 13b (35%), 14b (57%), and 12b (8%). The bands *f*, *g*, and *h* were combined with the corresponding bands from fractions 10 and 11. Band *d* and *e* were combined and separated into three distinct zones by TLC (seven developments with hexane:benzene=3:1). The upper band gave 8b (5 mg), mp 140-140.5°, $[\alpha]_D$ -52±3° (*c*, 0.44). Free sterol [8a], mp 154-157.5°, $[\alpha]_D$ -47±2° (*c*, 0.26). The middle band gave 9b (2.5 mg), mp 129-130°, $[\alpha]_D$ 0±3° (*c*, 0.23). Free sterol [9a], mp 132-133°, $[\alpha]_D$ 0±3° (*c*, 0.1). The lower band gave 10b (9 mg), mp 106°, $[\alpha]_D$ 0±3° (*c*, 0.77). Free sterol [10a], mp 121°, $[\alpha]_D$ +12±3° (*c*, 0.4).

Isolation of 24-norcholest-22-en-3β-ol [11a], *24-epibrassicasterol* [12a], *occelasterol* [13a], *and 22-trans-cholesta-5,22-dien-3β-ol* [14a]

Fractions 10 and 11 were separated into three bands by TLC with two developments ($CHCl_3$:hexane=2:3). The upper band (trace) was a mixture of 5b and 12b. The lower band was a mixture of 13b, 14b, and minor amount of 12b and 24-norcholesta-5,22-dien-3β-ol acetate [15b]. The middle band was composed of 11b and 12b and divided into two zones by TLC with the same solvent. The upper zone gave 11b (20 mg), mp 113-113.5°, $[\alpha]_D$ +1±2° (*c*, 1.9). Free sterol [11a], mp 124.5-128°, $[\alpha]_D$ +5±2° (*c*, 0.98). The lower zone gave 12b (150 mg), mp 154-155°, $[\alpha]_D$ -46° (*c*, 3.60). NMR δ 0.692 (18-Me), 0.816 (3H, d, *J*=6.7 Hz, 26 or 27-Me), 0.833 (3H, d, *J*=6.7 Hz, 27 or 26-Me), 0.909 (3H, d, *J*=7 Hz, 28-Me), 1.008 (3H, d, *J*=7 Hz, 21-Me), 1.023 (19-Me), 2.035 (OAc), 4.4-4.8 (1H, m, 3α-H), 5.12-5.26 (2H, m, 22,23-H), 5.38 (1H, m, 6-H). Free sterol [12a], mp 151-152°, $[\alpha]_D$ -40.9° (*c*, 3.74). IR (Nujol), 962, 970 cm^{-1}. Mass spectrum, *m/e* 398 (M^+), 383 (M^+-Me), 380 (M^+-H_2O), 365 (M^+-Me and H_2O), 355 (M^+-C_3H_7), 337 (M^+-C_3H_7 and H_2O), 300 (M^+- C-22 to C-28 and 1H), 271 (M^+-side chain and 2H), 255 (M^+-side chain and H_2O). The lower band was separated several times by TLC with $CHCl_3$ and hexane (2:3) into upper and lower zones, and both zones were purified several times with the same solvent. The upper zone gave 13b (30 mg), mp 145°, $[\alpha]_D$ -48° (*c*, 1.67). Free sterol [13a], mp 129-131°, $[\alpha]_D$ -43.5° (*c*, 1.27). NMR δ 0.69 (18-Me), 1.01 (19-Me), 0.825 (t, *J*=7 Hz, C-26 primary Me), 0.925 (3H, d, *J*=7 Hz, secondary Me at C-24), 1.00 (3H, d, *J*=7 Hz, 21-Me), 3.3-3.7 (1H, m, 3α-H), 5.1-5.2 (2H, m, 22,23-H), 5.35 (1H, m, 6-H). The lower zone gave 14b, mp 130-131°, $[\alpha]_D$ -54° (*c*, 1.46).

Isolation of 24-norcholesta-5,22-dien-3β-ol acetate [15b]

Fraction 15 was purified by TLC ($CHCl_3$:hexane=2:3, four stages) to 15b, mp 143-144.5°, $[\alpha]_D$ -54° (*c*, 1.18).

Isolation of 29-methylisofucosterol [18a] *and isofucosterol* [19a]

Fractions 21 to 23 were separated into two subfractions by TLC by several developments with benzene and hexane (3:5). The upper subfraction was composed of desmosterol acetate [16b] and fucosterol acetate [17b], and other less polar compounds. The lower subfraction was further separated in the same way into two narrow zones. The lower zone gave 19b (17 mg), mp 132-133°, $[\alpha]_D$ -33±2° (*c*, 1.54). Free sterol [19a], mp 133.5-135°, $[\alpha]_D$ -31±3° (*c*, 1.05). The upper zone was a mixture of 19b and 18b and purified in the same manner to pure 18b (5.5 mg), mp 107°, $[\alpha]_D$ -32±3° (*c*, 0.49). Free sterol, mp 113-114.5°, $[\alpha]_D$ -26±3° (*c*, 0.32).

Isolation of 24-methylenecholest-5-en-3β-ol acetate [20b] Fraction 3 was purified by TLC ($CHCl_3$:hexane=3:2) to a pure sample of 20b, mp 133-135°, $[\alpha]_D$ -46° (*c*, 0.82).

Conversion of the compound 12b to campesterol A solution of 200 mg of 12b in 15 ml of AcOEt and 5 ml of AcOH was shaken with 500 mg of 10% palladium-charcoal catalyst in an atomospheric pressure of H_2 for 2 day and the catalyst was filtered. The solvent was evaporated and the residue was separated by TLC ($CHCl_3$:hexane=1:3, two stages) to 2 bands. The upper band (Rf, 0.6) gave (24R)-24-methylcholestanol acetate (20 mg mp 138°. Free sterol, mp 145-146°. The lower band (Rf, 0.5) gave (24R)-24-methylcholest-5-en-3β-ol acetate (70 mg), mp 141.5-142°, $[\alpha]_D$ -38.2° (*c*, 2.42) (lit.(*33*), mp 138-139°, $[\alpha]_D$ -35°). Free sterol (campesterol), mp 159-160°, $[\alpha]_D$ -33.7° (*c*, 1.97). IR (Nujol), 3400, 840, 800 cm^{-1}. NMR δ 0.68 (18-Me), 0.78 (3H, d, *J*=6 Hz, 28-Me), 0.805 and 0.853 (6H in total, partially enveloped doublets, *J*=7 Hz, non-equivalent 26,27-Me), 0.98 (3H, d, *J*=7 Hz, 21-Me), 1.01 (19-Me), 3.3-3.7 (1H, m, 3α-H), 5.38 (1H, m, 6-H). Mass spectrum *m/e* 400 (M^+), 385 (M^+-Me), 382 (M^+-H_2O), 367 (M^+-Me and H_2O), 315 ($M^+-C_5H_7$ and H_2O), 289 ($M^+-C_7H_9$ and H_2O) (12).

REFERENCES AND NOTES

1. Part V, Kobayashi, M., and Mitsuhashi, H., STEROIDS, *24*, 399 (1974
2. Idler, D.R., and Wiseman, P., J. FISH. RES. BD, CANADA, *29*, 385 (1972); Teshima, S., and Kanazawa, A., NIPPON SUISAN GAKKAISHI, *38* 1299 (1972).
3. Kritchevsky, D., Tepper, S.A., Ditullo, N.W., and Holmes, W.N., J. FOOD SCI., *32*, 64 (1967).
4. (a) Idler, D.R., Wiseman, P., and Safe, L.M., STEROIDS, *16*, 451 (1970); (b) Idler, D.R., Safe, L.M., and McDonald, E.F., STEROIDS *18*, 545 (1971); (c) Idler, D.R., and Wiseman, P., COMP. BIOCHEM. PHYSIOL., *38*, 581 (1971).
5. (a) Alcaide, A., Viala, J., Pinte, F., Itoh, M., Nomure, T., and Barbier, M., C. R. HEBD. SEANCES, ACAD. SCI. SER. C, *273*, 1386 (1971); (b) Teshima, S., Kanazawa, A., and Ando, T., COMP. BIOCHEM PHYSIOL., *41*B, 121 (1972); (c) Erdman, T.R., and Thomson, R.H., TETRAHEDRON, *28*, 5163 (1972); (d) Kobayashi, M., Nishizawa, M., Todo, K., and Mitsuhashi, H., CHEM. PHARM. BULL. (Tokyo), *21*, 323 (1973).
6. Voogt, P.A., COMP. BIOCHEM. PHYSIOL., *41*B, 831 (1972); Voogt, P.A. and van Rheenen, J.W.A., EXPERIENTIA, *29*, 1070 (1973); Teshima, S. Kanazawa, A., and Ando, T., MEM. FAC. FISH, KAGOSHIMA UNIV., *20*, 131 (1971); Voogt, P.A., van De Ruit, J.M., and van Rheenen, J.W.A COMP. BIOCHEM. PHYSIOL., *48*B, 44 (1974).
7. Kobayashi, M., and Mitsuhashi, H., TETRAHEDRON, *30*, 2147 (1974).
8. Kita, M., and Toyama, Y., NIPPON KAGAKU ZASSHI, *81*, 485 (1960).
9. Lyon, A.M., and Bergmann, W., J. ORG. CHEM., *7*, 428 (1942).
10. Thompson, M.J., Dutky, S.R., Patterson, G.W., and Gooden, E.L., PHYTOCHEMISTRY, *11*, 1781 (1972).

11. Fieser, L.M., and Fieser, M., "STEROIDS", Reinhold Inc., New York, p. 172 (1959).

12. Knights, B.A., J. GAS CHROMATOGRAPHY, *5*, 273 (1967): Wyllie, S.G., and Djerassi, C., J. ORG. CHEM., *33*, 305 (1968).

13. Bhacca, N.S., and Williams, D.H., "APPLICATIONS OF NMR SPECTROSCOPY IN ORGANIC CHEMISTRY", Holden-Day Inc., San Francisco, p. 13 (1964)

14. Zurcher, R.F., HELV. CHIM. ACTA, *46*, 2054 (1963).

15. Metayer, A., and Barbier, M., C. R. HEBD. SEANCES, ACAD. SCI. SER.C *276*, 201 (1973).

16. Thompson, M.J., Cohen, C.F., and Lancaster, S.M., STEROIDS, *5*, 745 (1965); Barton, D.H.R., and Robinson, C.H., J. CHEM. SOC., 3045 (1954).

17. Rubinstein, I., and Goad, L.J., PHYTOCHEMISTRY, *13*, 485 (1974).

18. The same compound (mp 151-152°; acetate, mp 153-154°) was also isolated from *P. magellanicus* by Wainai *et al.* but they regarded it brassicasterol containing a small amount of its C-24 epimer. Wainai, T., Tamura, T., Truscott, B., and Idler, D.R., J. FISH. RES BD. CANADA, *21*, 1543 (1964).

19. Hutchins, R.F.N., Thompson, M.J., and Svoboda, J.A., STEROIDS, *15*, 113 (1970).

20. Fryberg, M., Oehlschlager, A.C., and Unrau, A.M., CHEM. COMUN., 1194 (1971).

21. Gibbons, G.F., Goad, L.J., and Goodwin, T.W., PHYTOCHEMISTRY, *7*, 983 (1968).

22. Idler, D.R., and Fagerlund, U.H.M., J. AM. CHEM. SOC., *77*, 4142 (1955).

23. Smith, A.G., Rubinstein, I., and Goad, L.J., BIOCHEM. J., *135*, 443 (1973).

24. Rosa, M.D., Minale, L., and Sodano, G., COMP. BIOCHEM. PHYSIOL., *46*B, 823 (1973).

25. Djerassi *et al.* recently reported the presence of 22-*cis*-dehydrocholesterol in sponge, *T. aurantia* (*26*), but the reported NMR chemical shifts were compatible with occelasterol and different from that of synthetic 22-*cis*-dehydrocholesterol (*19*) supplied by Dr. Svoboda.

26. Sheikh, Y.M., and Djerassi, C., TETRAHEDRON, *30*, 4095 (1974).

27. Kobayashi, M., Tsuru, R., Todo, K., and Mitsuhashi, H., TETRAHEDRON *29*, 1193 (1973).

28. Boutry, J.L., Alcaide, A., and Barbier, M., C. R. HEBD SEANCES, ACAD. SCI. SER. D, *272*, 1022 (1974).

29. Ferezou, J.P., Devys, M., Allais, J.P., and Barbier, M., PHYTOCHEMISTRY, *13*, 593 (1974).

30. There are numerous reports on the occurrence of brassicasterol in marine invertebrates (*2,4-6*, and references therein). Apparently, their elucidation is based on melting point and GLC retention time, and/or mass, NMR, and IR spectral data but the differentiation of brassicasterol (24R) and 24-epibrassicasterol (24S) by these means is not sufficient. Differentiation of these C-24 isomeric sterols by NMR signals was recently reported (*17*) though the disparity is not large enough to exceed the experimental error due to the operation conditions. In our opinion, the large discrepancy in the specific rotations (25°) is the most distinct difference between the two compounds. Similar difference was also noted between 5α,6-dihydroergosterol (24R) and stellasterol (24S) (*7,27, 34*). Up to the present, there are three examples of the sufficient identification of 24-epibrassicasterol from marine source. These are from an ophuroidea (*17*), an annelida (*1*), and a diatom (*17*) and the latter seems to be the primary source. Its Δ^7-isomer, stellasterol [24], which was conceivably derived by bioconversion from the ingested 24-epibrassicasterol (*23,31*), was also identified from starfish in various districts (*7,23,27,34*). In contrast, sufficient identification of brassicasterol is scanty in literature and in only one example, it was reported in a coelenterate (*35*).

31. Goad, L.J., Rubinstein, I., and Smith, A.G., PROC. ROY. SOC., *180*B, 223 (1972); Fagerlund, U.H.M., and Idler, D.R., CAN. J. BIOCHEM. PHYSIOL., *38*, 997 (1960).

32. Teshima, S., Kanazawa, A., and Ando, T., NIPPON SUISAN GAKKAISHI, *40*, 631 (1974).

33. Fernholz, E., and Ruigh, W.L., J. AM. CHEM. SOC., *40*, 1157 (1941).

34. Sheikh, Y.M., Kaishin, M., and Djerassi, STEROIDS, *22*, 835 (1973).

35. Gupta, K.C., and Scheuer, P.J., STEROIDS, *13*, 343 (1969).