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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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In the monounsaturated sterol fraction the main component was cholest-5-en-3β-ol acetate (20.65%), the mass spectrum of which coincided with that of a standard sample [10], while in the ^{1}H NMR spectrum there was the signal of an olefinic proton at 5.36 ppm that is typical for Δ^5-sterols [11]. The acetates of 24ξ-methylcholest-5-en-3β-ol (3.1%) and of 24ξ-ethylcholest-5-en-3β-ol (8.44%), were identified from their GLC characteristics and on the basis of the results of a study of mass spectra. Thus, the mass spectrum of the first of them had signals at m/z 382: (M^+-60; 100), 367 (40), 274 (28), 261 (40), 255 (11), 213 (11), and the second signals at m/z: 396 (M^+-60, 100), 381 (30), 288 (27), 275 (27), 255 (36), 229 (7), 228 (7), 215 (14), 213 (26). The mixture of the acetates of the C_{28}-Δ^5 and C_{29}-Δ^5 sterols after hydrogenation gave products shown by GLC to be identical with samples of ergostanol and stigmastanol acetates.

In addition to Δ^5 derivatives, this fraction contained small amounts of 24ξ-ethylcholest-7-en-3β-ol (2.11%), 5α-cholest-22-en-3β-ol (1.07%), and 24ξ-ethylcholest-22-en-3β-ol (2.82%). The main diagnostic peaks in the mass spectrum of the acetate of the Δ^7-sterol were peaks with m/z 456 (M^+) and 288 (detachment of the side chain and part of ring D) [4]. The spectrum had the following signals, m/z (%): 456 (M^+, 100), 441 (27), 396 (68), 381 (46), 315 (17), 288 (17), 273 (21), 255 (100), 229 (15) 213 (82).

Homologs of this compound — 5α-cholest-7-en-3β-ol and 24ξ-methyl-5α-cholest-7-en-3β-ol — were present in trace amounts in the fraction obtained.

In the mass spectra of the acetates of the Δ^{22}-sterols, signals with m/z 315, 257, 255, 215, and 213 confirmed that the double bond was present in the side chain, and a diagnostic peak with m/z 344 showed that its precise position was 22(23) [5, 13-15].

The fractions of diunsaturated sterols consisted of compounds of the $\Delta^{5,22}$ series. The main component of this fraction was the acetate of 24ξ-ethylcholesta-5,22-dien-3β-ol (10.72%); its mass spectrum had m/z (%): 394 (M^+ 60, 100), 379 (23), 351 (59), 323 (10), 296 (10), 282 (46), 255 (100), 253 (100), 228 (45), 213 (57), which coincides with the fragmentation described in the literature for these compounds [4]. After the hydrogenation of the sterol, a product was obtained which was shown by GLC to be identical with stigmastanol. The acetates of cholesta-5,22-dien-3β-ol, of 24ξ-methylcholest-5,22-dien-3β-ol, and of 24ξ-ethylcholesta-24 (28)-3β-ol, were also detected in this fraction, as trace amounts.

Thus, all the main steroid components obtained in the solvolysis of the sterol sulfates from the holothurian *Parathyona* sp. have been identified.

We have detected similar steroid metabolites in holothurians of other families (Stihopodidae and Synaptidae).

EXPERIMENTAL

The GLC—MS analysis of the steroid components was carried out on an LKB-9000S mass spectrometer using a column containing 1% of SE-30 on Chromosorb W, the temperature of the analysis being 270°C and the ionizing energy 70 eV. GLC analysis was performed on a Pye Unicam 104 chromatograph with steel columns containing 2% of SE-30 on Chromosorb W, the temperature of the analysis being 385°C and the carrier gas argon at the rate of 60 ml/min.

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker HX-90 E spectrometer in chloroform solution using tetramethylsilane as internal standard.

The animals were collected in February, 1978, by senior engineer V. V. Kiselev on the island of Monterrey (Republic of Cuba).

Isolation of the Sterol Sulfates and Desulfation of the Fraction Obtained. When the total ethanolic extract of the animals was chromatographed on L40/100 silica gel in the chloroform—methanol—water (60 : 30 : 3) system, the chromatographically homogeneous fraction of sulfated sterols was obtained. Desulfation was performed by a method described previously [3].

Acetylation of the Combined Sterols. The acetylation of 60 mg of the total sterol fraction was carried out with a mixture of 3 ml of dry pyridine and 2 ml of acetic anhydride by the usual method. The combined sterol acetates were separated on silica gel impregnated with 29% of silver nitrate in the hexane—benzene (1 : 1) system.

The hydrogenation of the sterol acetates was carried out with a PtO_2 catalyst in ethyl acetate for 8 h.

SUMMARY

We have isolated sulfated steroid alcohols from holothurians for the first time and have characterized them. It has been shown that these animals contain mainly sulfated saturated and Δ^5-monounsaturated sterols.

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MOLECULAR AND CRYSTAL STRUCTURE OF SOPHORIDINE N-OXIDE

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The spatial structure of natural sophoridine N-oxide has been studied by x-ray structural analysis. It has been shown that when sophoridine is oxidized, rings B and C, which have the boat form, are deformed in the direction of the twist form.

In the investigation of the spatial structures of the molecules of sparteine alkaloids and their N-oxides, it has been shown that the molecules of the alkaloids of this class tend to undergo a conformational transition on oxidation if they are labile or appreciably strained [1]. Sophoridine, which also has a labile molecule [2, 3], can radically change its conformation after oxidation. The results of a study by physicochemical methods of sophoridine N-oxide isolated from plants [4] and the N-oxide obtained by the synthetic oxidation of sophoridine show that the conformations of molecules of these compounds are not identical [5]. In order to elucidate the existence of a conformational transition on the oxidation of sophoridine and to determine the features of the molecules of the two modifications of the N-oxide, we have investigated the three-dimensional structure of natural sophoridine N-oxide and have proposed to perform a similar investigation of the synthetic N-oxide.

Sophoridine N-oxide crystallizes with two molecules (I and II) in the independent part of the elementary cell. The conformations and geometric parameters of molecules I and II are shown in Figs. 1 and 2.

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