SUMMARY

The new isoprenoid cycloasgenin A has been isolated from the roots of the plant *Astragalus taschkendicus* C. Bge.; it has the structure of 6α,11α,16β,25-tetrahydroxy-20(S),24(R)-epoxycycloartan-3-one.

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STEROID ALCOHOLS FROM THE ASCIDIAN *Halocynthia aurantium*

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The total sterols have been isolated from *Halocynthia aurantium* by column chromatography on silica gel. The following steroid alcohols have been identified in it with the aid of GLC, GLC-MS, and ^{1}H NMR: 5α-cholestan-3β-ol, 24ξ-methylcholestan-3β-ol, 24ξ-ethylcholestan-3β-ol, 4ξ-methyl-24ξ-ethyl-5α-cholestan-3β-ol, cholest-5-en-3β-ol, 24ξ-methylcholest-5-en-3β-ol, 24ξ-ethylcholes-5-en-3β-ol, 5α-cholest-22-en-3β-ol, 24-nor-5α-cholest-22-en-3β-ol, cholesta-5,22-dien-3β-ol, 24ξ-methylcholesta-5,22-dien-3β-ol, 24-norcholesta-5,22-dien-3β-ol, 24-ethylcholesta-5,24(28)-dien-3β-ol, and 24-methylcholesta-5,24(28)-dien-3β-ol.

Continuing a study of the steroid alcohols of ascidians (subtype *Tunicata*, class Ascidiae [1], we have investigated the sterols isolated from the Far Eastern species *Halocynthia aurantium*.

The combined sterols were obtained from a chloroform—methanol extract of the animals after column chromatography on silica gel.

To facilitate their subsequent analysis, the sterols were acetylated with acetic anhydride in pyridine (1 : 1), and were then first separated into groups according to degree of unsaturation by chromatography on a column containing silica gel impregnated with silver nitrate [2]. The separation was monitored by gas—liquid chromatography and thin-layer chromatography (plates coated with silica gel impregnated with silver nitrate) [3]. Each of the acetate fractions obtained was studied by the GLC, GLC-MS, and ^{1}H NMR methods. After the

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deacetylation of each fraction and its trimethylsilylation, the GLC—MS analysis was repeated first for the free sterols, and then for the TMS ethers.

The fraction of saturated sterols, consisting of four components, was eluted from the SiO_2—$AgNO_3$ column first. The sterols of this fraction gave molecular peaks in their mass spectra with m/z 388, 402, 416, and 430, respectively and are, consequently, C_{27}, C_{28}, C_{29}, and C_{30} stanols. The first of these components was identified by direct comparison (GLC, GLC—MS) with a standard sample of 5α-cholestan-3β-ol. In its mass spectrum (MS), the main signals were observed at m/z 233 ($M^+ - R - 42$, when R is the side chain), 234 ($M^+ - R - 41$), and 215 ($M^+ - R - 42 - H_2O$) for the free sterol; at 275, 276, and 215 for the acetate; and at 305, 306, and 215 for the TMS ether. These signals correspond to fragmentation with respect to the splitting out of part of ring D and the side chain [4]. Two of the other components of this fraction were identified as 24ξ-methylcholestan-3β-ol (M^+ 402), and 24ξ-ethylcholestan-3β-ol (M^+ 416) on the basis of their MS and GLC behavior (Table 1). The mass spectra of sterols 2 and 3, like that of sterol 1, had peaks with m/z 257, 233, 234, and 215. In the mass spectrum of the fourth component of this fraction (M^+ 430), in addition to signals at 257, 233, 234, and 215 m/z, peaks were observed with m/z 271 ($M^+ - R - H_2O$), 247 ($M^+ - R - 42$), 248 ($M^+ - R - 41$), and 229 ($M^+ - R - 42 - H_2O$), indicating the presence of an additional methyl group in the saturated steroid nucleus. The MS and GLC characteristics of the component agree with literature figures for synthetic 4α-methyl-24ξ-ethylcholestan-3β-ol [5]. This permitted us to assume that the sterol is 4ξ-methyl-24ξ-ethylcholestan-3β-ol.

The following fraction, more polar according to TLC, contained three sterols. The first of them (sterol 5) was the main component of the combined steroids from the ascidian. It was identified by direct comparison [MS, GLC, ^1H NMR (for the acetates)] as cholesterol. Only insignificant amounts of sterols 6 and 7 were present in the mixture. On the bais of MS and GLC behavior and order of elution from the SiO_2—$AgNO_3$ column, they were ascribed the structures of 24ξ-methylcholest-5-en-3β-ol and 24ξ-ethylcholest-5-en-3β-ol, respectively. The main ions corresponding to the splitting off of ring A and part of ring B in the mass spectrum of sterol 6 were observed at m/z 261, 289, and 315, and were shifted in the direction of large masses by 14 m/z as compared with the mass spectrum of sterol 5 (m/z 247, 275, 301), which shows the presence of an additional methyl group in the side chain [6]. In the mass spectrum of sterol 7, the corresponding signals were shifted by another 14 m/z (ethyl group in the side chain), having m/z 275, 303, and 329.

The presence of a 5,6-double bond in all the sterols of this fraction was confirmed by the absence of the peaks of the molecular ions from the mass spectra of the acetates and by the presence of strong signals at $[M - 129]^+$ and at m/z 129 in the mass spectra of the TMS ethers [6].

Then the minor sterol 8, identified as 5α-cholest-22-en-3β-ol, was eluted from the SiO_2—$AgNO_3$ column. The strong peak of the molecular ion at m/z 428 in the mass spectrum of this acetate showed that the compound under investigation was a monosubstituted C_{27} sterol. A signal at m/z 257 ($M^+ - R - H_2O$), which is characteristic for steroid saturated in the nucleus, permitted the assumption that the double bond was present in the side chain. The position of this double bond followed from the presence in the mass spectrum of its acetate of a strong peak with m/z 344, which is characteristic for the cleavage of the C_{20}—C_{22} bond in acetates of Δ^{22}-sterols [7].

The minor sterol 9 (M^+ 372), more strongly sorbed on the SiO_2—$AgNO_3$ column, was a C_{26}-stanol with one double bond in the side chain. As is well known, C_{26} monosaturated derivatives are eluted from the column on argentation chromatography later than their C_{27} analogs [2]. The mass spectra of sterol 9 and its derivatives had strong peaks of ions with m/z 257 characterizing a saturated steroid nucleus and also peaks of ions with m/z 302 (free sterol), 344 (acetate), and 374 (TMS ether) showing the presence of a 22,23-double bond. These facts, and also the retention times of the sterol itself and its derivatives on GLC permitted compound 9 to be identified as 24-norcholest-22-en-3β-ol [8].

The following fraction contained two compounds (sterols 10 and 11), which were identified on the basis of mass spectra, order of elution from the SiO_2—$AgNO_3$ column, and chromatographic characteristics as, respectively, cholesta-5,22-dien-3β-ol (M^+ 384, characteristic ions with m/z 300, 273, 271, 255, 229, and 213 for the free sterol), and 24ξ-methylcholesta-5,22-dien-3β-ol (M^+ 398, characteristic ions with m/z 300, 273, 271, 255, 229, and 213).

Sterol 12 (M^+ 370) had a similar series of ions in the mass spectrum (m/z 300, 273, 271, 255, 213). An intense peak with m/z 97 and the molecular ion showed the presence of a mono-

TABLE 1. Relative Retention Times of the Free Sterols and Their Amounts

Name	Relative retention time		Relative amount, %
	found	accord. to the literature	
1.* 5α-Chlorestan-3β-ol	1,00	1,00 [13]	1,3
2. 24ξ-Methylcholestan-3β-ol	1,33	1,34 [13]	<0,5
3. 24ξ-Ethylcholestan-3β-ol	1,56	1,64 [17]	<0,5
4. 4ξ-Methyl-24ξ-ethyl-5α-cholestan-3β-ol	1,83		<0,5
5. Cholest-5-en-3β-ol	1,00	1,00 [13]	45,5
6. 24ξ-Methylcholest-5-en-3β-ol	1,30	1,30 [13]	0,6
7. 24ξ-Ethylcholest-5-en-3β-ol	1,54	1,58 [14]	1,0
8. 5α-Cholest-22-en-3β-ol	0,94	0,91 [16]	<0,5
9. 24-Nor-5α-cholest-22-en-3β-ol	0,70	0,67 [16]	<0,5
10. Cholesta-5,22-dien-3β-ol	0,94	0,91 [13]	7,4
11. 24ξ-Methylcholesta-5,22-dien-3β-ol	1,11	1,12 [13]	0,6
12. 24-Norcholesta-5,22-dien-3β-ol	0,70	0,67 [16]	5,8
13. 24-Ethylcholesta-5,24(28)-dien-3β-ol	1,59	1,59 [14]	7,3
14. 24-Methylcholesta-5,24(28)-dien-3β-ol	1,23	1,26 [15]	28,0

*The serial number of the compound corresponds to the sequence of elution from the $SiO_2 - AgNO_3$ column.
†Relative to cholesterol.

unsaturated side chain shorter than those of the usual C_{27} sterols. The ^1H NMR spectrum of its acetate had signals at 5.39 ppm (1 H, m, C-6) and 5.25 ppm (2 H, m, C-22, 23), which are characteristic for $\Delta^{5,22}$-sterols and the signals of methyl groups at 0.68 ppm (3H, s, C-18) 0.93 (6H, d, J = 6 Hz, C-26, 27), 0.99 (3H, d, J = 7 Hz, C-21), and 1.02 ppm (3H, s, C-19), of an acetate group at 2.02 ppm, and the signals of proton at C-3, 4.64 ppm, which enabled sterol 12 to be identified as 24-norcholesta-5,22-diene-3β-ol [9].

Sterol 13 (M^+ 412) which was eluted from the column after sterol 12, was identified, by direct comparison with an authentic sample, as fucosterol (GLC, GLC—MS).

Sterol 14 (M^+ 398), one of the main components of the total steroid alcohols of the ascidians, was identified on the basis of the results of mass spectrometry (intense ions with m/z 314, 296, and 286 for the spectra of the free sterol, the acetate, and the TMS ether, respectively) and the ^1H NMR spectrum as 24-methylcholesta-5,24(28)-dien-3β-ol.

The minor sterol 15 could not be identified.

The combined steroids from *H. aurantium* have not been investigated in detail previously. although Kobayashi et al. [10] identified in it one of the unusual minor components — a C_{26} sterol — occelasterol. This sterol was not detected in our fraction. It is obvious that the C_{26} sterols, being exogenous compounds for ascidians, pass into them with their food. The amounts of the individual C_{26} components may vary greatly according to the season and living conditions.

The opinion of the exogenous nature of the C_{26} sterols for marine animals has been expressed previously by Barbier et al. [11]. On the whole, the sterol fraction from *H. aurantium* is close to the corresponding fractions from other ascidians studied previously [12]; cholesterol predominates in it and there are also stanols and sterol alcohols of the Δ^{22}, $\Delta^{5,22}$, and $\Delta^{5,24(28)}$series. On the other hand, monomethylsterols similar to the compound 4 that we have detected have not been identified previously in ascidians.

EXPERIMENTAL

The chromato—mass-spectrometric (GLC—MS) study of the steroid fractions was performed on an LKB-9000S spectrometer at an ionizing energy of 70 eV using a 300 × 0.5 cm column containing 1.5% of SE-30. The column temperature was 260°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 a 150 × 0.3 cm column containing 3% of SE-30 on Chromaton N-AW-HMDS at 280°C. The carrier gas was argon at the rate of 60 ml/min.

The ^{1}H NMR spectra were recorded on a Bruker HX-90E spectrometer in $CDCl_3$ using TMS as internal standard, δ scale.

Isolation of the Sterol Fraction. Freshly-collected biological material (containing water) was comminuted and covered with 5 ml of mixture of chloroform and methanol (2 : 1). After 48 h, the extract was poured off and when the phases had separated, the lower (chloroform) layer was taken off; the upper (aqueous—methanolic) was not used further. The residual tissue was re-extracted with ethyl acetate (4 × 1.5 liters). The extracts were combined and were concentrated in vacuum. The residue was chromatographed on a 22 × 2.5 cm column containing silica gel L (40/100 μ) with elution by chloroform. The fraction collected, which was enriched with sterols and had a polarity similar to that of cholesterol (TLC — fixed layer of KSK silica gel (100-200 mesh), chloroform system), was concentrated and was rechromatographed in an identical column under the same conditions in order to eliminate accompanying pigments. The weight of steroid fraction obtained was 669 mg.

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 an acetonitrile solution of silver nitrate. (The weight of $AgNO_3$ was 20% of the weight of the silica gel.) The sorbent obtained was kept in vacuum for 10 h. The column, protected from the light, was filled with a suspension of the sorbent in low-boiling petroleum ether. A solution of 205 mg of the acetates in the same solvent was deposited on the column and elution was carried out with a smooth gradient: 100 ml of petroleum ether → 100 ml of petroleum ether—benzene (70 : 30). Fractions with a volume of 5 ml were collected. The separation was monitored by TLC on plates with a fixed layer of silica gel L (5/40 μ) impregnated with 20% of silver nitrate [benzene—petroleum ether (2 : 1)]. The spots were observed after treatment with sulfuric acid vapor at 110°C.

Fractions 14, 18, 19, 24-26, and 30, each giving a single spot on TLC, and also the intermediate fractions 15-17 and 27-29, giving two spots each, were concentrated in vacuum and were used for GLC-MS analysis. Fractions 20-23, each giving three spots on TLC, were combined, concentrated in vacuum, and reseparated in an identical column with a smooth gradient: 100 ml of petroleum ether → 100 ml of petroleum ether —benzene (80 : 20). The mixtures of sterols obtained after reseparation, each giving one spot on TLC, were analyzed by the GLC-MS method.

Deacetylation. A solution of 10 mg of the acetates in 1 ml of dry ethanol was treated with 0.1 ml of 3% sodium ethanolate, and the mixture was left at room temperature for 4 h. To eliminate sodium ions, the mixture was stirred with a small amount of Amberlite IR 120 (H^+) until a neutral reaction had been obtained, after which the resin was filtered off and the solution was evaporated.

Silylation. The free sterols (5-7 mg) were dissolved in dry pyridine (0.5 ml). Then 0.05 ml of hexamethyldisilazane and 0.2 ml of chlorotrimethylsilane were added to the solution. The reaction mixture was kept at room temperature for 1 h and was then investigated by the GLC—MS method.

SUMMARY

The composition of the steroid alcohols of the ascidian *H. aurantium* has been studied with the aid of GLC, GLC-MS, ^{1}H NMR, and argentation chromatography.

It has been shown that the fractions studied consisted of a mixture of saturated, monounsaturated, and diunsaturated C_{26}, C_{27}, C_{28}, and C_{29} sterols. The main component was cholesterol. A sterol with an additional methyl group in the steroid nucleus — 4ξ-methyl-24ξ-ethylcholestan-3β-ol — has been identified in ascidians for the first time.

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SULFATED DERIVATIVES FROM MARINE ORGANISMS.

II. SULFATED STEROYL ALCOHOLS FROM THE HOLOTHURIAN *Parathyona* sp. (HOLOTHURIOIDEA, CUCUMARIIDAE)

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Sulfated steroid alcohols from marine invertebrates of the class *Holothurioidea* have been isolated and characterized for the first time.

Continuing a study of the steroid metabolites of holothurians [1, 2], we have isolated the sulfated sterols from the holothurian *Parathyona* sp. and have studied their structures. No such compounds have previously been identified in holothurians.

The fraction of sulfated sterols was obtained after column chromatography of an ethanolic extract of *Parathyona* sp. on silica gel L 40/100 in system 1 [chloroform–methanol–water (60 : 30 : 2)]. The fraction gave a single spot on thin-layer chromatography (fixed layer of silica gel, system 1; R_f 0.47). Its ^{13}C NMR spectrum contained a series of signals in the region from 12.02 to 57 ppm and one signal in the weaker field (78.6 ppm, C-3). The free steroid alcohols have similar spectra, but the C-3 signal appears in them at 71.35 ppm (cholest-5-en-3β-ol). This permitted the assumption that the fraction that we had isolated consisted of a mixture of steroid derivatives having an electronegative substituent, such as a sulfate group, at C-3. In actual fact, under the conditions for the solvolytic cleavage of sulfates [3], the product that we had obtained gave a mixture of free sterols. For their structural identification, the free sterols were first acetylated and the acetyl derivatives were separated on a column containing KSK silica gel impregnated with silver nitrate. The acetates of saturated, monounsaturated, and diunsaturated steroid alcohols so obtained was studied by the chromato–mass-spectrometric method.

The stanol acetate fraction contained three components having M^+ 430, 444, and 458 m/z. Signals with m/z, 275, 276, 257, and 215 in their mass spectra confirmed the presence of a saturated steroid nucleus [4]. The predominating component (23.19%) was identified by its mass and ^1H NMR spectra and its GLC behavior in comparison with a standard sample of 5α-cholestan-3β-ol acetate [5-7]. The other two sterols of this fraction were identified as 24ξ-methyl-5α-cholestan-3β-ol (5.37%); mass spectrum, m/z (%): M^+ 444 (25), 429 (9), 384 (39), 369 (40), 276 (44), 275 (42), 257 (21), 230 (34), 215 (100) and 24ξ-ethyl-5α-cholestan-3β-ol (5.8%) mass spectrum, m/z (%): M^+ 458 (29), 443 (7), 398 (50), 383 (40), 290 (16), 276 (69), 276 (50), 215 (100) [8, 9].

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