GLYCOSIDES OF MARINE INVERTEBRATES.

VIII. STEROID GLYCOSIDES OF THE HOLOTHURIAN Isostichopus badionotus

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The steroid glycoside fraction of Isostichopus badionotus, consisting of a mixture of D-xylosides of steroid alcohols, has been isolated by column chromatography on silica gel. The mixture of aglycones obtained after acid hydrolysis was separated with the aid of argentation chromatography into several fractions. GLC-MS analysis, and also the use of IR and ^{13}C NMR spectroscopy has shown that the steroid glycosides of I. badionotus are xylosides of Δ^0-, Δ^7-, Δ^{22}-, and $\Delta^{7,22}$-, C_{27}-, C_{28}-, and C_{29}-steroids of the cholestane series.

The steroid glycosides of marine invertebrates — holothurians (class Holothuroidea, type Echinodermata) — have been less investigated than their triterpene glycosides. However, their study is important both for establishing the general laws of the pathways of steroid metabolism in the animal organism and for studying the physiological activity. There is contradictory information in the literature concerning the chemical composition of the fractions of steroid glycosides of holothurians. Thus, according to Nomura et al. [1] the holothurian Stichopus japonicus Selenka contains monoxylosides of Δ^5-sterols. In later investigations [2] it was shown that the glycosides of Stichopus japonicus are xylosides of Δ^7-sterols. The present work on the steroid glycosides of the holothurian Isostichopus badionotus confirms the fact that the main components of the fractions of steroid glycosides of holothurians of the family Stichopodidae are sterols of the cholestane and the 24-methyl- and 24-ethylcholestane series and of the Δ^7-, Δ^{22}-, and $\Delta^{7,22}$-cholestanols corresponding to them.

A fraction of the steroid glycosides of the holothurian I. badionotus was isolated by the chromatography of an ethanolic extract of the holothurian on silica gel and consisted of a chromatographically homogeneous product. Its ultraviolet spectrum had no absorption band above 220 nm. In the IR spectrum there was a broad absorption band of hydroxy groups (3400 cm^{-1}). On acid hydrolysis of the glycoside fraction, the main monosaccharide found in the hydrolysate was xylose, which was identical with an authentic sample according to paper chromatography and the results of a comparison of the peracetates of the aldononitriles in the gas—liquid chromatography of the monosaccharide obtained and of D-xylose. The nature of the fragmentation of the aglycone on MS analysis shows that it consisted of a mixture of 3β-hydroxysterols, mainly of the C_{27}, C_{28}, and C_{29} series. Below we give the chemical shifts of the ring carbon atoms in the ^{13}C NMR spectra of the acetates of methyl α- and β-D-xylopyranosides [3] and the chemical shifts of the carbon atoms of the carbohydrate moiety of the acetylated glycoside fraction. Taking into account the influence of the aglycone on the chemical shifts of the C_1 and C_2 atoms [4], these figures show the β-configuration of the glycosidic bond:

Compound	C_1'	C_2'	C_3'	C_4'	C_5'
Methyl α-D-xyloside	97.0	71.1	69.7	69.4	58.2
Methyl β-D-xyloside	101.8	71.0	71.8	69.1	62.1
Carbohydrate moiety	99.4	71.3	71.9	69.1	62.1

The mixture of aglycones obtained on acid hydrolysis of the glycoside fraction, after acetylation, was separated on a column of silica gel impregnated with 20% of $AgNO_3$. Argen-

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tation chromatography enabled us to obtain several fractions of the acetates which corresponded to the acetates of saturated, monounsaturated, and diunsaturated C_{27}, C_{28}, and C_{29} sterols. The compositions of these fractions were determined with the aid of GLC-MS analysis.

The fractions of the saturated sterols consisted of three components, the acetates of which had molecular peaks with m/e 430, 444, and 458. In the nature of its fragmentation, the acetate of the first sterol corresponded to the acetate of 5α-cholestan-3β-ol [5]. Its mass spectrum contained ions with m/e (%): M^+ 430, 415 (6), 370 (30), 355 (25), 276 (30), 275 (30), 257 (6), 230 (30), 217 (30), 216 (45), 215 (100). The acetates of two other sterols had similar ions, and these were assigned to 24-methyl-5α-cholestan-3β-ol and 24-ethyl-5α-cholestan-3β-ol [6, 7]. The presence of a saturated steroid nucleus in the acetate of the aglycone was confirmed by the fact — in addition to the results of mass spectrometry — that in the ^{13}C NMR spectrum of the acetate of the aglycone there was the signal of a quaternary carbon atom at 35.5 ppm, corresponding to the C-10 signal in 5α-cholestanol [8].

The acetate of the fourth sterol had a molecular ion with m/e 428 corresponding to a monounsaturated C_{27}-sterol. The presence in its mass spectrum of a strong peak with m/e 257 (100%) characterizes a saturated steroid nucleus and indicates that the double bond is present in the side chain. A strong peak with m/e 344, which is present in the mass spectra of all Δ^{22}-sterols [5, 9-11], characterizes the cleavage of the C_{20}—C_{22} bond. Thus, this sterol is 5α-cholest-22-en-3β-ol. The mass spectrum of its acetate has peaks with m/e (%): 128 (26), 368 (15), 344 (53), 315 (34), 257 (100), 215 (26). The fraction of monounsaturated sterols also contained 24-methyl- and 24-ethyl-22-en-3β-ols, which were identified in the same way as for the sterol considered. In the ^{13}C NMR spectrum of the acetate of the mixture of aglycones and the acetate of the glycoside there were signals of methine carbon atoms at 136.1 and 131.8 ppm, the appearance of which is connected with the presence of a 21,24,25,25-tetramethylpent-22-enyl radical in the side chain, and signals at 138.1 and 125.1 ppm corresponding to the structure of a 21,25,25-trimethylpent-22-enyl radical [8]. In the ^{13}C NMR spectrum of the acetate of the aglycone and of the acetate of the glycoside in the region of signals of the carbon atoms with double bonds there are the signals of a quaternary carbon atom at 139.5 ppm and of a methine carbon atom at 117.3 ppm corresponding to the presence of a double bond in the steroid nucleus in the 7,8 position [12]. In addition, the mass spectra of the free sterols contain ions with m/e 271 (M^+ − s.c. − 2H) and 246 that are characteristic for Δ^7-sterols [13]. From the fraction of monounsaturated sterols with a double bond in the 7,8 position we isolated in the pure form a sterol, the mass spectrum of the acetate of which had M^+ 456 (23.4%), m/e (%): 441 (10.4), 396 (8.34), 255 (49.8), 229 (19.4), 213 (22). This sterol corresponds to 24-ethyl-5α-cholest-7-en-3β-ol [2].

The fraction of diunsaturated sterols with a dienic grouping contained compounds with molecular weights of 384, 398, and 412. The fragmentation of the acetates of the sterols was identical with the decomposition of 5α-cholesta-7,22-dien-3β-ol, 24-methyl-5α-cholesta-7,22-dien-3β-ol, and 24-ethyl-5α-cholesta-7,22-dien-3β-ol. The mass spectra of their acetates contained ions with m/e 342, 313, which are characteristic for this type of compound [9, 13, 14].

Of the minor components present in the mixture of aglycones, 24-methylene-5α-cholestan-3β-ol was characterized. The mass spectrum of its acetate contained ions with m/e (%): 422 (5.1), 427 (8.1), 358 (100), 343 (18), 315 (42), 255 (30), 229 (25), 215 (50). The presence of a strong peak with m/e 358 characterizing the decomposition of $\Delta^{24(28)}$-sterols by the McLafferty mechanism [5, 9] is evidence in favor of this structure. We also isolated a sterol with a molecular weight of 398 the mass spectral fragmentation of which was identical with that of 24-methylenecholest-7-en-3β-ol [14, 15]. Mass spectrum of its acetate: M^+ 440, m/e (%): 425 (15), 380 (7), 356 (30), 313 (100), 255 (31), 213 (29).

EXPERIMENTAL

GLC-MS analysis was performed on an LKB-9000 spectrometer. IR spectra were recorded on a JR-75 spectrophotometer. ^{13}C NMR spectra were obtained on a Brüker HX-90E spectrometer. The gas—liquid chromatography of the mixture of aglycones and of their acetates was performed on a Pye Unicam 104 chromatograph using glass columns containing SE-30 (2%) as stationary phase. The temperature of chromatography was 185°C.

The total ethanolic extract of the holothurian Isostichopus badionotus (100 mg) was separated on a column of SiO_2 in the chloroform–methanol–water (90:10:0.5) system. This gave 15 mg of a chromatographically homogeneous fraction of steroid glycosides. The fraction recrystallized from methanol had the following composition (%): C 71.94; H 10.31, mp 214°C $[\alpha]_D^{20}$ −10.7° (c 0.3; $CHCl_3$–EtOH (1:1)).

Acid Hydrolysis. The glycoside fraction was hydrolyzed with 12% HCl at 70°C for 2 h. The mixture of aglycones was obtained in the usual way.

Argentation chromatography of the mixture of acetates of aglycones was carried out on a column of SiO_2 impregnated with 20% of $AgNO_3$ in a gradient hexane–benzene system (10% → 100%). The column had dimensions of 20 × 1 cm and the support was Wöelm 1 silica gel.

The holothurian Isostichopus badionotus was collected by V. V. Kiselev in the island of Cuba. The mass spectra were recorded by O. F. Vereshchagin.

SUMMARY

It has been shown that the fraction of steroid glycosides of the holothurian Isostichopus badionotus consists of a mixture of the β-xylosides of Δ^0-, Δ^7-, Δ^{22}-, and $\Delta^{7,22}$-, C_{27}-, C_{28}-, and C_{29}-sterols of the cholestane series. Minor components of this mixture are glycosides the aglycones of which belong to the $\Delta^{24(28)}$- and $\Delta^{7,24(28)}$-sterols.

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