Sterols of the Oyster, *Crassostrea virginica*

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ABSTRACT

A commercial sample of the oyster, *Crassostrea virginica*, obtained from Maryland waters of the Chesapeake Bay, contained 31 desmethylesterols and at least eight 4-monomethylsterols. The combined gas liquid chromatography-mass spectra of the minor components showed the presence of 6 unusual sterols, 24-ethylcholest-22-en-3β ol, 4α-methyl-24-ethylcholestan-3β-ol, occelasterol, (24E)-24-propylidene-cholest-5-en-3β-ol, (24Z0-24-propylidene-cholest-5-en-3β-ol, and 24-methylene-cholestanol. The C-24 configuration of 24-ethylcholest-5-enol, 24-methyl-cholesta-5,22-dienol, and 24-ethyl-cholesta-5,22 dienol were elucidated by 220 MHz nuclear magnetic resonance spectrometry.

INTRODUCTION

Many investigations have pointed out that the bivalves are unique in containing a great diversity of Δ^5-sterols. However, the origin of sterols in the bivalves is not fully elucidated. Rubinstein and Goad (1) reported that the marine diatom, *Phaeodactylum tricornutum*, contained epibrassicasterol, (24S)-24-methyl-cholesta-5,22E-dienol, as a principal sterol, and suggested that diatoms probably are the primary source of this sterol which is one of the prominent sterols in the bivalves and some marine invertebrates (2-4). Kobayashi and Mitsuhashi (5) also have suggested that epibrassicasterol is far more abundant in marine environments than brassicasterol, (24R)-24-methyl-cholesta-5,22E-dienol. Thus, the C-24 configuration of C_{28}- and C_{29}-sterols has received great interest in relation to the origin of sterols in marine invertebrates. Except for a few studies, however, the C-24 configuration has not been sufficiently characterized in the sterols of marine invertebrates, especially with the stanols and monoene sterols with saturated side chains of C_9 and C_{10}. This primarily results from the difficulty in separating them by sophisticated chromatographic techniques.

Lipophilic Sephadex column chromatography has been used for the separation of scallop sterols containing a complex mixture of C_{26}-C_{30} sterols, and then assigned the C-24 configuration of C_{28}- and C_{29}-sterols by high resolution (220 MHz) nuclear magnetic spectrometry (NMR) (6,7). The sterols of the oyster, *Crassostrea virginica*, have been investigated by several workers (8,9). However, the characterization of minor components is not fully accomplished. Therefore, we have reexamined the sterols of the oyster, *C. virginica*, and paid careful attention to the presence of methylsterols and other minor desmethylsterols which will provide significant information on the biosynthesis and modification of exogenous sterols in the oyster and other bivalves.

MATERIALS AND METHODS

Chromatography

Gas liquid chromatography (GLC) was performed on a Glowall Chromalab A-110 equipped with an argon ionization detector and a glass column (1.8 m x 3.4 mm id) of 3.0% SE-30 at 250 C (10). Relative retention times (RRT) were relative to cholesteryl acetate unless specified. Thin layer chromatography (TLC) was carried out using the following adsorbents and solvent systems: adsorbent—Silica Gel G, 10% (w/w) $AgNO_3$-Silica Gel G, and 20% (w/w) $AgNO_3$-Silica Gel $HF_{254+366}$; solvents—chloroform (commercial reagent grade) (system I), chloroform/methanol (98:2) (system II), chloroform/methanol (65:35) (system III), ethanol/free chloroform (system IV), and hexane/benzene (5:2) (system V). Sterols were detected under ultraviolet (UV) light after spraying Rhodamine 6G or by heating after spraying 10% H_2SO_4 in ethanol. Sterols or steryl acetates on the TLC plates were eluted with ether. Column chromatography of lipids on Silica Gel 60 (150 g) eluted with 150 ml each of 0 and 2% methanol in chloroform and 750 ml each of 6, 10, 15, 25 and 50% methanol in chloroform was carried out to separate steryl esters, free sterols and steryl sulfates; the eluates were checked by TLC on Silica Gel G with system III. Column chromatography of unsaponifiable matter on alumina (Woelm, grade II-III) was performed by increasing the proportions of ether in hexane (11). Column chromatography on 20% (w/w) $AgNO_3$-impregnated silicic acid (Bio-Sil® A,

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100-220 mesh, Bio-Rad Laboratories, Richmond, CA) ($AgNO_3$-column chromatography) was conducted by increasing the proportions of benzene in hexane (12). Column chromatography on a lipophilic Sephadex, the hydroxyalkoxypropyl derivative (13), was carried out according to the Patterson et al. method (6).

Physicochemical and General Methods

Mass spectra were obtained on a LKB 9000 mass spectrometer equipped with a Varian SS100 Data System. For combined GLC and mass spectrometry (GLC-MS), the LKB 9000 mass spectrometer was coupled with a column of 0.75% SE-30 (1.8 m x 4 mm id) and operated under the following conditions: GLC column temperature 230 C, electron energy 70 eV, accelerating voltage 3.5 kV, ion source temperature 290 C and electron multiplier 3.7 kV. NMR spectra were measured on a Varian HR-200 spectrometer (220 MHz, sweep width 250 Hz in $CDDl_3$. Acetylation of sterols was carried out with dry pyridine/acetic anhydride (1:1, v/v) at room temperature. Hydrolysis of steryl acetates was done with 5% KOH in ethanol at 70 C for 1 hr. Steryl sulfates were solvolyzed to free sterols by refluxing with 2% acetic acid in dioxane at 80 C for 24 hr.

Isolation of Sterols

The oysters, *C. virginica*, 12.0 kg in fresh weight, were obtained from a commercial source, September 21, 1979. Lipids (117.5 g) were extracted with chloroform/methanol/water by the Bligh and Dyer method (14). Aliquots of lipids (14.0 g) were separated into steryl ester, free sterols and steryl sulfate fractions by column chromatography on Silica Gel 60. Steryl esters, free sterols and steryl sulfates were mainly eluted with 0, 2 and 10-15% methanol in chloroform, respectively. The steryl ester and free sterol fractions were separately saponified, and the unsaponifiable matters were chromatographed on alumina (50 g) with 200 ml each of 0, 10, 20, 30, 40, 50 and 70% ether in hexane and 100% ether. Sterols were eluted with 30-40% ether in hexane. The steryl sulfate fraction was solvolyzed, and the free sterols obtained were purified by alumina column chromatography and TLC on Silica Gel G with system I. The percentage composition of sterols from the 3 sterol fractions was determined by GLC.

The remainder of lipids (103.5 g) was saponified, and the unsaponifiable matters were chromatographed on alumina (1 kg) with 2,000 ml each of 0, 10, 20, 30, 40 and 70% benzene in hexane and 100% benzene. In this alumina column chromatography, methylsterols and desmethylsterols were mainly eluted with 10% and 20-50% ether in hexane, respectively. The desmethylsterols (6.5 g) were acetylated and the acetate derivatives purified by alumina column chromatography. Recrystallization from methanol afforded desmethylsterol acetates (7.5 g). The methylsterol fraction obtained by the initial alumina column chromatography was contaminated with a small amount of desmethylsterols. Rechromatography of the crude methylsterol fraction on alumina afforded the mixture of methylsterols free of desmethylsterols. The isolated methylsterols (296 mg) gave a single spot in TLC on Silica Gel G with system II, but they were shown to be heterogenous by GLC. The methylsterols were less polar than common desmethylsterols but more polar than dimethylsterols such as lanosterol, so they were suspected to be a mixture of 4α-methylsterols.

Fractionation and Characterization of Desmethylsterols

The desmethylsterol acetates (7.5 g) were chromatographed on 20% (w/w) $AgNO_3$-silicic acid (440 g) with 1,500 ml each of 0, 10, 15, 20, 27, 29, 33, 36, 40, 50, 60 and 75% benzene in hexane and 100% benzene. Seventy-one fractions (each fraction, 300 ml or 250 ml) were collected and monitored by GLC and/or GLC-MS.

Fraction 15. Fractions 1-14 gave no steryl acetate. Fraction 15, eluted with 15% benzene in hexane, afforded a mixture of Δ^0- and Δ^5-steryl acetates. The 10% $AgNO_3$-TLC of fraction 15 with system V gave 3 bands. The less polar band was composed of cholestan-3β-yl (4a), 24-methylcholestan-3β-yl (7a), 24-ethylcholestan-3β-yl (10a) and a C_{30}-steryl (RRT 1.89) acetate (10d). The C_{30}-steryl acetate was deduced to be a 4α-monomethylsteryl acetate (see Results). GLC-MS: 4a (RRT 1.03), m/e 430 (M^+, 42%), 415 (M^+-CH_3, 7%), 370 (M^+-AcOH, 49%), 355 (M^+-AcOH-CH_3, 35%), 316 (M^+-C-1 to C-4, 9%), 290 (M^+-R-27, R = side chain, 13%), 276 (M^+-R-41, 50%), 275 (M^+-R-42, 35%), 257 (M^+-R-AcOH, 14%), 230 (M^+-R-27-AcOH, 41%), and 215 (M^+-R-42-AcOH, 100%); 7a (RRT 1.34), m/e 444 (M^+, 39%), 429 (M^+-Ch_3, 6%), 384 (M^+-AcOH, 32%), 330 (M^+-C-1 to C-4, 6%), 290 (7%), 276 (7%), 275 (36%), 257 (9%), 230 (36%), and 215 (100%); 10a (RRT 1.67), m/e 458 (M^+, 36%), 443 (M^+-CH_3, 4%), 398 (M^+-AcOH, 27%), 383 (M^+-AcOH-CH_3, 23%), 344 (M^+-C-1 to C-4, 5%), 290 (16%), 276 (29%), 257 (13%), 230 (40%), and 215 (100%). The middle band

contained small amounts of a C_{29}-steryl acetate (9a) (RRT 1.41) (see Results). The polar band was composed of a mixture of Δ^5-steryl acetates.

Fractions 16-19. These fractions, eluted with 20% benzene in hexane, afforded a mixture of cholest-5-en-3β-yl (4b), 24-methylcholest-5-en-3β-yl (7b) and 24-ethylcholest-5-en-3β-yl (10b) acetates. The mass spectra and RRT of these compounds corresponded with those reported for the authentic materials (10,15). The free sterols from the mixture of 4b, 7b, and 10b were chromatographed on lipophilic Sephadex for separation into individual components. 24-Ethylcholest-5-enol was isolated as a pure compound (a single peak in GLC), purified by recrystallization from methanol, and analyzed by NMR (220 MHz) to determine the C-24 configuration. Insufficient 24-methylcholest-5-enol was available for NMR analysis.

Fraction 20. Fraction 20, eluted with 20% benzene in hexane, afforded the mixture of 4b and 24-ethylcholesta-5,22-dien-3β-yl acetate (9b). The RRT and the GLC-MS of 9b corresponded to that of authentic 24-ethylcholesta-5,22-dien-3β-yl acetate (10,15). 9b was separated from 4b by 10% $AgNO_3$-TLC with system IV, and the C-24 configuration of 9b was evaluated by NMR spectrometry (see Results).

Fractions 21 and 22. These fractions, eluted with 25% benzene in hexane, gave 24-methylcholesta-5,22-dienyl acetate (6b) and a small amount of 4 compounds, 1b (RRT 0.64), 3b (RRT 0.91), 9b and 2b. The RRT and the GLC-MS were those of 24-methylcholesta-5,22-dienyl acetate (10,15). 6b was purified by 10% $AgNO_3$-TLC with system V and the C-24 configuration was elucidated by NMR spectrometry (see Results). The extremely minor component 2b was deduced to be occelasteryl acetate (see Results).

Fractions 23-26. These fractions, eluted with 25% benzene in hexane, afforded cholesta-5,22E-dien-3β-yl (3b) and 24-norcholesta-5,22-dien-3β-yl (1b) acetates, both of which were identified by GLC and GLC-MS.

Fractions 27-31. These fractions, eluted with 27% benzene in hexane, contained large amounts of 1b and (24E)-24-ethylidenecholest-5-en-3β-yl acetate (11b) and small amounts of desmosterol acetate (5b), plus 2 unknown compounds (RRT, 1.54 and 1.88). One of the unknown compounds (RRT 1.88) was characterized as (24E)-24-propylidenecholest-5-en-3β-yl acetate (13b), by GLC-MS (see Results). GLC-MS: 11b (RRT 1.63), m/e 394 (M^+-AcOH, 71%), 379 (M^+-AcOH-CH_3, 13%), 296 (M^+-C-23 to C-29 in 1H-AcOH, 100%), 281 (m/e 296-CH_3, 53%), 267 (7%), 255 (14%), 253 (29%), 228 (29%), and 213 (41%).

Fractions 32-34. These fractions, eluted with 27-29% benzene in hexane, yielded a large amount of (24Z)-24-ethylidenecholest-5-en-3β-yl acetate (12b) and small amounts of 5b and 2 unknown compounds (RRT 0.92, 1.93). GLC-MS: 5b (RRT 1.09), m/e 366 (M^+-AcOH, 80%), 351 (M^+-AcOH-CH_3, 23%), 342 (M^+-C-22 to C-27-1H, 1%), 255 (13%), 253 (32%), 228 (7%), 296 (M^+-C-23 to C-29-1H-AcOH, 100%), 281 (58%), 267 (5%), 255 (5%), 253 (13%), 228 (12%), and 213 (20%). One of the unknown compounds (RRT 1.93) was characterized as (24Z)-24-propylidene-cholest-5-en-3β-yl acetate (14b) by GLC-MS (see Results). Another unknown compound (RRT 0.92) was assumed to be a C_{27}-diene steryl acetate with the chemical structure closely related to 22-dehydrocholesteryl acetate.

Fractions 35-41. These fractions, eluted with 29-33% benzene in hexane, gave 2 major components (RRT 1.18 and 1.30) and 2 very minor components (RRT 0.92 and 1.05). One of them (RRT 1.30) was identified as 24-methylenecholestan-3β-yl acetate (8a) (see Results). Other compounds were not characterized in this study.

Fractions 42-44. These fractions afforded 24-methylenecholest-5-en-3β-yl acetate (8b) as a sole component.

Fractions 45-71. These fractions, eluted with 45-75% benzene in hexane, afforded 7 compounds (RRT 0.69, 0.95, 1.09, 1.20, 1.43, 1.55 and 1.76) which were deduced to be $\Delta^{5,7}$-steryl acetates and an unknown compound (RRT 1.69). The characterization of these compounds will be the subject of a later report.

RESULTS AND DISCUSSION

Sterol Composition

This study showed that the oyster, *C. virginica,* contains steryl esters besides the large amounts of free sterols (Tables I and II). Also, careful examination demonstrated the presence of steryl sulfates as a very minor constituent in the oyster. Some difference in the percentage composition of sterol components was observed among the 3 sterol fractions. The percentages of 22-dehydrocholesterol and cholesterol were higher in steryl esters than in free and sulfated sterols, whereas those of C_{29}-sterols, e.g., 24-ethylcholesta-5,22-dienol, 24-ethylcholest-5-enol and 24-ethylidenecholest-5-enol, were higher in the latter 2 sterol fractions than in steryl esters. The proportions of C_{28}-sterols did

TABLE I

Lipid and Sterol Content in the Oyster

Fraction	Composition (%)	
Lipids (% of fresh oysters)	0.98	
Unsaponifiable matters (% of lipid)	22.90	
Total sterols (% of lipid)	6.57	
Desmethylsterols	6.29	(95.7%)[a]
Methylsterols	0.28	(4.3%)
Esterified sterols	0.98	(14.9%)
Free sterols	5.58	(84.9%)
Sulfated sterols	0.01	(0.2%)

[a]Numbers in parentheses indicate the percentage of total sterols.

not differ markedly among the 3 sterol fractions.

The steryl sulfate is known to play an important role as a precursor of steroid hormone (16) and as an excretory material in mammals and insects (17,18). With respect to the marine invertebrates, the starfish, *Asterias rubens*, has been shown to contain steryl sulfates in a comparatively high concentration (19-21). Although the function of steryl sulfates in the oyster, *C. virginica*, is obscure, the occurrence of steryl sulfates will be of interest from the viewpoint of sterol metabolism in marine mollusks.

Alumina column chromatography of the unsaponifiable matters afforded a small amount of methylsterols, besides large amounts of desmethylsterols. The methylsterols were composed of one major component (RRT 1.14, 68% of total methylsterols) and 6 minor compounds. The characterization of these methylsterols is in progress at our laboratory.

The sterol components of desmethylsterols were characterized by GLC, GLC-MS, or NMR after the fractionation of $AgNO_3$-column chromatography. As a result, the desmethylsterols of the oyster, *C. virginica*, were shown to be a more complex mixture than reported in earlier studies (8,9). The desmethylsterols of the oyster are composed of Δ^5-sterols and small amounts of Δ^0- and $\Delta^{5,7}$-sterols. The prominent components of desmethylsterols were cholesterol (34.0%), 24-methylcholesta-5,22-dienol (15.6%), 24-methylenecholesterol (12.6%), 22-dehydrocholesterol (10.2%), 24-norcholesta-5,22-dienol (4.0%), 24-methylcholest-5-enol (2.6%), 24-ethylcholesta-5,22-dienol (2.0%), and the unidentified 7 $\Delta^{5,7}$-sterols (6.6%) (Table III). In addition to these sterols, 15 sterols were detected as a minor component (less than 1.0% of total desmethylsterols). Some of them were characterized by GLC-MS (Table IV).

Unconventional Sterols

Six unusual sterols were identified as very minor constituents. On $AgNO_3$-column chromatography, fraction 15 contained 2 unknown compounds, 9a (RRT 1.41) and 10d (RRT 1.89). The GLC-MS of 9a gave the molecular ion at m/e 456 corresponding to a C_{29}-monoene steryl acetate and other prominent ions at m/e 413 (M^+-43), 353 (M^+-43-AcOH), 344 (M^+-C-22 to C-29-1H), 329 (M^+-C-22 to C-29-1H-CH_3), 315 (M^+-R-2H), 284 (M^+-C-22 to C-29-1H-AcOH), 269 (M^+-C-22 to C-29-1H-AcOH-CH_3), 257 (M^+-R-AcOH), 255 (M^+-R-2H-AcOH), and 215 (M^+-R-42-AcOH) (Table IV). The intense ion at m/e 257 and the ion at m/e 215 established that 9a involved the side chain of $C_{10}H_{19}$ (MW = 139) containing one double bond. The presence of the high fragment ion at m/e 315, together with the ions at m/e 413, 353, 284 and 269, place the side chain double bond at Δ^{22} (15,22). On the basis of these data, this compound was identified as 24-ethylcholest-22-en-3β-yl acetate (9a). 24-Ethylcholest-22-en-5β-ol has been isolated from the slime mold, *Distyostelium discoideum*, by

TABLE II

Composition of the Sterols Isolated from the Esterified, Free and Sulfated Sterol Fractions (%)

GLC		Composition (%)			
Peak	RRT	Steryl ester	Free Sterol	Steryl sulfate	Main constituent
1	0.64	7.7	3.4	5.0	24-Norcholesta-5,22-dienol
2	0.91	17.1	9.1	8.4	22-Dehydrocholesterol
3	1.00	41.4	33.8	26.8	Cholesterol
4	1.12	12.6	16.1	11.5	24-Methylcholesta-5,22-dienol
5	1.29	11.8	14.0	10.2	24-Methylenecholesterol
6	1.41	0	8.2	9.3	24-Ethylcholesta-5,22-dienol
7	1.67	9.4	14.9	28.7	24-Ethylcholest-5-enol and 24-Ethylidenecholest-5-enol

Heftmann et al. (23). Erdman and Thomson (24) also have found monoene sterols with the unsaturated double bond in the side chain in the sponge, *Hymeniacidon perleve*. However, 24-ethylcholest-22-en-3β-ol has not been found in other marine environments.

Another compound (RRT 1.89) gave the molecular ion at m/e 472, corresponding to a C_{30} fully saturated stanyl acetate, and prominent ions at m/e 412 (M^+-AcOH), 397 (M^+-AcOH-CH_3), 290 (M^+-R-41), 271 (M^+-R-AcOH), 244 (M^+-R-27-AcOH), 230 (M^+-R-AcOH-41), and 229 (M^+-R-AcOH-42). The intense ions at m/e 229 (the highest above m/e 200) and 230, together with the molecular ion at m/e 412, established that this compound has the steroid ring with an extra methyl group and the side chain of $C_{10}H_{21}$ (MW = 141). Since

TABLE III

Sterol Components of Desmethylsterols from the Oyster

Sterol		RRT	%	Sterol		RRT	%
(1b)	24-Norcholesta-5,22-dienol	0.64	4.0	(8a)	24-Methylenecholestanol	1.30	0.5
	Unknown[a]	0.69	0.5	(7a)	24-Methylcholestanol	1.34	<0.1
(2b)	Occelasterol	0.87	<0.1	(9a)	24-Ethylcholest-22-enol	1.41	<0.1
(3b)	(*trans*)-22-Dehydrocholesterol	0.91	10.2	(9b)	24-Ethylcholesta-5,22-dienol	1.42	2.0
	Unknown	0.92	<0.1		Unknown[a]	1.43	0.2
	Unknown[a]	0.95	1.0		Unknown	1.54	<0.1
(4b)	Cholesterol	1.00	34.0		Unknown[a]	1.55	0.8
(4a)	Cholestanol	1.03	0.3	(10b)	24-Ethylcholest-5-enol	1.63	3.7
	Unknown	1.05	<0.1	(11b)	(24E)-24-Ethylidenecholesterol	1.63	1.5
(5b)	Desmosterol	1.09	0.2	(10a)	24-Ethylcholestanol	1.67	<0.1
	Unknown[a]	1.09	1.8	(12b)	(24Z)-24-Ethylidenecholesterol	1.69	4.6
(6b)	24-Methylcholesta-5,22-dienol	1.12	15.6		Unknown	1.69	0.2
	Unknown	1.18	0.4		Unknown[a]	1.76	0.2
	Unknown[a]	1.22	2.1	(13b)	(24E)-24-Propylidene-cholesterol	1.88	<0.1
(8b)	24-Methylenecholesterol	1.26	12.6	(14b)	(24Z)-24-Propylidenecholesterol	1.93	0.2
(7b)	24-Methylcholest-5-enol	1.30	3.7				

[a]$\Delta^{5,7}$-Sterols: 6.6% of total desmethylsterols.

TABLE IV

Mass Spectral Data for the Acetates of the Unusual Sterols in the Oyster

	Sterols					
Fragmentation	9a	10d	2b	13b	14b	8a
M^+	456(12)[a]	472(11)	–	–	–	442(8)
M^+-CH_3	–	–	–	–	–	427(16)
M^+-43	413(16)	–	–	–	–	–
M^+-43-AcOH	353(23)	–	–	–	–	–
M^+-AcOH	–	412(23)	366(47)	408(42)	408(42)	382(3)
M^+-AcOH-CH_3	–	379(18)	351(5)	393(7)	393(7)	367(13)
M^+-(cleavage at C-20, 22 + 1H): a	344(51)	–	–	–	–	–
M^+-a-CH_3	329(14)	–	–	–	–	–
M^+-a-AcOH	284(8)	–	282(11)	–	–	–
M^+-a-AcOH-CH_3	269(17)	–	267(3)	–	–	–
M^+-(cleavage at C-22,23 + 1H): b	–	–	–	–	–	358(100)
M^+-b-CH_3	–	–	–	–	–	343(29)
M^+-b-AcOH	–	–	–	296(100)	296(100)	298(21)
M^+-b-AcOH-CH_3	315(88)	–	–	281(58)	81(58)	283(23)
M^+-R-2H	–	–	–	–	–	315(76)
M^+-R-41	–	290(9)	–	–	–	–
M^+-R-42	–	–	–	–	–	275(25)
M^+-R-AcOH	257(100)	271(7)	255(34)	255(2)	255(12)	257(12)
M^+-R-2H-AcOH	255(21)	–	253(8)	253(8)	253(30)	255(35)
M^+-R-27-AcOH	–	244(9)	228(4)	228(11)	228(39)	230(25)
M^+-R-41-AcOH	–	230(34)	–	–	–	–
M^+-R-42-AcOH	215(22)	229(40)	213(12)	213(24)	213(45)	215(92)
Other ions	–	43(100)	43(100)	–	–	229(34)

[a]Relative intensity

the mass spectrum of this compound did not yield a high peak at m/e 441 (M^+-CH_3) characteristic of a sterol with a 14 α-methyl group (25), the extra methyl group in the steroid ring was conceived to be located at C-4α from biogenetic grounds. Ballantine et al. (26) and Steudler et al. (27) have found 24-propylcholestanol in an oceanic sponge, *Synops* sp., and 4-methylgorgostanol in the zooxanthellae of the gorgonian, *Briareum asbestinum*, respectively. However, the possibility of these 2 C_{30}-stanols was clearly denied by the mass spectral pattern. Therefore, this steryl acetate was identified as 4α-methyl-24-ethylcholestan-3β-yl acetate (10d) which also has been found in the scallop, *Patinopecten yessoensis* (28).

The very minor component (2b, RRT 0.87) was present in the fractions eluted with 25% benzene in hexane. The mass spectrum of this compound gave prominent ions at m/e 366 (M^+-AcOH), 351 (M^+-AcOH-CH_3), 282 (M^+-C-22 to C-28-1H-AcOH), 267 (M^+-C-22 to C-28-1H-AcOH-CH_3), 255, 253, 228, 213 and 43. The mass spectral cracking pattern of this compound was similar to that of 22-*trans*-22-dehydrocholesteryl acetate (3B). However, this compound gave a shorter RRT in GLC and eluted faster in $AgNO_3$-column chromatography than 3b. These data showed that this compound was occelasteryl acetate, 22-*trans*-24-nor-(24S)-methylcholesta-5,22-dien-3β-yl acetate, which was first isolated from the marine annelid, *Pseudopotamilla occelata* (5).

The fractions, eluted with 27-29% benzene in hexane, afford 2 C_{30} steryl acetates, 13B (RRT 1.88) and 14b (RRT 1.93). The mass spectra of 13b and 14b were similar and gave the prominent ions at m/e 408 (M^+-AcOH), 393 (M^+-AcOH-CH_3), 296 (M^+-C-23 to C-30-1H-AcOH), 281 (M^+C23 to C-30-1H-AcOH-CH_3), 255, 253, 228 and 213. The absence of molecular ion peak showed that both compounds were C_{30}-Δ^5-steryl acetates. The ions at m/e 255 and 253, together with the ion at m/e 408, revealed the presence of unsaturated side chain of $C_{11}H_{21}$ (15). The intense ions at m/e 296 and 281 (22) were indicative of the $\Delta^{24(28)}$ bond. Considering the RRT in GLC and the mobility in $AgNO_3$-column chromatography besides the MS data, 13b and 14b were identified as (24E)-24-propylidenecholest-5-en-3β-yl and (24Z)-24-propylidene-cholest-5-en-3β-yl acetates, respectively. Both compounds have been first found in the scallop, *Placopecten magellanicus* (29,30).

Fractions 35-41, eluted with 29-33% benzene in hexane, contained the unknown compound 8a with a RRT of 1.30 as a major component. The mass spectrum of 8a gave the molecular ion at m/e 442 corresponding to a C_{28}-monoene steryl acetate and the prominent ions at m/e 427 (M^+-CH_3), 382 (M^+-AcOH), 367 (M^+-AcOH-CH_3), 358 (M^+-C-23 to C-28-1H), 343 (M^+-C-23 to C-28-1H-CH_3) 298 (M^+-C-23 to C-28-1H-AcOH), 283 (M^+-C-23 to C-28-1H-AcOH-CH_3), 315 (M^+-R-2H), 275 (M^+-R-42), 257 (M^+-R-AcOH), 255, 230, 229 and 215. The ions at m/e 315, 257 and 215, together with the low intensity of the molecular ion at m/e 442, showed the presence of the ring-saturated steryl acetate with one double bond in the side chain. The ions at m/e 358, 343, 298 and 283 were conceived to be formed by a McLafferty rearrangement (22) and indicated the $\Delta^{24(28)}$ bond. From these data, this compound was identified as 24-methylenecholestanyl acetate (8a) which had been first isolated from the sponge, *Hymeniacidon perleve* (24).

C-24 Configuration of C_{28} and C_{29}-Sterols

The C-24 configuration of 3 steryl acetates from the oyster was evaluated by the 220 MHz NMR at a sweep width of 250 Hz (Table V). The NMR spectra of epibrassicasterol (24S/24α) and brassicasterol (24R/24β) have been shown to be similar, but a significant downfield shift of the C-21 methyl proton signal in comparison of the spectra of the 2 compounds has been observed in the brassicasterol (7,31). This difference is useful for the discrimination of the C-24 configuration of both sterols. In the acetates of the 2 sterols, a similar difference has been seen (1,31). As shown in Table V, the NMR spectrum of 24-methylcholesta-5,22-dienyl acetate from the oyster showed that the isolated compounds were a mixture of both the 24S and 24R epimers. Khalil et al. (7) also have pointed out the concurrence of brassicasterol and epibrassicasterol in the sterols of the scallop, *Placopecten magellanicus*.

The C-24 epimers of C_{29}-$\Delta^{5,22}$-sterols have been observed to be not easily differentiated as compared with those of C_{28}-$\Delta^{5,22}$-sterols (31-33). However, since the C-21 and C-29 signals are deshielded in the 24R/24β epimer (poriferasterol) more than in the 24S/24α epimer (stigmasterol), both isomers are differentiated by the discrepancy in the shapes of peaks in the δ 0.77-0.86 region (31,32). The NMR spectrum of 24-ethylcholesta-5,22-dienyl acetate from the oyster indicated that the isolated steryl acetate contained predominantly poriferasteryl acetate (Table V).

The C-24 epimers of C_{29}-Δ^5-sterols also have been realized to be differentiated by the

TABLE V

Methyl Group Chemical Shifts of C_{28}- and C_{29}-Sterols Isolated from the Oyster

Sterols	C-24 Config.	Chemical shift (δ) of methyl group						
		C-18	C-19	C-21	C-26	C-27	C-28	C-29
Sterols from the oyster								
24-Methylcholesta-5,22-dienol acetate	*	0.690	1.020	1.002	0.833	0.815	0.908	–
24-Ethylcholesta-5,22-dienol acetate	*2	0.693	1.020	1.026	0.844	0.791	–	0.808
24-Ethylcholest-5-enol	*2	0.674	1.005	0.920	0.831	0.806	–	0.846
Standards								
Brassicasterol acetate*3	R/β	0.693	1.023	1.003	0.832	0.815	0.909	–
Epibrassicasterol acetate*3	S/α	0.693	1.018	1.001	0.833	0.816	0.907	–
Stigmasterol acetate*3	S/α	0.693	1.017	1.017	0.842	0.791	–	0.799
Poriferasterol acetate*3	R/β	0.695	1.020	1.025	0.841	0.791	–	0.808
β-Sitosterol*3	R/α	0.680	1.007	0.919	0.833	0.813	–	0.841
Clionasterol*4	S/β	0.683	1.001	0.928	0.834	0.814	–	0.855

* Predominantly the S/α isomer; δ of C-21 methyl of R/β isomer = 1.007.
*2Predominantly the R/β isomer.
*3Cited from the data of Rubinstein et al. (31).
*4Cited from the data of Khalil et al. (7).

signals of C-29 methyl group; the C-29 methyl signal of the 24S/24β epimer (clionasterol) appeared in the deshielded region compared with that of the 24R/24α epimer (β-sitosterol). The NMR spectrum of 24-ethylcholest-5-enyl acetate from the oyster showed that the isolated steryl acetate was almost completely composed of the 24S/24β epimer, clionasteryl acetate (Table V).

Khalil et al. (7) have shown the concurrence of the 24α- and 24β-epimers in the C_{28}- and C_{29}-sterols isolated from the scallop, *P. magellanicus*. This study also revealed the presence of both 24α- and 24β-epimers in 24-methylcholesta-5,22-dienyl acetate from the oyster. However, in C_{29}-sterols, e.g., 24-ethylcholesta-5,22-dienyl acetate and 24-ethylcholest-5-enyl acetate, the oyster contained, almost exclusively, the 24β-epimer.

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