α-Ecdysone 2,3,25-Triacetate 22-Sulfate (III). A solution of 70 mg of α-ecdysone 22-sulfate (I) in 2 ml of pyridine was acetylated with 2 ml of acetic anhydride at room temperature for 3 days. Then the reaction mixture was diluted with water and extracted with ethyl acetate. The reaction products were chromatographed on a column of silica gel. Elution of the system with chloroform–methanol (9:1) yielded 20 mg of the triacetate (III), $C_{33}H_{49}O_9SO_3Na$, with mp 152-154°C (benzene–hexane), $[\alpha]_D^{20}$ +3.4 ± 2° (c 0.33; methanol); ν_{max}^{KBr} (cm^{-1}); 3450 (OH); 1755, 1260 (acetate group); 1675 (Δ^7-6-keto grouping); 1230 (sulfate group).

Mass spectrum, m/z (%): 512 (5), 494 (49), 479 (5), 438 (4), 414 (15), 392 (9), 385 (12), 384 (41), 383 (15), 281 (14), 255 (10), 269 (10), 249 (10), 242 (14), 225 (10), 222 (9), 173 (8), 172 (9), 109 (75), 99 (74), 81 (100), 69 (50).

SUMMARY

A new ecdysteroid, α-ecdysone 22-sulfate, has been isolated from the roots of the plant *Silene brahuica* Boiss.

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STEROL SULFATES FROM THE FAR EASTERN HOLOTHURIAN *Cucumaria japonica*

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A chloroform–methanol extract of the musculocutaneous sac of the Far-Eastern holothurian *C. japonica* has yielded a fraction of sterol sulfates (13% of the weight of the extract, 0.8% of the weight of the dry biomass), the main components of which were derivatives of cholest-5-en-3β-ol, 24-methylene-, 24-ethyl-, and 24-ethylidenecholest-5-en-3β-ols, 5α-cholestan-3β-ol, and 24-methyl- and 24-methylene-5α-cholesten-3β-ol; among the minor components were found the sulfates of 24-ethyl-5α-cholestan-3β-ol of cholesta-5,22-dien-3β-ol, of a Δ^5-C_{30} sterol, and also of dienic and trienic C_{26} sterols.

Analysis of a lipid extract of the musculocutaneous sac of the Far-Eastern holothurian *Cucumaria japonica* with the aid of GLC using specific reagents for the detection of the substances showed that the extract included two fractions the components of which contained sterol residues. One of them (fraction A) had an acidic nature and the other (B) was neutral

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and contained carbohydrate residues. Below we describe the isolation and chemical characterization of fraction A.

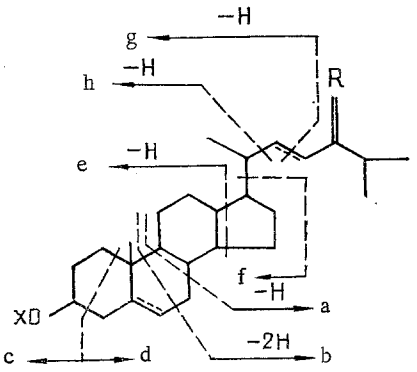
The comminuted and freeze-dried musculocutaneous sac of the holothurian was extracted with mixtures of chloroform and methanol. The extract was subjected to alkaline methanolysis under mild conditions, and the lipophilic part of the methanolysate was chromatographed on a column of DEAE-cellulose. A neutral solvent system of low polarity eluted a mixture of fatty acid methyl esters, cerebrosides, and fraction B. Fraction A was not desorbed from the DEAE-cellulose by neutral solvents and acetic acid, but was eluted only by a system containing aqueous ammonia. This fact indicates that the molecules of the components of fraction A contained a strongly acidic grouping. The fraction A obtained in this way migrated in the form of a homogeneous zone when subjected to TLC on silica gel in neutral, basic, and acidic solvent systems. After isolation it did not change its mobility in TLC and, consequently, its components had undergone no structural transformations whatever during the operations described.

Fraction A made up 13% of the extracted lipids (0.8% of the weight of the dry biomass). From the results of elementary analysis, it contained about 6.4% of sulfur. The action on fraction A of dioxane containing traces of sulfuric acid at 20°C [1] gave a mixture of substances with mobilities in TLC practically identical with that of cholesterol, and no other organic degradation products whatever were detected. When fraction A was treated with chlorotrimethylsilane and hexamethyldisilazane in pyridine at 120°C [2], a mixture of trimethylsilyl derivatives (TMSDs) of sterols was formed. These facts indicate that the fraction under consideration consists of a mixture of acid sulfates of sterols. This was confirmed by the presence in the IR spectrum of the fraction of a strong band of symmetrical O=S=O stretching vibrations at 1195 cm^{-1}. To identify the sterols of which the sulfates were present, fraction A was subjected to the solvolytic desulfation process described above. The free sterols were acetylated and the mixture of acetates was separated into subfractions of saturated, monoenic, and dienic compounds with the aid of chromatography on silica gel impregnated with silver nitrate. The subfractions were isolated by the GLC-mass spectrometric method. To obtain additional information on the structures of the sterols, their TMSDs were anlayzed by the same method.

The subfraction of the acetates of saturated sterols (10% of the combined sterols of fraction A) consisted of two compounds present in a ratio of 2:1. The mass spectra of the major and minor components did not differ with respect to the positions of the main peaks from the spectra of the acetyl derivatives of 5α-cholestan-3β-ol and of 24-methyl-5α-cholestan-3β-ol [3-6], respectively. Furthermore, when the subfraction to which an authentic sample of 5α-cholestan-3β-ol acetate had previously been added was subjected to GLC, the main component was eluted simultaneously with this acetate. On the basis of the facts given, the structures mentioned above were ascribed to the saturated components of fraction A.

The subfraction of acetates of monoenic sterols (70% of the combined sterols of fraction A) consisted of a mixture of mainly three compounds with molecular weights of 428, 422, and 456 in a ratio of 6:1:2. The component with the lowest molecular weight had a mass spectrum and a retention time on GLC identical with those of cholesterol acetate. On the analysis of TMSDs of the monoenic sterols, likewise, the retention time and mass spectra of the low-molecular-weight component and of the TMSD of cholesterol were found to coincide. Consequently, the alcohol corresponding to the acetate weight of 428 was characterized as cholest-5-en-3β-ol. The position of the double bond at $C_{(5)}$ in its molecule was confirmed by the presence in the mass spectrum of the acetate of characteristic peaks with m/z 247 and 260 (ions *a* and b; see scheme) [7] and in the case of the TMSD by peaks at m/z 129 (ion c) and 329 (ion d) [8-10], which were the most intense in the spectrum. The mass spectrum of the second acetate subfraction (Table 1) contained the peak of the M^+ (m/z 442), [M − Me·] (427), [M − AcOH] (382), and [M − Me· −AcOH] (367) ions corresponding to the acetate of a monoenic C_{28} sterol. The mass numbers of the ions e (m/z 275) and of [e − AcOH] (215) showed the saturated nature of the cyclic part of the molecule, and the appearance of the fragments [f − 2H] (m/z 315) and [f − 2H − AcOH] (255) with a comparatively low intensity of the peak of the ion showed that the double bond was present in the aliphatic chain. The peak of ion g with m/z 358 had the maximum intensity. A high intensity of ions of this type is characteristic for the mass spectra of $\Delta^{24(28)}$-sterols [3, 11], and their appearance is a consequence of the cleavage of the $C_{(22)}$—$C_{(23)}$ allyl bond and the migration of the H

atom to the neutral fragment. The peak of ion g (m/z 388) was also one of the strongest in the mass spectrum of the corresponding TMSD. Thus, the compound under discussion had the structure of 24-methylenecholestan-3β-ol acetate. The presence of an exomethylene group in its molecule was confirmed by the IR spectrum of the subfraction, in which strong $\nu_{C=C}$ and ν_{CH} bands (at 1645 and 890 cm^{-1}, respectively) and also a band δ_{CH} at 1412 cm^{-1}, characteristic for the $R'R''C=H_2$ grouping, were observed.



X = Ac or Me_3Si

R = CH_2, MeCH, H_2, Me + H or Et + H

The mass spectrum of the third monoenic acetate contained strong peaks of the ions M^+ (m/z 456) [M − Me·] (441), [M − AcOH] (396), and [M − Me· − AcOH] (381), corresponding to the acetate of a C_{29} monoenic sterol. The peaks of ions f with m/z 315 and [f − AcOH] with m/z 255 showed the saturated nature of the side chain and the position of the double bond in the steroid nucleus. This was also indicated by the peaks of ions e (m/z 273) [e − AcOH] (213). The localization of the double bond at $C_{(5)}$ followed from the presence in the spectrum of peaks of ions of types *a* (m/z 275) and b (288), homologous to fragments *a* and b mentioned above in the mass spectrum of cholesterol; furthermore, in the spectrum of the corresponding TMSD the maximum peak was that of ion c (m/z 129), and the peak third in intensity that of the ion d (m/z 327). In the region of high mass numbers there were the peaks of the ions [M − $C_2H_5^·$] and [M − $C_3H_7^·$] of low intensity but important from the analytical point of view. The appearance of these ions may be connected with the presence on one of the C atoms of the side chain of C_2 and C_3 substituents. On the basis of what has been said above, the third monoenic sterol of fraction A was characterized as 24-ethylcholest-5-en-3β-ol.

The subfraction of dienic sterols consisted of two main components present in a ratio of 2:1. In the mass spectra of the predominating component the mass numbers of the ions M^+ [M − Me·], [M − AcOH], and [M − Me· − AcOH] (m/z 454, 439, 394, and 379, respectively) corresponded to the acetate of a C_{29} dienic sterol. The formation of fragments of the type [f − 2H] with m/z 313 and [f − 2H − AcOH] with m/z 253 in the fragmentation of the molecular ion showed that there was one double bond in the cyclic part of the molecule and one in the side chain. This was also shown by the mass numbers of the ions e and [e − AcOH] − m/z 273 and 213. In the mass spectrum of the corresponding TMSD the peaks of ions c and d (m/z 129 and 325) were among the main ones, from which it followed that the endocyclic double bond was present at $C_{(5)}$. The peak third in intensity in the mass spectrum of acetate was that of a type g ion with m/z 356, and the maximum peak was that of the fragment [g − AcOH] with m/z 296 (see [5]); the origin of the latter from the ion [M − AcOH] was shown by the peak of a metastable ion with m* = 266.8. The system also contained the peaks of metastable ions characterizing the further breakdown of fragment g, which led to the ions [g − Me·] (m/z 341), [g − Me· − AcOH] (281), and [f − 2H] (313). The peak of fragment g (m/z 386) was one of the most intense in the spectrum of the TMSD of the sterol under discussion. As already mentioned, the intensive formation of ions of types g serves as an indication of the presence of a $C_{(24)}$–$C_{(28)}$ double bond in the molecule of a sterol. Therefore the main component of the dienic subfraction was ascribed the structure of 24-ethylidenecholest-5-en-3β-ol acetate.

A minor component of the same subfraction was characterized as 24-methylene-cholest-5-en-3β-ol acetate on the basis of the following characteristics of its mass spectrum. The spectrum contained the peaks of the ions M^+, [M − Me·], [M − AcOH], and [M − Me· − AcOH] at m/z 440, 425, 380, 365, respectively, corresponding to the acetate of a dienic C_{28} sterol.

TABLE 1. Characteristic Ions in the Mass Spectra of the Acetates of Unsaturated Sterols from Fraction A

Type of ion	m/z of the ions (I_{rel}, %) in the mass spectra of the acetates of the sterols (the substituents in the positions of the double bonds in the structure of 3β-acetoxy-5α-cholesterol are shown)			
	Δ^5	24-ethyl-Δ^5	24-ethylidene-Δ^5	24-methylene-Δ^5
M^+	428 (100)	456 (100)	454 (6)	442 (7,5)
M—Me·	413 (17)	441 (19)	439 (6)	427 (10)
M—AcOH	368 (57)	396 (67)	394 (26)	382 (7)
M—Me·—AcOH	353 (16)	381 (17)	379 (7)	367 (7,5)
a	247 (12)	275 (19)		
b	260 (11)	288 (12)		
e	273 (11)	273 (9)	273 (8)	275 (16)
e—AcOH	213 (28)	213 (25)	213 (31)	215 (46)
f	315 (10)	315 (12)		
f—AcOH	255 (55)	255 (52)		
f—2H			313 (98)	315 (42)
f—2H—AcOH			253 (18)	255 (24)
g			356 (56)	358 (100)
g —AcOH			296 (100)	298 (7)
h				

Type of ion	24-methylene-Δ^5	$\Delta^{5,22}$	nor-$\Delta^{5,22}$	nor-$\Delta^{5,22}$, x
M^+	440 (13)	426 (100)	412 (18)	410 (56)
M—Me·	425 (7)	411 (22)	397 (5,5)	395 (17)
M—AcOH	380 (100)	366 (63)	352 (39)	350 (100)
M—Me·—AcOH	365 (1)	351 (31)	337 (6)	335 (18)
a	259 (8)			
b				
e	273 (10)	273 (16)	273 (7)	271 (7)
e—AcOH	213 (40)	213 (42)	213 (18)	211 (27)
f				
f—AcOH				
f—2H	313 (47)	313 (85)	313 (46)	311 (50)
f—2H—AcOH	253 (28)	253 (51)	253 (100)	251 (19)
g	356 (48)			
g —AcOH	296 (12)			
h		342 (20)	342 (31)	340 (15)

The ions of the [f − 2H] and [f − 2H − AcOH] types had m/z 313 and 253, and the fragments of types e and [e − AcOH] had m/z 273 and 213. Fragments of types g (m/z 356), [g − AcOH] (296), and [g − Me· − AcOH] (281) were assigned to the main fragmentary ions. In the mass spectrum of the TMSD of the sterol the peak at m/z 129 was the maximum peak and that of the ion d (m/z 311) was third in intensity.

Thus, the predominating components of fraction A were the acid sulfates of 5α-cholestan-3β-ol, of 24-methyl-, and 24-methylenecholestan-3β-ols, of cholest-5-en-3β-ol, and of 24-methylene-, 24-ethyl-, and 24-ethylidenecholest-5-en-3β-ols.

Among the minor sterol components of fraction A (total content about 3%) we detected 24-ethyl-5α-cholestan-3β-ol, a C_{30} monoenic sterol, cholesta-5,22-dien-3β-ol, and C_{26} dienic and trienic sterols. The mass spectra of the acetate and of the TMSD of the first of them coincided satisfactorily with the spectra of the same derivatives of stigmastanol.

The mass spectrum of the TMSD of the C_{30} sterol contained the peaks of the ions M^+ (m/z 500), [M − Me·] (485), [M − Me_3SiOH] (410), [M − Me· − Me_3SiOH] (395), f (345), [f − Me_3SiOH] (255), c (129), and d (371). The high intensities of the peaks of the last two ions indicated the location of the double bond at $C_{(5)}$, and the mass numbers of the fragments f and [f − Me_3SiOH] led to the conclusion that the side chain of the molecule had the composition $C_{11}H_{23}$. The presence in the spectrum of the ions [M − C_2H_5] and [M − C_5H_{11}] made it possible to suggest that the sterol under consideration had the structure of 24,26,-27-trimethylcholest-5-en-3β-ol.

The mass spectrum of the acetate of the third minor component (see Table 1) showed that it consisted of a C_{27} dienic sterol in the molecule of which one double bond was present in the steroid nucleus and the second one in the side chain. This second bond must have been located at $C_{(22)}$, as was shown by the high intensity in the spectrum of the ion h with m/z

342 formed as the result of the cleavage of the $C_{(20)}$—$C_{(22)}$ bond with the migration of a H atom to the neutral fragment (vinyl decomposition, cf. [3]). The intense peaks of the ions c and d (m/z 129 and 327) permitted the conclusion that the endocyclic double bond was present at $C_{(5)}$.

The last two of the minor components mentioned above were C_{26} sterols the acetates of which had molecular weights of 412 and 410 (see Table 1). As follows from the mass numbers of the ions e, [e — AcOH], [f — 2H], and [f — 2H — AcOH] in the spectra of the acetyl derivatives, both sterols contained a common steroid nucleus, while in one of them (M 412) this part of the molecule was monounsaturated and in the other (M 410) it was diunsaturated; in both compounds the side chain had the composition C_7H_{13}. The presence of the intense peak of the h ion in the spectrum of the acetate of the dienic sterol (M 412), and also the high intensity of the peaks of fragments c and d in the mass spectrum of the corresponding TMSD showed that the double bonds were located at $C_{(5)}$ and $C_{(22)}$. It followed from similar results in relation to the trienic sterol that two of the double bonds in its molecule had the same locations. So far as concerns the third double bond, it was probably present between $C_{(8)}$ and $C_{(14)}$, since the mass spectrum of the acetate of the sterol (M 410) contained the strong peak of an ion with m/z 286 the appearance of which may be connected with the elimination form the molecular ion of the side chain and of two carbon atoms of ring D. Such a fragmentation is characteristic for the molecular ions of Δ^7- and $\Delta^{8(14)}$-steroids, but the localization of the third double bond at $C_{(7)}$ was excluded, since the UV spectrum of the subfraction of dienic sterols where the compound under discussion was detected completely lacked the absorption of a conjugated dienic chromophore. The determination of the structures of the side chains in the $C_{(26)}$ did not appear possible on the basis of the results obtained.

Acid sulfates of sterols have been found in marine invertebrates of various taxonomic groups: in a sea lily [12], in brittlestars [12], in sea urchins [12, 13], in starfish [14, 15], and also in holothurians [16] and, in particular, in *Parathyona* sp. [16], belonging to the Cucumariidae family like the *C. japonica* which we have investigated. The qualitative compositions of the sulfate-forming steroids in the two species largely agree, but there are substantially qualitative differences. Thus, in *Parathyona* sp. about 35% of the total steroids is represented by saturated compounds, including 23.19% of 5α-cholestan-3β-ol; in the analogous fraction of *C. japonica* the amount of the latter was only 6.5%, and the sum of the saturated sterols about 10%. The main type of sterols in the above-described fraction A consisted of monoenic compounds (70% of the sum of the sterols), and cholesterol was predominating (40%). In *Parathyona* sp. cholesterol was again one of the main steroid alcohols of the sulfatide fraction, but it amounted to only 20.6% and the total amount of monoenic steroids to about 32%. A characteristic difference of the sterols of fraction A from *C. japonica* consisted in the absence of Δ^{22}-compounds from among the main components and the presence of a considerable amount of $\Delta^{24(28)}$-sterols: 24-methylenecholestan-3β-ol, 24-methylene, and 24-ethylidenecholest-5-en-3β-ols. In *Parathyona* sp. of sterols of this type only 24-ethylidenecholest-5-en-3β-ol was found, in small traces, while the amount of compounds with a double bond at $C_{(22)}$ exceeded 10%.

The information in the literature on the distribution and biochemical properties of sulfated lipids give grounds for a number of hypotheses concerning the possible biological function of sterol sulfates. For example, it has been established that the concentration of cerebroside sulfates in the tissues of animals depends directly on the intensity of Na^+—K^+ metabolism and the activity of Na^+,K^+-ATPase [17-19]. It is considered that these sulfates act as cofactors of the enzyme [20] and transfer mainly potassium ions to it, since the sulfate group is characterized by a higher affinity precisely for these cations than for Na^+ [21, 22] (in contrast to the phosphate group of membrane phospholipids, which bind Na^+ predominantly [21, 22]). It is also known that the structural lipids of extremely halophilic bacteria living in salt waters contain acid sulfate groups (see, for example [20, 23] and the papers cited in these communications). It is assumed that the high concentration of these lipids in membranes ensures the selective passage of potassium ions into the cell and the antiport of sodium ions under the conditions of an extremely high concentration of the latter in the surrounding medium [20]. Sulfated and sulfonated lipids are widely distributed among unicellular marine organisms. Thus, the diatom-alga *Nitzschia alba* contains considerable amounts of a N-acyl-1-deoxysphingenine-1-sulfonate and a sulfoquinovosyldiglyceride, and also of 24-methylenecholest-5-en-3β-ol sulfate [24], which has been found in *C. japonica*

and other marine invertebrates [12]. Marine phytoflagellates of the genus *Ochromonas* synthesise lipids with two sulfate groups — docosane-1,14-diel disulfates [25].

Cholesterol sulfate is present in many tissues of mammals as an intermediate in the cholesterol metabolism [26], but its level is far lower than that of similar substances in the tissues of marine invertebrates. By analogy with the samples given above it may be assumed that in the cells of marine organisms the sterol acid sulfates fulfill the function of regulators of the cation metabolism under the conditions of a high concentration of inorganic salts in the habitat.

EXPERIMENTAL

Materials and General Methods. The Far Eastern holothurian *C. japonica* was collected by V. A. Vnukov and A. V. Zakharychev in October, 1979, in Peter the Great Bay, Sea of Japan. The internal organs of the animals were separated, the musculocutaneous part was comminuted with the aid of a mincing machine, freeze-dried, and extracted with a mixture of chloroform and methanol by a method described previously [27].

For the isolation of fraction A we used DEAE-cellulose (Reanal, Hungary) previously treated as described by Rouser et al. [28]. TLC was performed on plates with dimensions of 9 × 6 cm prepared by Svetashev and Vaskovsky's method [29]. The substances were revealed on the chromatograms by nonspecific reagents — 50% H_2SO_4 and 5% molybdophosphoric acid solution in 96% ethanol (the plates were heated for 20-25 min at 180-200°C and at 95-100°C, respectively) — and also by specific reagents for steroids — the carbazole reagent [30] and the Lieberman–Burchard reagent [31].

For the GLC of the acetates and the TMSDs of the sterols we used a Perkin-Elmer model 910 chromatograph fitted with a flame-ionization detector and a column (1000 × 3 mm) containing 1% of silicone SE-30 on Gas Chrom Q (75-90 mesh). Temperature regime of the column: 10 min at 200°C, then a linear rise in the temperature (6 deg/min) to 300°C, and 10 min at 300°C; rate of flow of helium 30 ml/min. In the analysis of the sterol derivatives by the GLC-mass-spectrometric method, chromatography was performed under the same conditions. The analysis was carried out on a LKB 9000 chromato-mass spectrometer at an energy of the ionizing electrons of 70 eV and an accelerating voltage of 3.5 kV. IR spectra were obtained on a Perkin-Elmer model 180 spectrograph in KBr.

Isolation of Fraction A. The extracted lipids (3.2 g) were subjected to alkaline methanolysis [27], the lipophilic fraction of the methanolysate was dissolved in 30 ml of $CHCl_3$–MeOH (9:1; here and below the volume ratio of the solvents is given), and the solution was deposited on a column (25 × 3.5 cm) filled with DEAE-cellulose (AcO^- form) in the same solvent mixture. Elution was performed successively with 800 ml of this mixture, 600 ml of $CHCl_3$–MeOH (2:1), 600 ml of MeOH, 800 ml of AcOH, 600 ml of MeOH, and 700 ml of $CHCl_3$–MeOH (2:1) containing 5% of concentrated aqueous ammonia. The eluate obtained on elution with the last solvent mixture was evaporated to dryness and the residue was dried at 30°C/0.02 mm for 8 h, giving 4.6 mg of fraction A in the form of the ammonium salt; R_f 0.3 [$CHCl_3$–MeOH–water (80:20:2)], 0.15 [$CHCl_3$–MeOH–concentrated NH_4OH (85:15:1.5)], 0.7 [$CHCl_3$–MeOH–AcOH–water (80:15:2:1)]. IR spectrum, ν_{max}^{KBr} (cm^{-1}): 3360 (νNH), 3025 (νCH of a vinyl bond), 1645 (νC=C in $R'R''C=CH_2$), 1412 (δCH in $R'R''C=CH_2$), 1380 and 1367 (δCH in CMe_2), 1195 (νSO_2), 890 ($\gamma CHR'R''C=CH_2$).

Sterol Acetates. A solution of 25 mg of fraction A in 5 ml of dioxane was treated with 15 μl of 0.05 N H_2SO_4. The mixture was kept at 20°C for 2 h and was then neutralized with Dowex 2 × 8 (CO_3^{2-} form) and evaporated. This gave 20 mg of a mixture of sterols with R_f 0.6 (benzene–ethyl acetate (3:2)) and 0.7 [$CHCl_3$–hexane–isopropanol (5:5:2)], which was acetylated in the usual way (Ac_2O–Py (1:1), 20°C, 12 h).

Fractionation of the Sterol Acetates. A solution of 20 mg of the sterol acetates in 2 ml of hexane was deposted on a column (17 × 1.8 cm) filled in hexane with silica gel impregnated with 35% of $AgNO_3$ [32]. The column was washed with 80 ml of hexane and then it was eluted with a hexane–ether system with a linearly increasing concentration of ether of from 1 to 25%. The eluate was collected in 5-ml fractions, and there were analyzed with the aid of TLC on silica gel impregnated with 20% of $AgNO_3$ in the hexane–ether (9:1) system. The fractions containing chromatographically homogeneous saturated, monoenic, and dienic compounds, respectively, were grouped and evaporated. Inhomogeneous fractions were reseparated

by the method described above. In total, 2 mg of saturated, 14.5 mg of monoenic, and 3.5 mg of dienic sterol acetates were obtained.

To obtain the TMSDs, aliquots of the subfractions of sterol acetates isolated were subjected to alkaline deacetylation (0.1 M solution of MeONa in MeOH, 20°C, 30 min), and the free sterols were silylated with a mixture of pyridine, hexamethyldisilazane, and chlorotrimethylsilane (4:2:1, 20°C, 2 h).

SUMMARY

A fraction of acid sterol sulfates has been isolated from a lipid extract of the musculocutaneous sac of the Far Eastern holothurian *Cucumaria japonica*, and its main components have been identified as derivatives of cholest-5-en-3β-ol, of 24-methylene-, 24-ethyl-, and 24-ethylidenecholest-5-en-3β-ols, of 5α-cholestan-3β-ol, and of 24-methyl- and 24-methylene-5α-cholestan-3β-ols; among the minor components were detected the sulfates of 24-ethyl-5α-cholestan-3β-ol, of cholesta-5,22-dien-3β-ol, of a Δ^5-monoenic C_{30} sterol, and of $\Delta^{5,22}$-dienic and $\Delta^{5,22,X}$-trienic C_{26} sterols.

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