STEROID COMPOUNDS OF MARINE SPONGES.

I. STEROLS OF *Esperiopsis digitata*

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From extracts of the Far Eastern sponge *Esperiopsis digitata*, by column chromatography on silica gel we have isolated three fractions of steroid compounds. The fractions isolated have been analyzed with the aid of TLC, GLC-MS, PMR, and ^{13}C NMR. The least polar fraction (1) had a R_f value identical with that of cholesterol and represents the total free steroids. After argentation column chromatography, 13 components were identified in it, the main one being cholesterol. The more polar fraction (2) consisted of a combination of 3β-hydroxychol-5-en-24-al and 3β-hydroxy-24-norchol-5-en-23-al. The most polar of the fractions isolated — fraction (3) — consisted of a single component which was identified as cholesta-5,25-diene-3β,24ξ-diol.

We have isolated three fractions of steroid compounds by column chromatography from ethanolic and ethanolic-chloroformic extracts of the Far Eastern sponge *Esperiopsis digitata*.

The least polar of them (0.3% of the dry weight of the animals) had a R_f value identical with that of cholesterol on TLC and consisted of the sum of the free sterols. To facilitate subsequent analysis, this fraction was acetylated and separated on a column of silica gel impregnated with 20% of $AgNO_3$. The structural identification of the components isolated was was carried out by chromato-mass spectrometry and with the aid of analysis of the PMR spectra taking into account the sequence of elution from the SiO_2-$AgNO_3$ column and the relative retention times in GLC. The results obtained are given in Table 1.

For compound (VII) (Table 1) it was possible to determine the configuration of C-24 asymmetric center. For this purpose, by catalytic hydrogenation was obtained a dihydro derivative the PMR spectrum of which coincided with the spectrum given in the literature of (24R)-methylcholest-5-en-3β-ol [1].

Compounds (VIII) and (IX) could not be separated. They are close to one another in their chromatographic behavior on an SiO_2-$AgNO_3$ column and in GLC characteristics. The mass spectra and also the relative retenion times (Table 1) permitted the assumption that we were dealing with a mixture of cholesta-5,22-dien-3β-ol and 24-methyl-27-norcholesta-5,22-dien-3β-ol. A comparison of the high-resolution PMR spectra with literature figures [1, 2] confirmed this hypothesis.

The main components of the mixture being analyzed proved to be cholesterol (IV) and 24-methylcholesta-5,24(28)-dien-3β-ol (XIII). It differed from the sterol fractions of the fungus *E. digitata* studied previously by its high amount of desmosterol (XII). Minor components were stanols, the C-28, and C-29 homologs of cholesterol, and fucosterol (XI).

The more polar fraction (according to TLC) (0.009% of the dry weight of the animals) consisted of two components (XIV) and (XV), differing from one another by 14 mass units. The mass spectrum of this fraction showed the peaks of molecular ions with m/z 358 and 344.

Absorption at 1724 cm^{-1} in the IR spectrum permitted the assumption that compounds obtained contained an aldehyde group. This was also indicated by a triplet signal in the PMR spectrum at 9.76 ppm (J = 1.5 Hz) and a doublet at 203 ppm in the ^{13}C NMR spectrum.

Reduction by KBH_4 in aqueous dioxane led to the disappearance of the signal of the aldehyde group in the PMR spectrum and to an increase in the relative intensity of the signal at 3.64 ppm that is characteristic for carbinyl protons.

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TABLE 1. Sterols of *Esperiopsis digitata*

Compound*	Amt. %	RRT†	Main ions in the mass spectrum of the acetate	Lit. cited	Main signals in the PMR spectrum of the acetate	Lit. cited
(I) 5α-Cholestan-3β-ol	0,54	1,0	430 (M^+), 415, 370, 355, 316, 276, 275, 262, 257, 255, 230, 217, 215	[3]	0,645 (3H, s C-18); 0,815 (3H, s. C-19); 0,893 (3H, d J=6,5 Hz C-21); 0,854 (6H, d, J=6,5 Hz C-26, 27)	[4]
(II) 24ξ-Methylcholestan-3β-ol	Tr.	1,25	444 (M^+) 429, 384, 369, 330, 276, 257, 257, 255, 230, 217, 215	[3]		
(III) 24ξ-Ethylcholestan-3β-ol	Tr.	1,56	458 (M^+), 443, 398, 383, 344, 290, 276, 275, 257, 255, 230, 217, 215	[3]		
(IV) Cholest-5-en-3β-ol	43,78	1,0	368 (M^+—60), 253, 260, 255, 247, 228, 213	[5]	0,68 (3H, s C-18); 1,017 (3H, s C-19); 0,98 (3H, d J=6,5 Hz C-21); 0,855 (6H, d, J=6,5 Hz C-26,27)	[1]
(V) 24ξ-Methylcholest-5-en-3β-ol	Tr.	1,25	382 (M^+—60), 367, 274, 261, 255, 228, 213	[5]		
(VI) 24ξ-Ethylcholest-5-en-3β-ol	Tr.	1,54	396 (M^+—60), 381, 288, 275, 255, 228, 213	[5]		
(VIII)‡ 24(S)-Methylcholesta-5,22-dien-3β-ol	8,54	1,08	380 (M^+—60), 365, 337, 282, 255, 253, 228, 213	[6]	0,692 (3H, s, C-18); 1,02 (3H, s, C-19); 1,002 (3H, d, J=6,5 Hz C-21); 0,818 (3H, d, J=6,5 Hz, C-26); 0,835 (3H, d, J=6,5 Hz C-27); 0,909 (3H, d, J=6,8 Hz, C-28); 5,165 (2H, m, C-22,23)	[1]
(VIII)‡ 24-Methyl-27-norcholesta-5,22-dien-3β-ol	1,69	0,91	366 (M^+—60), 351, 337, 282, 267, 255, 228, 213	[6]	0,692 (3H, s, C-18); 1,004 (3H, s, C-19); 0,926 (3H, d J=6,6 Hz C-28), 0,995 (3H, d J=7 Hz, C-21); 0,831 3H, t, J=7,2 Hz C-26); 5,13 (2H, m, C-22,23)	[2]
(IX)‡ Cholest-5,22-dien-3β-ol	4,5	0,91	366 (M^+—60), 351, 323, 282, 267, 255, 228, 213	[7]	0,692 (3H, s C-18); 1,02 (3H, s C-19); 1,008 (3H, d J=6,5 Hz C-21); 0,858 (3H, d J=6,6 Hz C-26); 0,862 (3H, d J=6,6 Hz C-27); 5,25 (2H, m C-22,23)	[1]
(X) 24-Norcholesta-5,22-dien-3β-ol	6,86	0,69	352 (M^+—60), 337, 282, 255, 244, 231, 228, 213	[7]	0,69 (3H, s C-18); 1,017 (3H, s C-19); 1,00 (3H, d J=6,5 Hz C-21); 0,937 (6H, d, J=6,5 Hz C-26,27), 5,22 (2H, m, C-22,23)	[8]

TABLE 1. (Continued)

Compound	Amt. %	RRT†	Main ions in the mass spectrum of the acetate	Lit. cited	Main signals in the PMR spectrum of the acetate	Lit. cited
(XI) 24ξ-Ethylcholesta-5,24(28)-dien-3β-ol	Tr.	1.50	394 (M^+—60), 379, 296, 281, 255, 253, 228, 215, 213	[4]		
(XII) Cholesta-5,24-dien-3β-ol	9,28	1,08	366 (M^+60), 351, 296, 283, 282, 258, 255, 253, 245, 228, 213	[9]	0,68 (3H, s, C-18); 1,017 (3H, s, C-19); 0,935 (3H, s J=6,5 Hz C-21); 1,58 (3H, s C-26); 1,66 (3H, d C-27); 5,2 (1H, m C-23)	[8]
(XIII) 24-Methylcholesta-5,24(28)-dien-3β-ol	22,35	1,23	380 (M^+—60), 365, 296, 281, 255, 253, 228, 213	[7]	0,68 (3H, s C-18); 1,02 (3H, s, C-19); 0,95 (3H, d J=6,5 Hz C-21); 1,022 (6H, d, J=6,5 Hz C-26,27); 4,66 (1H, m C-28); 4,71 (1H, m, C-28)	[8]

*The numbers of the compounds correspond to the sequence of elution from a SiO_2-$AgNO_3$ column

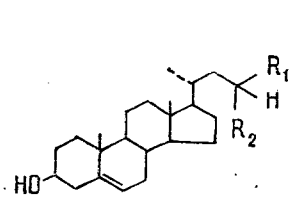
†Relative to cholesterol acetate.

‡The PMR spectra were recorded on a Bruker WM-250 instrument.

The mass spectrum of one of the diols obtained (XVI) has peaks with m/z 360 (M^+); 345 (M^+ − 15); 342 (M^+ − 18); 327 (M^+ − 33) and signals with m/z 275 (M^+ − 85), 273, 255, 249 (M^+ − 111), 231, 213 which were characteristic for 3β-hydroxy-Δ^5-monounsaturated steroids.

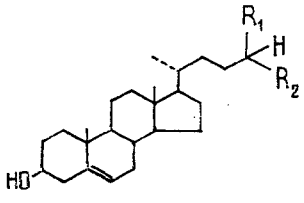
The mass spectrum of the other diol (XVII) contains peaks with m/z 346 (M^+), 331 (M^+ − 15), 328 (M^+ − 18), 313 (M^+ − 33), 273; 261 (M^+ − 85), 255, 235 (M^+ − 111), 213.

The results obtained, and also the coincidence of the signal of C_1-C_{20} atoms in the ^{13}C NMR spectrum with the corresponding signals of the spectra of other Δ^5-monounsaturated steroids (10) showed that fraction (II) consisted of a mixture of 3β-hydroxychol-5-en-24-al (XIV) (70%) and of 3β-hydroxy-24-norchol-5-en-23-al (XV) (30%).



XV. R_1R_2=O

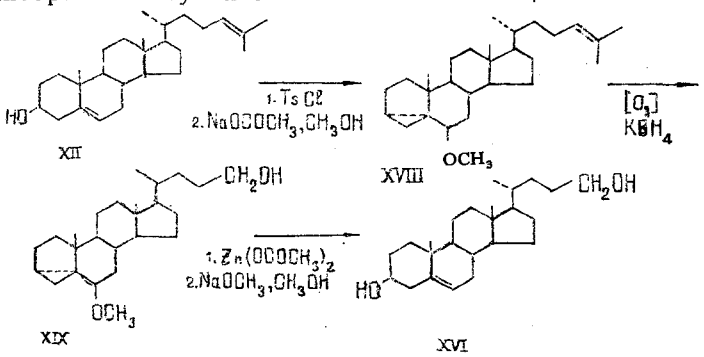
XVII. R_1=H; R_2=OH



XIV. R_1 R_2=O

XVI. R_1=H; R_2=OH

We carried out an independent synthesis of chol-5-ene-3β-24-diol (XVI) by the following scheme:



XII XVIII XIX XVI

The compound synthesized was, according to GLC and mass spectrometry, identical with the diol (XVI) obtained by the tetrahydroborate reduction of fraction (2).

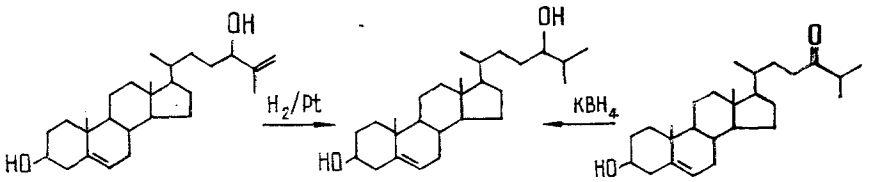
The most polar of the fractions isolated (0.009% of the dry weight of the animals) consisted of a single component, which was identified as cholesta-5,25-diene-3β,24ξ-diol (XX).

The mass spectrum of this compound contained the peak of the molecular ion with m/z 400 and the peaks of fragmentary ions showing that (XX) was a C-27 diunsaturated diol.

The ^{13}C NMR and PMR spectra and the conversion of the substance into cholesterol on dehydration under the action of p-toluenesulfonic acid followed by catalytic hydrogenation showed the presence in (XX) of a cholestane skeleton, a 3β-hydroxy group, and a double bond in the 5(6) position.

The 25(26) position for the second double bond followed from the IR spectrum (absorption bands at 899 and 1646 cm^{-1}; >C=CH_2), from the ^{13}C NMR spectrum (111.3 ppm, t, C-26), and from the PMR spectrum (4.82 ppm, 1 H, br.s, C-26); 4.90 ppm, (1 H, br.s, C-26); 1.71 ppm (3 H, s, C-27).

The catalytic hydrogenation of (XX) gave the dihydro derivative (XXI), which was also obtained by independent synthesis through the tetrahydroborate reduction of 24-oxocholesterol.



XX XXI XXII

Thus, it has been shown that the second hydroxy group occupies position 24.

The steroid derivatives oxidized in the side chain that we have isolated – (XIV), (XV), and (XX) – have not previously been found in sponges. However, Ikekawa et al. [11] have identified cholesta-5,25-diene-3β-24ξ-diol by the GLC-MS method in a mixture of sterols from the red alga *Rhodymenia palmata*. This compound had not previously been obtained in the individual state.

The biogenesis of compounds (XIV), (XV), and (XX) is not clear. They are possibly formed through the oxidation, taking place *in vivo*, of sterols precursors and as the result of the oxidation of the sterols of *E. digitata* in the process of extraction and chromatography.

EXPERIMENTAL

The sponges were collected in Peter the Great Bay at a depth of 40-50 m in August, 1980. The chromato-mass spectrometric study was carried out on a LKB 9000S spectrometer at an ionizing voltage of 70 V using a 300 × 0.5 cm column with 1.5% of SE-30. The column temperature was 265°C and the carrier gas was helium at the rate of 30 ml/min.

GLC analysis was performed on a Pye-Unicam 104 chromatograph with 150 × 0.5 cm columns containing 3% of SE-30 at 280°C. The carrier gas was argon at 60 ml/min.

The ^{13}C and PMR spectra were determined on Bruker HX-90E and Bruker WM-250 instruments in deuterochloroform with tetramethylsilane as internal standard.

IR spectra were recorded on a Specord IR-75 spectrophotometer in chloroform.

Isolation of the Sterols. Freshly collected animals (dry weight 568 g) were comminuted and extracted with ethanol. Then the extract was concentrated in a vacuum to an aqueous residue and this was extracted repeatedly with ethyl acetate. The residual tissues were extracted twice with chloroform–methanol (2:1) for 48 h.

The ethyl acetate and chloroform–methanol extracts were combined and concentrated in a vacuum to dryness. The residue was chromatographed repeatedly on columns containing silica gel L (40/100 μ) in the benzene–ethyl acetate (5:1) system. Three fractions each giving one spot in TLC (fixed layer of silica gel L (5/40 μ) benzene–ethyl acetate (5:1) system; spots revealed by treating with sulfuric acid vapors at 110°C) were isolated.

Fraction (1) consisted of the sum of the free sterols; 1.6 g, mp 143-148°C (from MeOH), $[\alpha]_D^{20}$ −34° (c 1.12; $CHCl_3$).

Fraction (2) was a mixture of 3β-hydroxychol-5-en-24-al (XIV) and 3β-hydroxy-24-norchol-5-en-23-al (XV); m/z 358, 344, 343, 340, 329, 326, 273, 259, 255, 246, 233, 231, 229, 215, 213.

Fraction (3) consisted of cholesta-5,25-diene-3β,24ξ-diol (XX): 53 mg, mp 199-201°C (from MeOH), $[\alpha]_D^{20}$ −36 (c 0.6; $CHCl_3$). IR spectrum, cm^{-1}: $\lambda_{max}^{CHCl_3}$ 3629 (O—H), 1646 (C=C), 899 (C—H).

Mass spectrum, m/z: 400 (M^+), 382, 367, 349, 315, 300, 271, 255, 229, 232, 213.

PMR spectrum ($CDCl_3$), δ, ppm): 0.68 (3 H, s, C-18); 1.00 (3 H, s, C-19); 0.93 (3 H, d, J = 6.5 Hz, C-21); 1.71 (3 H, s, C-27); 3.7 (1 H, m, C-3); 3.99 (1 H, m, C-24); 4.82 (1 H, br.s, C-26); 4.90 (1 H, br.s, C-26); 5.3 (1 H, m, C-6).

Separation of the Sterol Acetates on a SiO_2-$AgNO_3$ Column. To prepare the sorbent, silica gel L (40/100 μ) was impregnated with a solution of silver nitrate (20% of the weight of the silical gel) in acetonitrile. The resultant sorbent was left in a vacuum at 50°C for 8 h. A column (90 × 3 cm) protected from the light was filled with a suspension of the sorbent in hexane. A solution of 1.6 g of the sterol acetates in hexane was charged onto the column and elution was carried out with a stepwise hexane—benzene gradient. The separation was monitored with the aid of GLC. Fractions giving a single peak were concentrated and used for GLC-MS and PMR analysis.

The intermediate and final fractions each giving two and more peaks in GLC were concentrated and analyzed by the GLC-MS method.

Reduction of Fraction (2). A solution of 10 mg of the substance in 1.5 ml of dioxane with treated with a few drops of water and 2.5 mg of KBH_4, and the mixture was stirred at 20°C for 3 h. The course of the reaction was monitored with the aid of TLC. Then the reaction mixture was neutralized with a small amount of 3% HCl, and boric acid was eliminated in the form of methyl borates by adding methanol to the solution in portions and evaporating. The reaction products were freed from the initial compounds by column chromatography on silica L 40/100 μ in the benzene—ethyl acetate (4:1) system. GLC analysis showed the presence of a mixture of two substances. Mass spectrum, m/z:

(XVI): 360 (M^+), 345, 342, 327, 309, 300, 275, 273, 255, 249, 231, 229, 213;

(XVII): 346 (M^+), 331, 328, 313, 295, 273, 261, 255, 235, 213.

Preparation of Chol-5-ene-3β-24-diol (XVI). A solution of 50 mg of desmosterol (XII) in 1 ml of dry pyridine was treated with 48 mg of p-TsCl (mp 68-69°C), and the mixture was left at 4°C. After 40 h, 5 ml of a solution of $NaHCO_3$ cooled to 0°C was added. The resulting precipitate was filtered off, washed with water, and dried in vacuum.

The pulverulent substance obtained was added to a boiling solution of 25 mg of $NaOCOCH_3$ in absolute methanol. The mixture was heated under reflux for 4.5 h and was then evaporated. Column chromatography on silica gel in the benzene—ethyl acetate (7:1) system yielded 25 mg of the methyl ether of i-desmosterol (XVIII). Mass spectrum, m/z: 366 (M^+ − MeOH), 351, 255; 253; 245; 228; 213. PMR spectrum ($CDCl_3$, δ, ppm): 0.71 (3 H, s, C-18), 1.02 (3 H, s, C-19); 0.925 (3 H, d, J = 6.5 Hz, C-21); 1.61 (3 H, s, C-26); 1.69 (3 H, s, C-27); 2.76 (1 H, m, C-6); 3.32 (3 H, s, OCH_3); 5.12 (1 H, m, C-24).

Ozone was passed (at 10-12 μmole/min) through a solution of 10 mg of (XVIII) in a mixture of 2 ml of CH_2Cl_2 and 0.7 ml of MeOH for 50 min. The substance obtained was reduced with KBH_4 as described above.

To remove the i-methyl protection, 2 ml of CH_3COOH and 7 mg of $Zn(OCOCH_3)_2$ was added to the reduced product and the mixture was heated under reflux for 6 h. Then the cooled reaction solution was diluted with water (t = 0°C), and the precipitate was filtered off and washed on the filter with water to neutrality. The resulting product was deacetylated with 3% sodium methanolate in methanol at 20°C for 12 h. Column chromatography on silica gel in the benzene-ethyl acetate (5:1) system yielded 2 mg of chol-5-ene-3β,24-diol (XVI).

Mass spectrum, m/z: 360 (M^+); 345; 342; 327; 309; 300; 275; 273; 255; 249; 231; 229; 213.

PMR spectrum ($CDCl_3$, δ, ppm): 0.68 (3 H, s, C-18), 1.01 (3 H, s, C-19); 0.94 (3 H, d, J = 6.5 Hz, C-21); 3.6 (3 H, m, C-3, C-24); 5.36 (1 H, m, C-6).

Dehydration of Cholesta-5,25-diene-3β,24ξ-diol. A solution of 5 mg of cholesta-5,25-diene-3β,24ξ-diol in 1 ml of dry benzene was treated with 1 mg of p-toluenesulfonic acid. The mixture was heated under reflux with a Dean-Stark trap for 4 h. The substance, which was isolated by column chromatography on silica gel, was hydrogenated over Adams catalyst in ethyl acetate for 8 h. According to GLC and GLC-MS, the resulting product was identical with cholesterol.

Preparation of Cholest-5-ene-3β,24ξ-diol (XXI). I. The hydrogenation of 6 mg of cholesta-5,25-dien-3β,24ξ-diol (XX) was carried out in solution for 2 ml of ethyl acetate over 1.5 mg of Adams catalyst at 20°C for 10 h. This gave cholest-5-ene-3β,24ξ-diol (XXI).

Mass spectrum, m/z: 402 (M^+); 384; 396; 366; 351; 273; 271; 255; 253; 245; 231; 229; 213.

PMR spectrum ($CDCl_3$, δ, ppm) 0.68 (3 H, s, C-18); 1.01 (3 H, s, C-19); 0.93 (9 H, d, J = 6.5 Hz, C-21, -26, -27); 3.31 (1 H, m, C-24); 3.6 (1 H, m, C-3); 5.31 (1 H, m, C-6).

II. A solution of 2 mg of 24-oxocholesterol in 1 ml of aqueous dioxane was treated with KBH_4, and reduction was carried out for 10 h as described above. The resulting product was identified as cholest-5-ene-3β-24ξ-diol (XXI) by a comparison of its mass and PMR spectra with the spectra of the product obtained by method I.

SUMMARY

The steroid fraction of the Far Eastern sponge *Esperiopsis digitata* has been studied. It has been shown that its main components are C-26, C-27, and C-28 mono- and diunsaturated sterols.

Minor steroid derivatives are 3β-hydroxychol-5-en-24-al, 3β-hydroxy-24-norchol-5-en-23-al, and cholesta-5,25-diene-3β,24ξ-diol.

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