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STEROID COMPOUNDS OF MARINE SPONGES.

IV. NEW STEROLS WITH UNUSUAL SIDE CHAINS FROM THE FUNGUS *Halichondria sp*$_1$

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Five new steroid alcohols have been isolated in the form of their acetates from extracts of the sponge *Halichondria sp*$_1$ by column chromatography on silica gel and they have been identified as 24-isopropyl-5α-cholest-22Z-en-3β-ol, 24-isopropylcholesta-5,22Z-dien-3β-ol, 24,24,26,26-tetramethylcholesta-5,25(27)-dien-3β-ol, 24,24,26,26-tetramethylcholesta-5,22E,25(27)trien-3β-ol, and 24-isopropenyl-25-methylcholesta-5,22E-dien-3β-ol. The structures were established by an analysis of spectral characteristics.

Continuing a study of sponge steroids [1] we have isolated five new sterols and have established their structures. The compounds, isolated in the form of their acetates (I)-(V) were the main components of the fraction of free sterols from a Vietnamese collection (Scientific-Research Ship "Professor Bogorov," 1982) of the sponge *Halichondria sp*$_1$. Their structures were established by an analysis of the results of chromato-mass spectrometry and a study of NMR spectra using experiments with differential spin decoupling and the recording of nuclear Overhauser effects (NOEs).

The sterol acetate (I) had a mass spectrum practically coinciding with that of the corresponding spectrum of the known [2] 24-isopropylcholesta-5,22E-dien-3β-ol acetate (VI). The values of the CH_3-18, CH_3-19, and H-6 chemical shifts in the ^{1}H NMR spectra of the two compounds also coincided. At the same time, the chemical shifts and the natures of the multiplicities of the H-22 and H-23 signals in the ^{1}H NMR spectra of (I) and (VI) differed. This gave grounds for assuming that (I) was the 22-cis isomer of the sterol (VI). In actual fact, on catalytic hydrogenation under the same conditions the two compounds gave the same derivative – 24-isopropyl-5α-cholestan-3β-ol acetate (VII). The 22, 23- position of the double bond in (I) was confirmed by experiments with differential spin decoupling (^{1}H NMR). When H-22 or CH_3-21 was irradiated, an H-20 multiplet (2.36 ppm) appeared. Irradiation at 2.36 ppm led to the degeneration of the CH_3-21 doublet into a singlet. Chemical shifts and multiplicities of the H-24, H-25, and H-28 signals in the ^{1}H NMR spectrum were determined similarly (Table 2). An analysis of the spin–spin coupling constants in the ^{1}H NMR spectrum of sterol (I) confirmed the hypothesis of the cis configuration of the 22,23- double bond. In actual fact, $J_{22,23}$ proved to be 11.5 Hz, while for related sterols with a trans-22,23-double bond this constant is usually

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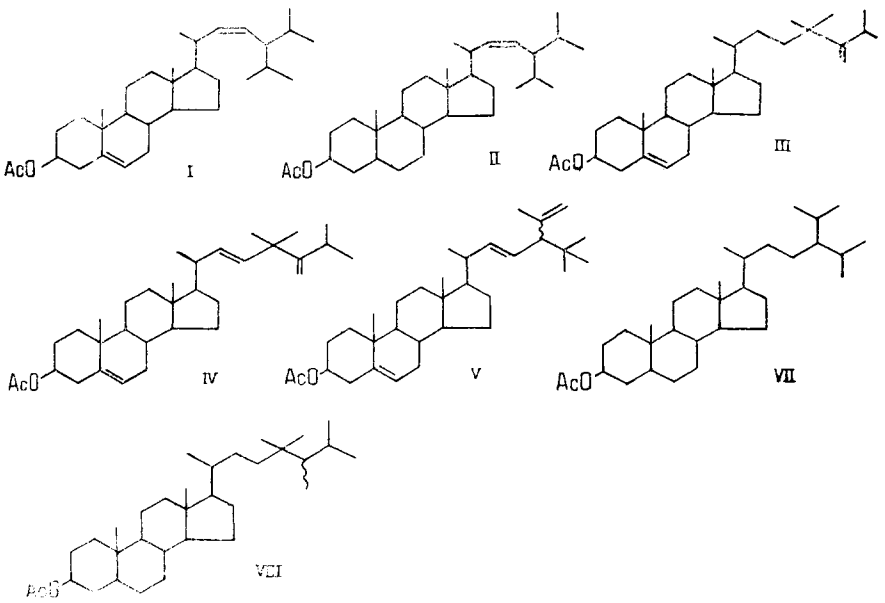
15.1 Hz [3]. In this way, it was established that (I) was 24-isopropylcholesta-5,22Z-dien-3β-ol acetate.



The sterol acetate (II) had characteristic peaks in its mass spectrum at, m/z: 470 (M^+), 344 (cleavage of the C-20–C-21 bond), 257 (M^+ – side chain – CH_3COOH), and 215 (M^+ – side chain – 42 – CH_3COOH). The peak of the molecular ion and the fragmentation characteristic showed that (II) was the acetate of a C_{30} 3β-hydroxysteroid saturated in the steroid nucleus and with a 22,23- double bond in the side chain. The chemical shifts of the CH_3-18 and CH_3-19 signals in the ^1H NMR spectrum of (II) (Table 1) confirmed the hypothesis of the presence of a 5α-cholestane polycyclic system in (II). The closeness of the values of the CH_3-21, CH_3-26, CH_3-27, CH_3-29, H-22, and H-23 chemical shifts in the corresponding spectra of (I) and (II) (Tables 1 and 2) and the value of 11.5 Hz for $J_{22,23}$ showed that the side chains in (II) and (I) were identical.

On the basis of these facts, it was concluded that (II) was 24-isopropyl-5α-cholest-22Z-en-3β-ol acetate.

The sterol acetate (III) was the main component of the fraction of acetates of the free sterols from *Halichondria sp*$_1$ that was studied. In its ^{13}C NMR spectrum the signals of the C-1–C-20 atoms coincided with the corresponding signals for the acetates of 3β-hydroxysteroids of the cholest-5-ene series [4], which indicates the presence in it of a cholestane nucleus with a 5,6- double bond. It follows from the mass and ^{13}C NMR (Table 3) spectra of (III) that this had a 12-carbon side chain. As was shown by experiments with incomplete suppression of interaction with protons, the C-21–C-31 signals in the ^{13}C NMR spectrum of (III) belonged to five CH_3, two –CH_2, and one –CH groups, one quaternary carbon atom, and one unsymmetrically disubstituted double bond.

In the ^1H NMR spectrum doublets at 1.034 ppm (3 H, J = 6.8 Hz) and 1.038 ppm (3 H, J = 6.8 Hz) were assigned to an isopropyl group, and singlets at 1.012 ppm (3 H) and 0.999 ppm (3 H) to a gem-dimethyl group (Table 1). The presence of a 25(27)- double bond in (III) was shown by the presence of signals at 4.8 ppm (1 H, t, H-27) and 4.85 ppm (1 H, d, H-27') in the ^1H NMR spectrum (Table 2) and an intense peak at m/z 310 (McLafferty rearrangement) in the mass spectrum. A distinction between H-27 and H-27' signals was made from the results of measurements of the NOE signals on the irradiation of H-27 (enhancement of the CH_3-28 and CH_3-29 signals) and of H-27' (enhancement of the CH_3-30 and CH_3-31 signals). On the basis of the results obtained, sterol (III) was assigned the structure of 24,24,26,26-tetramethylcholesta-5,25(27)-dien-3β-ol acetate.

The sterol acetate (IV) differed from compound (III) by the presence of an additional double bond [mass spectrum, m/z: 420 (M^+ – CH_3COOH); ^1H NMR spectrum: 5.21 ppm (2 H, m; Table 2)].

The 22,23-position of this double bond was established in the same way as for sterol (I). Its trans configuration followed from the value of the spin–spin coupling constant between the C-22 and C-23 protons ($J_{22,23}$ = 15.7 Hz).

TABLE 2. Chemical Shifts (ppm) and Spin—Spin Coupling Constants (Hz) of the Protons of the Sterol Acetates (I)-(V) in First-Order Approximation

Compound	H	δ	H	δ	H, H	J
I	H-3 2H-4 H-6 H-20	4,60m 2,32m 5,38m 2,36m	H-22 H-23 H-24 H-25 H-28	5,27Add 4,96B dd 1,86 ddd 1,73 m 1,72 m	20,22 22,23 23 24 24,25(28)	10,0 11,5 10,9 6,6
II	H-3 H-20	4,60m 2,32m	H-22 H-23	5,26Add 4,95E dd	20,22 22,23	10,0 11,5
III	H-3 2H-4 H-6	4,60 m 2,32 m 5,38 m	H-26 H-27 H-27′	2,36 m 4,80 t 4,85 d	26,27 27,27′ 26,30(31)	1,1 1,0 6,8
IV	H-3 2H-4 H-6 H-20	4,60m 2,32m 5,38m 2,04m	H-22 H-23 H-26 H-27 H-27′	5,05Bdd 5,24Add 2,28 sept. 4,90t 4,81d	22,23 22,20 23 20 26,27 27,27′	15,7 6,9 1,3 1,0 0,9
V	H-3 2H-4 H-6 H-20	4,60 m 2,38 m 5,38 m 2,08 m	H-22 H-23 H-24 H-29′ H-29	5,21Bdd 5,46Add 2,37d 4,65m 4,78 m	20,22 22,23 23,24 29 29′ 30,29′ 30,29	8,1 15,2 9,4 2,3 0,9 1,3

On catalytic hydrogenation under the same conditions, (IV) and (III) gave one and the same derivative - 24,24,26,26-tetramethyl-5α-cholestan-3β-ol acetate (VIII).

On the basis of the results obtained, compound (IV) was identified as 24,24,26,26-tetramethylcholesta-5,22E,25(27)-trien-3β-ol acetate.

The mass spectrum of the sterol acetate (V) had signals characterizing it as a 3β-acetoxycholest-5-ene derivative with a diunsaturated twelve-carbon side chain. In actual fact, signals were observed in the spectrum at, m/z: 420 ($M^+ - CH_3COOH$), 255 and 213. One of the two double bonds in the side chain must occupy the 22,23- position (intense peak at m/z 282 in the mass spectrum). The presence of an isopropyl fragment in the side chain was shown by signals at 1.732 ppm (3 H, dd), 4.65 ppm (1 H, m), and 4.78 ppm (1 H, m) in the ^{1}H NMR spectrum (Tables 1 and 2). A nine-proton singlet at 0.88 ppm in the ^{1}H NMR spectrum and an intense peak at m/z 364 ($M^+ - CH_3COOH - 56$) in the mass spectrum showed the presence of a tertiary butyl group in (V).

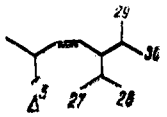
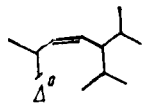
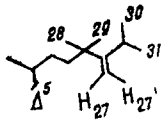
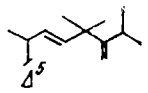
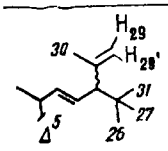
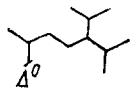
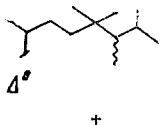
From an analysis of spectral characteristics, it was possible to suggest for (V) the structure of 24-isopropenyl-25-methylcholesta-5,22E-dien-3β-ol acetate, which was confirmed by spin-decoupling and NOE experiments. The results of these experiments permitted assignments to be made for the signals of all the protons in the side chain (Tables 1 and 2).

Thus, the five new sterols isolated from extracts of the sponge *Halichondria sp*, have unusual side chains with additional alkyl substituents as compared with side chains of the zoo- and phytosterols of terrestrial origin. Many unique steroid alcohols have been found previously in sponges [5], but at the same time sterols formed as the result of double biomethylation at C-26 (or C-27) had not been found. The only structural analog of compounds (III) and (IV) is a trisulfated derivative of a polyhydroxysteroid, the so-called sokotrasterol sulfate, which we have recently isolated from one species of sponge [6].

Another rare structural feature for steroids is the cis-22,23-double bond that we have detected in compounds (I) and (II).

Sterols with tertiary butyl groups in the side chains have recently been described by Djerassi et al. [7]. The 22,23-dihydro analog of compound (V) has been isolated from a sponge *Pseudoaxinyssa sp.*

TABLE 1. Methyl Regions of the ^{1}H NMR Spectra of Compounds

Compound	CH_3-18	CH_3-19	CH_3-21	CH_3-26
 I	0.709 s	1,025 s	0,970 d J=6,5 Hz	0,885 d J=6,8 Hz
 II	0,678 s	0 822 s	0,953 d J=6,5Hz	0,882 d J=6,8 Hz
 III	0,662 s	1,012 s	0,902 d J=6,6 Hz	
 IV	0,689 s	1,020 s	1,007 d J=6,5 Hz	
	0,691 s	1,018s	1,030 d J=6,5 Hz	0,880 s
 VII	0,644 s	0,815s	0,924 d J=6,8 Hz	0,821† d J=6,8 Hz
 VIII ‡	0,644	0,817	0,898	

*The signals of the acetate methyl groups in all the compounds appeared at 2.03 ppm.
†Assignment ambiguous.
‡Mixture of the 25R and 25S isomers.

(I)-(V), (VII), and (VIII)*

CH_3-27	CH_3-28	CH_3-29	CH_3-30	CH_3-31
0,794 d J=6,8 Hz		0,820 d J=6,8 Hz	0,904 d J=6,8 Hz	
0,790 d J=6,8 Hz		0,816 d J=6,8 Hz	0,901 d J=6,8 Hz	
	1,012 s	0,999 s	1,038 d J=6,8 Hz	1,034 d J=6,8 Hz
	1,096 s	1,096 s	1,001 d J=6,8 Hz	1,001 d J=6,8 Hz
0,880 s			1,732 dd J=1,3; 0,9 Hz	0,880 s
0,835 †d J=6,8 Hz		0,859 † d J=6,5 Hz	0,859 † d J=6,5 Hz	
0,704 d J=7 Hz 0,71 d J=7 Hz	0,807† s	0,783† s	0,780† d J=6,6 Hz	0,872† d J=6,6 Hz

TABLE 3. ^{13}C NMR Spectrum of Compound (III)

Atom	ppm	Atom	ppm	Atom	ppm	Atom	ppm
C 1	37,1	C 9	50,1	C 17	56,2	C 25	164,1
C 2	27,9	C 10	36,7	C 18	11,9	C 26	28,4*
C 3	74,1	C 11	21,1	C 19	19,4	C 27	106,0
C 4	38,2	C 12	39,8	C 20	36,5	C 28	27,4*
C 5	139,8	C 13	42,5	C 21	18,9	C 29	26,8*
C 6	122,7	C 14	56,8	C 22	30,8	C 30	25,6*
C 7	31,9	C 15	24,3	C 23	37,1	C 31	25,5*
C 8	31,9	C 16	28,2	C 24	39,8		

*Assignment ambiguous.

EXPERIMENTAL

The sponge was collected in May, 1982, in the Vietnam littoral at a depth of 2-5 m during the expedition of the Scientific Research Ship "Professor Bogorov."

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

The chromato-mass spectrometric study was performed on an LKB 9000S spectrometer at an ionizing voltage of 70 V using a 300 × 0.5 cm column with 1.5% of SE-30. The temperature was 265°C and the carrier was helium at the rate of 30 ml/min. ^1H NMR spectra were determined on a Bruker WM-250 instrument and ^{13}C NMR spectra on a Bruker WM-90E instrument in deuterochloroform with tetramethylsilane as internal standard.

Melting points were measured on the stage of Boëtius type, and optical rotations on a Perkin-Elmer 141 polarimeter.

Isolation of the Sterols. The frozen animals (dry weight 245 g) were comminuted and extracted three times with chloroform–methanol (2:1). The extracts were combined and concentrated in vacuum to dryness. Column chromatography on silica gel L (40/100 μm) in the benzene–ethyl acetate (5:1) system led to the isolation of 300 mg (0.123% on the dry weight of the animal) of the total sterol fraction, which was acetylated under the usual conditions.

The separation of the sterol acetates with the aid of chromatography on columns of silica gel impregnated with 20% of $AgNO_3$ was performed by a procedure described previously [8].

The following individual compounds were isolated (in order of their issuance from the column; the relative retention times (RRTs) are given in relation to cholesterol acetate):

24-Isopropyl-5α-cholest-22Z-en-3β-ol acetate (II), 6 mg, RRT 1.67, mp 153-155°C (from EtOAc), $[\alpha]_D^{20}$ –20° (c 0.2; chloroform). Mass spectrum, m/z: 470 (M^+, 13), 427 (16), 367 (100), 344 (24), 315 (43), 273 (22), 257 (89), 255 (27), 229 (8), 215 (19). For the ^1H NMR spectrum, see Tables 1 and 2;

24-Isopropylcholesta-5,22Z-dien-3β-ol acetate (I), 35 mg, RRT 1.64, mp 123-125°C (from EtOAc), $[\alpha]_D^{20}$ – 62° (c 0.5; chloroform). Mass spectrum, m/z: 408 (M^+ – 60, 100), 393 (5), 365 (52), 282 (10), 255 (60), 229 (5), 213 (18). For the ^1H NMR spectrum, see Tables 1 and 2;

24,24,26,26-Tetramethylcholesta-5.25(27)-dien-3β-ol acetate (III), 100 mg, RRT 1.97, mp 139-141°C (from EtOAc), $[\alpha]_D^{20}$ – 35.5° (c 0.5; chloroform). Mass spectrum, m/z: 422 (M^+ – 60.95), 407 (14), 310 (100), 255 (43), 228 (28), 213 (38). For the ^1H NMR spectrum, see Tables 1 and 2. For the ^{13}C NMR spectrum, see Table 3;

24,24,26,26-Tetramethylcholesta-5,22E,25(27)-trien-3β-ol acetate (IV), 40 mg, RRT 1.68, mp 170-172°C (from EtOAc), $[\alpha]_D^{20}$ – 29° (c 0.8; chloroform). Mass spectrum, m/z: 420 (M^+ – 60, 100), 405 (7), 377 (11), 296 (37), 283 (67), 255 (85), 228 (11), 213 (22). For the ^1H NMR spectrum, see Tables 1 and 2;

24-isopropenyl-25-methylcholesta-5,22E-dien-3β-ol acetate (V), 15 mg, RRT 1.65, mp 124-127°C (from MeOH), $[\alpha]_D^{20}$ – 49° (c 0.2; chloroform). Mass spectrum, m/z: 420 (M^+ – 60.6), 364 (45), 313 (51), 282 (100), 255 (58), 253 (76), 228 (6), 213 (21). For the ^1H NMR spectrum, see Tables 1 and 2.

In addition, 100 mg of a mixture of sterols was isolated.

Preparation of 24-Isopropyl-5α-cholestan-3β-ol Acetate (VII). At room temperature, 4 mg of (I) was hydrogenated over Adam's catalyst in ethyl acetate with the addition of 5 μl of $HClO_4$ for 8 h. The solution was neutralized with a small amount of $NaHCO_3$ and filtered, and the filtrate was concentrated in vacuum. This gave 3 mg of 24-isopropyl-5α-cholestan-3β-ol acetate (VII). mp 140-142°C (from EtOAc), $[\alpha]_D^{20}$ +11° (c 0.25; chloroform). Mass spectrum, m/z: 472 (M^+, 31), 457 (5), 412 (28), 397 (21), 369 (5), 275 (31), 257 (13), 230 (23), 215 (100). For the ^1H NMR spectrum, see Table 1.

The same compound was obtained by the hydrogenation under similar conditions of 24-isopropylcholesta-5,22E-dien-3β-ol acetate.

Preparation of 24,24,26,26-Tetramethyl-5α-cholestan-3β-ol Acetate (VIII). The hydrogenation of 4 mg of (III) was carried out in the same way as for (I). This gave 4 mg of 24,24,26,26-tetramethyl-5α-cholestan-3β-ol acetate (VIII). mp 135-137°C (from EtOAc), $[\alpha]_D^{20}$ +12° (c 0.1; chloroform).

Mass spectrum, m/z: 486 (M^+, 2), 426 (28), 411 (20), 355 (100), 315 (30), 298 (22), 285 (22), 275 (15), 257 (59), 215 (70).

The same compound was obtained by the hydrogenation of compound (IV) under the same conditions.

SUMMARY

The composition of the sterol fraction from the sponge *Halichondria sp*. has been investigated. Five new steroid alcohols have been obtained in the form of their acetates and have been identified as 24-isopropyl-5α-cholest-22Z-en-3β-ol, 24-isopropylcholesta-24-isopropyl-5α-ol, 24,24,26,26-tetramethylcholesta-5,25(27)-dien-3β-01, 24,24,26,26-tetramethylcholesta-, -55,22Z-dien-3β- 3β-ol, and 24-isopropenyl-25-methylcholesta-5,22E-dien-3β-ol.

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