MARINE STEROLS X.* MINOR CONSTITUENTS OF THE STEROLS OF THE SOFT CORAL SARCOPHYTON GLAUCUM

Masaru Kobayashi, Takashi Ishizaka, and Hiroshi Mitsuhashi

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

Received 7-30-82

ABSTRACT

The sterol mixture of the southern Japan soft coral Sarcophyton glaucum was found to contain a variety of minor components overlooked in a previous study. Five 4α-methylsterols (1 to 5) and three 4-demethylsterols (6 to 8) were isolated and their structures were confirmed.

INTRODUCTION

Our previous study showed that the southern Japan soft coral Sarcophyton glaucum contains eleven sterols, with 22,23-dihydrobrassicasterol and gorgosterol being the predominant components (1). Two new sterols, sarcosterol (9) (1a) and 23-methyl-22-dehydrocholesterol (10) (1b), were isolated and their structures were characterized. Kokke and coworkers have reported the occurrence of 10 and its 4α-methyl 5α,6-dihydro derivative in cultured zooxanthella from the zoanthid Zoanthus sociatus (2) and also peridinosterol, 4α,23,24-trimethyl-5α-cholest-17E(20)-en-3β-ol, from the dinoflagellate Peridinium foliaceum (3). In addition, many 4α-methylsterols have been found in a variety of gorgonians and dinoflagellates (4). Discovery of these sterols shows the ability of dinoflagellates to elaborate various 4-demethyl- and 4α-methylsterols with conventional or biogenetically modified structures and to accumulate these sterols in themselves or host organisms.

Soft corals should contain 4α-methylsterols derived from their symbiont dinoflagellates, although no examples have been found. In our previous study (1), the content of sterols other than 4-demethylsterols was too low for isolation and characterization. Thus, we

obtained a large quantity of crude sterol mixture from S. glaucum and reexamined its sterol composition for minor components, especially 4α-methylsterols.

RESULTS AND DISCUSSION

The crude sterol mixture (29.2 g) was obtained from a lipid extract (840 g) of S. glaucum utilizing chromatography and crystallization of the mother liquor to minimize loss of minor components. The gas chromatogram (GC) of the mixture was almost the same as that found in the previous study (1). Although the 4α-methylsterols are less polar than 4-demethyl sterols in ordinary silica gel chromatography (5), its low concentration in S. glaucum sterols precluded initial separation from the 4-demethyl sterols. The sterol mixture was first subjected as acetate to chromatography over silver nitrate-impregnated silica gel with a gradient mixture of hexane and benzene (Fig. 1). Examination of each fraction by GC revealed a significant number of unidentified components present in small amounts. The fractions which contained unknown components in isolable amounts were then separated according to the scheme shown in Fig. 2. Classical triangular recrystallization of the predominant less polar sterol fraction was found to be efficient for the initial concentration of less polar minor components. This fraction was then separated into less and more polar fractions by $AgNO_3$-silica gel chromatography and the unknown compounds 1 to 4 were isolated by hydrolysis followed by ordinary silica gel chromatography, and finally by reversed phase partition chromatography using a Lipidex 5000 column. Similarly, compounds 5 to 8 were obtained from the more polar fractions as shown in Fig. 2.

Compound 1 was found to be 4α,24S-dimethyl-5α-cholestan-3β-ol (1).

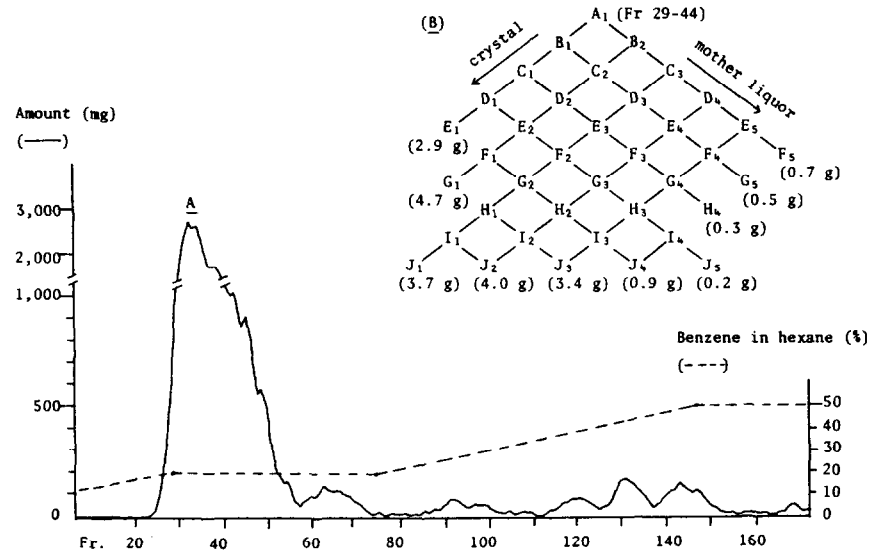


Figure 1. $AgNO_3$-silica gel chromatography of the sterol acetate mixture from S. glaucum (A) and triangular recrystallization of fractions 29-44 (B). Composition of fractions, see EXPERIMENTAL.

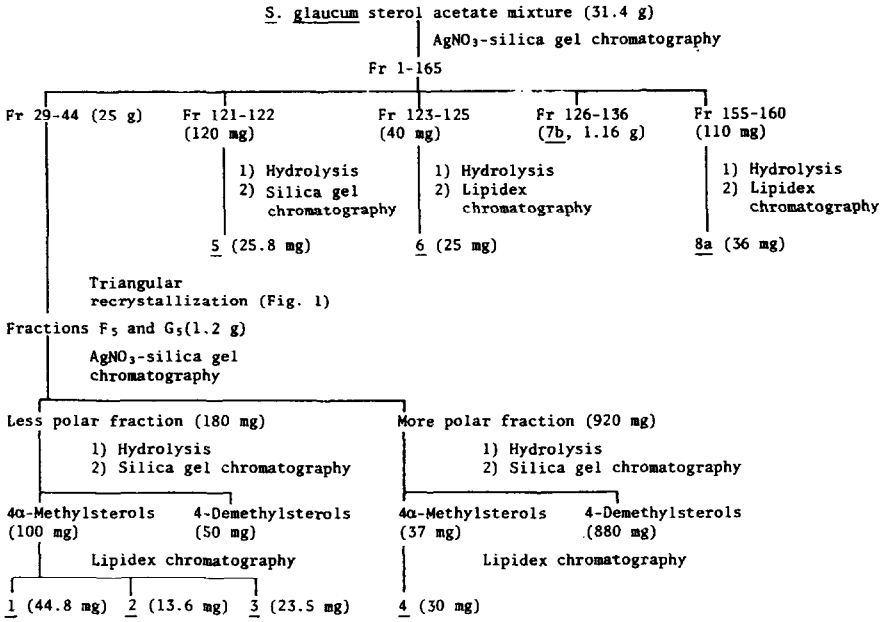


Figure 2. Separation of compounds 1 to 8

The mass spectrum showed the weak ions derived from the side chain cleavage at m/z 289 and 271 and strong ions due to ring D cleavage at m/z 247 and 229 (other ions and interpretations, see Table 2) (14). One nuclear methyl group is linked at 4α since the hydroxy-methine signal at δ 3.1 appears as a sextet (J=10, 10, 5Hz) with ax-ax-eq coupling (6). The proton magnetic resonance (PMR, Table 1) chemical shifts of the methyl signals in the side chain agreed with those of ergostanol (24S) and differed from those of its C-24 epimer campestanol, which is supposed to show C-21 and C-27 signals at δ 0.891 and 0.801, respectively (7). The 4α-methyl signal occurred at δ 0.945 (d, J=6.4Hz) and was the common signal in other 4α-methylsterols 2 to 5 (vide infra).

Compound 2 was a monounsaturated C_{30} sterol with a saturated 4α-methyl steroid ring (Table 2, m/z 289, 271, 247, 229: Table 1, δ 0.945, 3H, d, J=6.4Hz) and a side chain having a trisubstituted olefinic bond (δ 4.88, 1H, d, J=9.5Hz). The mp and specific rotation (Table 1) suggested that it is identical with dinosterol, the first member of biogenetically important 23-methylsterols found in the toxic dinoflagellate Golyaulax tamarensis (8). The PMR chemical shifts and fragmentation pattern in the mass spectrum of 2 agreed completely with those reported for dinosterol (2) (4d, 8).

Compound 3 was also a monounsaturated C_{31} sterol with a saturated 4α-methylsteroid ring (m/z 289, 271, 247 and 229; δ 0.945, d, J=6.6Hz). The gorgostane-type side chain was indicated by the presence of corresponding signals such as those for a cyclopropane ring and four secondary methyl groups (Table 1) and also from the mass spectrum which shows a strong ion at m/z 330 due to the cleavage at the cyclopropane ring (Table 2). This sterol, 4α-methylgorgostanol, was first

Table 1. PMR chemical shifts (ppm) and coupling constants (Hz) of the sterols from S. glaucum (200 MHz, in $CDCl_3$)

	1	2	3	4	5	6	7a	8a
mp	192-194°	219-220°	227-229°	187.5-193°	174°	123-124°	149-150°	135-136.5°
lit.	188-189°(19)	220-222°(8)	225.5-226°(4b)					
$[\alpha]_D$(c)	+12°(0.77)	-6°(0.52)	+10°(0.57)	-9°(0.32)	+21°(0.92)	+15°(1.23)	-44°(0.75)	-47°(0.89)
lit.	+13°(19)	±5°(8)						
C-18	0.642 s	0.680 s	0.621 s	0.659 s	0.650 s	0.652 s	0.671 s	0.690 s
C-19	0.822 s	0.827 s	0.818 s	0.823 s	0.824 s	0.802 s	1.004 s	1.006 s
C-21	0.900 d J=6.6	0.918*d J=6.4	0.851*d J=6.6	0.993 d J=6.6	0.931 d J=6.4	0.931 d J=6.4	0.910 d J=6.1	1.010 d J=6.6
C-26	0.851 d J=6.8	0.838 d J=6.2		0.830 d J=6.6	1.025 d J=6.8	1.024 d J=6.8	1.634 t J=1.0	1.673 broad s
C-27	0.778 d J=6.8	0.778 d J=6.4	0.933*d J=7	0.815 d J=6.4	1.020 d J=6.8	1.020 d J=6.6	4.66 2H broad s	4.701 2H broad s
C-28	0.770 d J=6.8	0.928*d J=6.6	0.966**d	0.906 d J=6.8	4.709 s 4.656 s	4.709 s 4.655 s	0.990 d J=6.8	1.077 d J=6.7
4α-Me	0.945 d J=6.4	0.945 d J=6.4	0.945*d 6H J=6.6	0.942 d J=6.8	0.947 d J=6.3			
3α-H	3.09 m	3.08 m	3.09 m	3.10 m	3.10 m	3.6 m	3.6 m	3.52 m
		1.496 3H d J=1.1 (23-Me)	0.890 s (23-Me)	5.15-5.2 2H m (22, 23-H)			5.36 1H m (6-H)	5.35 1H m (6-H)
		4.877 1H d J=9.5 (22-H)	-0.139 1H dd J=5.62, 4.4	0.1-0.32 2H m	0.445 1H dd J=4.2, 9.0			5.2-5.25 2H m (22,23-H)
			(cyclopropane ring and one methine which is shielded by cyclopropane ring)					2.705 1H m (24-H)

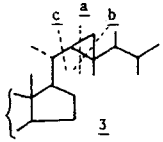
* These signals may be interchanged

** Better resolution obtained by 400 MHz PMR showed that this doublet signal appears as almost one line at δ 0.99 since the coupling counterpart methine proton resonates at almost the same position by the shielding effect of the neighbouring cyclopropane ring.

Table 2. Mass spectra (m/z) of the sterols from S. glaucum (relative abundance, %)

	1	2*	3	4*	5	6	7a	8a
Molecular ion (M^+)	416(100)	428(8)	442(36)	414(46)	414(7)	400(6)	398(100)	396(23)
-Me	401(22)	413(1)	427(5)	399(6)	399(12)	385(11)	383(24)	381(6)
$-H_2O$	398(5)	410(0.5)	424(2)	396(3)	396(1)	382(1)	380(18)	378(7)
$-Me-H_2O$	383(12)		409(2)	381(3)	381(3)	367(3)	365(23)	363(5)
$-C_3H_7$		385(6)	399(3)	371(5)				
$-C_3H_7-H_2O$		367(3)		353(12)	353(1)	339(1)		
$-C_5H_{11}$		357(2)	371(12)					123(100)
$-C_5H_{11}-H_2O$		339(2)	353(26)					(side chain)
M^+-side chain	289(6)	289(5)	289(22)	289(17)	289(4)	275(4)	273(13)	273(15)
$-H_2O$	271(8)	271(16)	271(36)	271(46)			255(19)	255(54)
-42	247(49)	247(3)	247(21)	247(6)	247(18)	233(21)	231(18)	231(7)
$-H_2O-42$	229(54)	229(4)	229(30)	229(11)	229(24)	215(22)	213(35)	213(17)
M^+-side chain-2H		287(32)	287(90)	287(46)	287(50)	273(46)	271(53)	271(80)
$-H_2O$		269(2)	269(6)	269(8)	269(7)	255(7)	253(11)	253(9)
M^+-C-22 to C-27-H		316(17)	316(36)	316(42)				300(35)
-Me		301(6)	301(16)	301(15)				285(12)
M^+-C-23 to C-27-H					330(100)	316(100)	314(31)	
-Me					315(25)	301(23)	299(29)	
	Cleavage at a		330(100)		M^+-C-24 to C-27-H		328(11)	
	-Me		315(29)			-Me	313(15)	
	$-Me-H_2O$		297(7)			$-H_2O$	310(4)	
	Cleavage at b		344(2)			$-Me-H_2O$	295(5)	
	Cleavage at c		317(18)					
	$-H_2O$		299(40)					

* Base peak: m/z 69

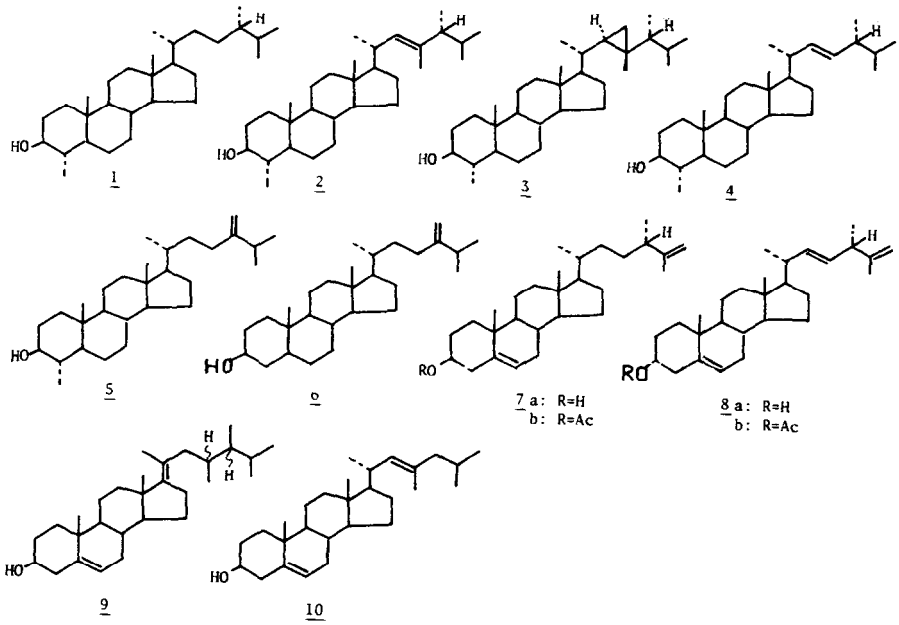


3

detected in the zooxanthellae of the Caribbean gorgonian Briareum asbestinum (4a), and was later isolated and characterized from the cultured dinoflagellate Peridinium foliaceum (4b). The mp 227-229° of compound 3 in the present study agreed with that (mp 225.5-226°) of synthetic 4α-methylgorgostanol (4b).

Compound 4 was a monounsaturated 4α-methyl C_{29} sterol with a side chain having a trans-disubstituted double bond (IR, 970 cm^{-1}). The ion at m/z 316 was assumed to be derived from the cleavage at C-20 and C-22 with 1H transfer and this is the characteristic fragmentation of Δ^{22}-sterols (9). Indeed the side chain signals were similar to those of brassicasterol or its C-24 epimer 24-epibrassicasterol (7). The difference of the side chain signals in the brassicasterol/24-epibrassicasterol pair is too small to make a judgement on the stereochemistry at C-24 in 4. It was hydrogenated to the fully saturated sterol 4α,24S-dimethyl-5α-cholestan-3β-ol, which was identical with compound 1. Thus, compound 4 was identified as 4α,24R-dimethyl-5α-cholest-22E-en-3β-ol (24R/S convention changes by saturation at C-22).

Compound 5 was also a monounsaturated C_{29} sterol with a saturated 4α-methylsteroid ring as in 1 to 4 (Table 1 and 2). One double bond was located at the terminal methylene group in the side chain at C-24. This was supported by the presence of signals at δ 4.656 and 4.709 and terminal isopropyl signals at δ 1.020 and 1.025 in PMR (Table 1). The mass spectrum showed the intense ion at m/z 330 derived from the McLafferty-type cleavage at C-22 and C-23 with 1H transfer from C-20 (9) Thus, this compound is 4α-methyl-24-methylene-5α-cholestan-3β-ol which was recently found in four Caribbean gorgonians including B. asbestinum (10).



Compound 6 was a monounsaturated 4-demethyl C_{28} sterol. The PMR and mass spectra were essentially the same as those of 5 (Table 1 and 2) except that major ions appeared at 14 mass units less than those of 5. This sterol, 24-methylene-5α-cholestan-3β-ol (6), was found in the sponge Hymeniacidon perleve by Thomson et al. (11).

Compound 7a was a diunsaturated C_{28} sterol with a 3β-hydroxy-Δ^5-steroid ring. This was supported by the PMR and mass spectra which satisfy the necessary conditions (chemical shifts of angular methyl groups (12); broad hydroxy-methine at δ 3.5 (13); C-6 olefinic proton at δ 5.35; very weak molecular ion in the acetate 7b (14)). The PMR spectrum showed signals of an isopropenyl group (δ 4.66, 2H, s; 1.63, 3H, t, J=1Hz) and a secondary methyl group at C-24 (δ 0.99, d). The mass spectrum showed the McLafferty-type cleavage ion at m/z 328 and also at m/z 314 which arose from the isomerization at C-25 (9). These observations are reminiscent of codisterol (24S-methylcholesta-

5,25-dien- 3β-ol, 7a), from a green alga Codium fragile (15), or its C-24 epimer 24-epicodisterol. Both of the epimers are known to occur in the sponge Verongia cauliformis (16). The PMR spectrum in the present study did not show the olefinic methyl signal at δ 1.651 which corresponds to 24-epicodisterol. Hydrogenation of 7b by a reported procedure (15) yielded 22,23-dihydrobrassicasterol acetate showing that 7a is the 24S isomer codisterol. The mp of the acetate 7b (142-143°) was clearly different from the reported mp 118-120° for codisterol acetate (15). However, we purified the authentic codisterol acetate and found that the mp of the authentic sample was actually 142-143°. It is noteworthy that this rare sterol occurs in S. glaucum as one of the major components (3.7% of total sterol mixture). This compound was formerly obtained as a mixture with its isomer 24-methylenecholesterol because of their similar natures in $AgNO_3$-silica gel chromatography and mass spectroscopy, and also in GC (relative retention time (RRT) to cholesterol: codisterol, 1.32; 24-methylenecholesterol, 1.35 on OV-17 column at 260°).

Compound 8a was triunsaturated C_{28} sterol. The 3β-hydroxy-Δ^5-steroid ring was deduced on the same grounds as that for 7a. The presence of the terminal isopropenyl group was confirmed by PMR chemical shifts which were almost identical with 7a (Table 1). The ion at m/z 300 (cleavage at C-20 and C-22 with 1H transfer) showed that another double bond was located at C-22 as in 4. No strong UV absorption was observed. The 28-methyl signal at δ 1.077 changed to a singlet upon irradiation of the doubly allylic methine (δ 2.705) at C-24. These observations indicate that the only possible structure for 8a is 24-methylcholesta-5,22,25-trien-3β-ol. The stereochemistry at C-24

was found to be S by the hydrogenation and correlation to the fully saturated compound ergostanol. Compound 8a (22-dehydrocodisterol) was also recently reported from a sponge Pseudaxinella lunacharta collected on the Senegalese coast by Sjöstrand et al. (17).

Nineteen sterols were characterized from S. glaucum including eleven 4-demethyl-Δ^5-sterols in the previous study. The 4-demethyl-Δ^5-derivatives of compounds 1 to 5 were the major components (1). Examination of other fractions by GC showed that still other minor components are present in trace amounts.

The remarkable feature of the sterol composition of S. glaucum is that in contrast to the high proportion of 24-methylcholestane derivatives, it does not include any 24-ethylcholestane derivatives such as 24-ethylcholesterol, 24-ethyl-22-dehydrocholesterol, or fucosterol. This finding is consistent with the results reported by Kanazawa et al. (18) who surveyed twelve species of southern Japan soft corals and found that only two species contain 24-ethyl-22-dehydrocholesterol and fucosterol in trace amounts. This suggests that although S. glaucum (or its zooxanthella) can alkylate at C-23 to produce such unusual sterols as 23-methyl-22-dehydrocholesterol, gorgosterol, and sarcosterol it hardly causes alkylation at C-28 of 24-methylenecholesterol which is the normal biosynthetic pathway in many living organisms.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. L. J. Goad of the University of Liverpool for the sample of codisterol acetate and to Dr. Koji Hayashi of Hokkaido University, for his helpful discussions.

EXPERIMENTAL

Melting points were determined on a Kofler hot stage and are uncorrected. PMR spectra were recorded in $CDCl_3$ soln. with TMS as an internal standard on a JEOL FX200 spectrometer operating at 200 MHz. Optical rotations were measured in $CHCl_3$ soln. Mass spectra were

determined on a JEOL JMS D-300 mass spectrometer. IR spectra were taken on a Hitachi 215 spectrometer in Nujol mull. GC was carried out on a Shimadzu GC3BF gas chromatograph using a glass column (2m x 3 mm I.D.) packed with 1.5% OV-17 on 80-100 mesh Shimalite W at 260°, with N_2 carrier gas at a flowing rate of 60 ml/min. Recrystallization of sterols was carried out from methanol.

Isolation and fractionation of sterols The lipid extract (840 g) obtained in the previous study (1a) was partitioned with a mixture of solvents, hexane-methanol-H_2O (20:10:2) and separated into upper (690 g) and lower exctracts (151 g). Sterol and other nonpolar compounds were extracted in the upper layer while the lower layer contained polyhydroxysterols and other polar compounds. TLC showed that the amount of sterols in the lower layer was negligible. The nonpolar extract which contained the sterol fraction was saponified by a usual procedure (1a) and the nonsaponifiable matter was extracted with ether. Chromatography of the ether extract over a column of silica gel (1.7 kg) with benzene gave a fraction which contained sterols. Crystallization from methanol gave 25.1 g of sterol mixture. Evaporation of the mother liquor gave 52.5 g of oil which was chromatographed in the same way and the sterols (3.92 g) were obtained by crystallization. The mother liquor (6.5 g) was chromatographed again and gave 0.17 g of sterols. The combined sterol mixture (29.2 g) was acetylated in the usual manner giving 31.36 g of crude sterol acetate mixture.

The sterol acetate mixture was chromatographed over a column of 15% $AgNO_3$-impregnated silica gel (200 to 300 mesh, 1.7 kg) and eluted (1 liter/fraction) with a gradient mixture of benzene and hexane (Fig.1) The major components reported in the previous study were eluted in the following fractions: cholesterol acetate, 22,23-dihydrobrassicasterol acetate and 23-demethylgorgosterol acetate (Fr 24-53), gorgosterol acetate (Fr 24-57), 23-methyl-22-dehydrocholesterol acetate and 23,24-dimethyl-22-dehydrocholesterol acetate (Fr 45-53), brassicasterol acetate (Fr 45-83), 22-dehydrocholesterol acetate (Fr 84-112), desmosterol acetate (Fr 113-122), sarcosterol acetate (Fr 84-116), 24-methylene-cholesterol acetate (Fr 137-154).

4α,24S-Dimethyl-5α-cholestan-3β-ol (1), dinosterol (2), 4α-methylgorgostanol (3), and 4α,24R-dimethyl-5α-cholest-22-en-3β-ol (4)

Fractions 29-44 (25 g) was submitted to triangular recrystallization from methanol (Fig. 1). The more soluble acetate mixture (Fr. F_5 and G_5, 1.2 g) was chromatographed over 15% $AgNO_3$-silica gel (900 g) with 15% benzene in hexane and separated into less polar (180mg) and more polar (920 mg) sterol fractions. Each fraction was hydrolyzed with 2.5% KOH in methanol and chromatographed in several portions over a column of silica gel (100 g) with 5% ether in $CHCl_3$. A mixture of 1, 2, and 3 (100 mg) was obtained from the less polar fraction while a mixture (37 mg) of 4 was obtained from the more polar fraction. Each mixture was dissolved in a minimum volume of a mixture of hexane-methanol (5:95) (ca. 0.5 mg/ml for mixture of 1, 2 and 3 and 1.5 mg/ml for the fraction which contained 4. Compound 3 was sparingly soluble in contrast to its acetate). Each 15 ml of solution was charged on a column of Lipidex 5000 (Packard, 2.2 x 45 cm) and eluted with the same solvent. The fractions (15 ml) were monitored by GC and combined accordingly. In the mixture of 1, 2 and 3, the elution order was 2, 1, and 3. For mp, $[\alpha]_D$, and mass and PMR spectra, see Tables 1 and 2.

4α,24S-Dimethyl-5α-cholestan-3β-ol (1) from 4 Hydrogenation of 4 (3.4 mg) in methanol with 5% Pd-C overnight gave a compound, mp 186-188°, identified as 1 by PMR which showed an identical spectrum.

4α-Methyl-24-methylene-5α-cholestan-3β-ol (5) Fractions 121-122 was an oily mixture (120 mg) contaminated with small amounts of 6 and unknown compound (RRT, 1.34). The main impurity was a non-steroid substance with a short retention time on GC. The mixture was hydrolyzed with 2.5% KOH in methanol. Column chromatography of the hydrolyzate over silica gel with 5% ether in $CHCl_3$ gave 25.8 mg of pure 5. For mp, $[\alpha]_D$, mass and PMR spectra, see Tables 1 and 2.

24-Methylene-5α-cholestan-3β-ol (6) Fractions 123-125 (40 mg) was a mixture of acetate of 6(90%), 5(3%), and an unknown compound (RRT, 1.16, 5%). It was hydrolyzed with 2.5% KOH in methanol and the free sterol fraction (38.7 mg) was chromatographed on a column of silica gel with 5% ether in $CHCl_3$. The 4-demethylsterol fraction (31.8 mg) was subjected to Lipidex 5000 chromatography and gave a mixture (5 mg) of 6 and unknown compound (RRT, 1.16) and 25 mg of pure 6. For mp, $[\alpha]_D$, PMR and mass spectra, see Tables 1 and 2.

Codisterol (7a) Recrystallization of combined fractions 126-136 gave 1.16 g of codisterol acetate (7b), mp 142-143°, $[\alpha]_D$ -47° (c, 0.81, $CHCl_3$). IR: 1640, 890 cm^{-1}. Hydrolysis of the acetate 7b gave the free sterol (7a). For mp, $[\alpha]_D$, and PMR and mass spectra, see Tables 1 and 2.

22,23-Dihydrobrassicasterol acetate from codisterol acetate (7b) A solution of 7b (80 mg) in 15 ml of benzene was hydrogenated according to the reported procedure (15) with tris-(triphenylphosphine) rhodium chloride and gave 62.7 mg of 22,23-dihydrobrassicasterol acetate, mp 148-149°, $[\alpha]_D$ -52° (c, 1.21, $CHCl_3$), which had identical PMR and mass spectra with the authentic sample (mp 149-150°)in the previous study(1a)

24S-Methylcholesta-5,22,25-trien-3β-ol (8a) Fractions 155-160 contained a large amount of non-steroid impurities which were removed by chromatography over a column of silica gel with benzene-hexane (1:1). The sterol fraction was composed of 8b (95%) and 24-methylenecholesterol acetate (5%). The mixture was submitted to Lipidex 5000 chromatography with 5% hexane in methanol and gave pure 8b (36.2 mg), mp 154°, $[\alpha]_D$ -53.7° (c, 1.08, $CHCl_3$). Hydrolysis of 8b gave the free sterol (8a). For mp, $[\alpha]_D$, and PMR and mass spectra, see Tables 1 and 2.

24S-Methyl-5α-cholestan-3β-ol from 8a A solution of 8a (3 mg) in 5% acetic acid in methanol (3 ml) was hydrogenated with 5 mg of PtO_2 overnight. Filtration and crystallization from methanol gave the hexahydroderivative, mp 136-142°. PMR: δ 0.645 (C-18), 0.802 (C-19), 0.771 (3H, d, J=6.4Hz, C-28), 0.780 (3H, d, J=6.8Hz, C-27), 0.851 (3H, d, J=6.8Hz, C-26), 0.990 (3H, d, J=6.8Hz, C-21), identical chemical shifts reported for ergostanol (7).

REFERENCES AND NOTES

* Part IX, Kobayashi, M., Hayashi, T., Nakajima, F., and Mitsuhashi, H., STEROIDS, 34, 285 (1979).

1. (a) Kobayashi, M., Tomioka, A., and Mitsuhashi, H., STEROIDS, 34,

273 (1979).
From the soft coral Sarcophyton elegans, which is closely related to S. glaucum, Kanazawa et al. isolated a diunsaturated C_{29} sterol to which they assigned the structure 23,24-dimethylcholesta-5,23-dien-3β-ol (Kanazawa, A., Ando, T., and Teshima, S., NIPPON SUISAN GAKKAISHI, 43, 83 (1977)). However, their assignment was not based on adequate evidence. The mass spectrum showing several characteristic ions was mistakenly interpreted: m/z 341 (cleavage of the double bond at C-23 with 1H transfer), 314 (M^+-C_7H_{13}-H), 301 (M^+-C_8H_{15}), 300 (cleavage at C-20 and C-22 with 1H transfer), 299 (cleavage at C-20 and C-22 with 2H transfer), 230 (M^+-side chain -42-1H). These ions show that at least the $\Delta^{5,23}$-structure should be excluded since the Δ^{23}-sterols would not decompose in this way (9). The possible ion is m/z 301 by allylic cleavage in the side chain (9) but the published mass spectrum showed a very weak ion at m/z 301 and it was attributable to the isotope peak of the stronger ion at m/z 300. The PMR data (in $CDCl_3$) was also ambiguous. Although the presence of a tetrasubstituted double bond was definite from the mass and PMR spectra, the assignment of a heap of composite signals at δ 1.82 for one of the vinylic methyl is unreasonable since it occupies an unusually deshielded position for such a non-conjugated olefinic methyl. For these reasons and the complete accordance of the reported PMR and mass spectra and RRT with those of sarcosterol, we conclude that the assignment by Kanazawa et al. is incorrect and the compound is in fact sarcosterol (9).
(b) Kobayashi, M., Tomioka, A., Hayashi, T., and Mitsuhashi, H., CHEM. PHARM. BULL.(Tokyo), 27, 1951(1979).
From the clam Tapes philippinarum, Teshima et al. isolated a diunsaturated C_{28} sterol to which they assigned the structure 24-methylcholesta-5,22Z-dien-3β-ol (Teshima, S., Kanazawa, A., and Ando, T., COMP. BIOCHEM. PHYSIOL., 47B, 507 (1974)). The assignment was based mainly on the mass spectral fragmentation pattern which was similar to brassicasterol, a very small IR absorption at 680 cm^{-1}, and ill-defined PMR signals at δ 4.15-4.22 which they claimed to be due to one of the olefinic protons of Δ^{22}. However, we think their description is inaccurate. In contrast to the trans double bond, the IR bands of cis-disubstituted double bond is variable. It usually occurs between 670 and 750 cm^{-1} with medium intensity and is not the specific absorption. For example, both the 22E and 22Z isomers of 22-dehydrocholesterol show similar bands between 650 and 750 cm^{-1} (Hutchins, R.F.N., Thompson, M.J., and Svoboda, J.A., STEROIDS, 15, 113 (1970)). Also, the signals at δ 4.15 to 4.22 are at an abnormally high field for such an olefinic proton (1b) and should be regarded as other signals due to impurities. Moreover, they neglected the strong signal at ca. δ 1.6 in the published PMR spectrum which may well be assigned to a vinylic methyl. Since the PMR and mass spectra, and the RRT on GC (OV-17 column) reported for their 24-methylcholesta-5,22Z-dien-3β-ol were identical with those of 10, we conclude that the assignment of Teshima et al. is wrong and the compound is in fact 23-methyl-22-dehydrocholesterol (10).

2. Kokke, W.C.M.C., Withers, N.W., Massey, I.J., Fenical, W., and Djerassi, C., TETRAHEDRON LETTERS, 3601 (1979).
3. Swenson, W., Tagle, B., Clardy, J., Withers, N.W., Kokke, W.C.M.C., Fenical, W., and Djerassi, C., TETRAHEDRON LETTERS, 4663 (1980).

4. (a) Steudler, P.A., Schmits, F.J., and Ciereszko, S.S., COMP. BIOCHEM. PHYSIOL., 56B, 385 (1977). (b) Withers, N. W., Kokke, W.C.M.C., Rohmer, M., Fenical, W.H., and Djerassi, C., TETRAHEDRON LETTERS, 3605 (1979). (c) Alam, M., Schram, K.H., and Ray, M., TETRAHEDRON LETTERS, 3517 (1978). (d) Withers, N.W., Tuttle, R.C., Holz, G.G., Beach, D.H., Goad, L.J., and Goodwin, T.W., PHYTOCHEMISTRY, 17, 1987 (1978). (e) Teshima, S., Kanazawa, A., and Tago, A., MEM. FAC. FISH., KAGOSHIMA UNIV., 29, 319 (1980). (f) Withers, N.W., Tuttle, R.C., Goad, L.J., and Goodwin, T.W., PHYTOCHEMISTRY, 18, 71 (1979). (g) Withers, N.W., Goad, L.J., and Goodwin, T.W., PHYTOCHEMISTRY, 18, 899 (1979). (h) Alam, M., Martin, G.E. and Ray, S.M., J. ORG. CHEM., 44, 4486 (1979).
5. Barton, D.H.R., Kempe, U.M., and Widdowson, D.A., J. CHEM. SOC., Perkin I, 513 (1972).
6. Knapp Jr, F.F., and Schroepfer Jr., G.J., STEROIDS, 26, 339 (1975).
7. Rubinstein, I., Goad, L.J., Clague, A.D.H., and Mulheirn, L.J.M., PHYTOCHEMISTRY, 15, 195 (1976).
8. Shimizu, Y., Alam, M., Kobayashi, A., J. AM. CHEM. SOC., 98, 1059 (1976).
9. Wyllie, S.G., and Djerassi, C., J. ORG. CHEM., 33, 305 (1968).
10. Kokke, W.C.M.C., Gohlin, L., Fenical, W., and Djerassi, C., PHYTOCHEMISTRY, 21, 881 (1982).
11. Erdman, R.R., and Thomson, R.H., TETRAHEDRON, 28, 5163 (1972).
12. Zurcher, R.F., HELV. CHIM. ACTA, 46, 2054 (1963).
13. Bridgeman, J.E., Cherry, P.C., Clegg, A.S., Evans, J.M., Jones, R.H., Kasal, A., Kumar, V., Meakins, G.D., Morisawa, Y., Richards, E.E., and Woodgate, P.C., J. CHEM. SOC., (C), 250 (1970).
14. Knights, B.A., J. GAS CHROMATOGR., 273 (1967).
15. Rubinstein, I., and Goad, L.J., PHYTOCHEMISTRY, 13, 481 (1974).
16. Kokke, W.C.M.C., Pak, C.S., Fenical, W., and Djerassi, C., HELV. CHIM. ACTA, 62, 1310 (1979).
17. Sjöstrand, U., Kornprobst, J.M., and Djerassi, C., STEROIDS, 38, 355 (1981).
18. Kanazawa, A., Teshima, S., and Ando, T., COMP. BIOCHEM. PHYSIOL., 57B, 317 (1977).
19. ÕDochaí, R., Flanagan, P.J., and Thomson, J.B., J. CHEM. SOC., 1142 (1964).