GLYCOSIDES OF MARINE INVERTEBRATES.

XXVI. HOLOTHURIN A FROM THE PACIFIC OCEAN HOLOTHURIAN *Holothuria squamifera*.

ISOLATION OF THE NATIVE AGLYCONE

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The known triterpene tetraoside holothurin A has been isolated from the Pacific Ocean holothurian *Holothuria squamifera*. By using two independent methods — enzymatic cleavage and two-stage Smith degradation — 22,25-epoxyholost-9(11)-ene-3β,12α,17α-triol, $C_{30}H_{46}O_6$, which is the native aglycone of holothurin A, was obtained. The structure of the native aglycone has been established on the basis of the results of IR, mass, and PMR spectroscopy.

Continuing a study of the glycosides of marine invertebrates [1] we have obtained a glycoside fraction from the Pacific Ocean holothurian *Holothuria squamifera* (Aspidochirota, Holothuriidae) after the chromatography of a butanol extract on Polikhrom-1. According to the ^{13}C NMR spectra, this fraction consisted of the sum of the sulfated triterpene tetraosides with carbohydrates chains of identical structure but with different aglycones. By reversed-phase chromatography on Polikhrom-1 we succeeded in isolating the main component of the fraction, which was found to be identical with the previously known holothurin A isolated by Kitagawa et al. from the holothurian *Holothuria leucospilota* [2, 3]. In actual fact, on acid hydrolysis the glycoside obtained gave the artifactual genin 22,25-epoxyholosta-7,9(11)-diene-3β,17α-diol (2) [4], sulfuric acid, and the monosaccharides D-glucose, D-xylose, D-quinovose, and 3-O-methyl-D-glucose, and the characteristic signals in its ^{13}C NMR spectra coincided with those given in the literature for (1) [3].

It is known that the native aglycone of holothurin A is extremely labile under the conditions of cleaving glycosidic bonds, and attempts to obtain it by mild acid hydrolysis and enzymatic hydrolysis have been unsuccessful [4, 6].

By using two independent methods — enzymatic cleavage of the glycosidic bonds and two-stage Smith degradation [7] — we obtained 22,25-epoxyholost-9(11)-ene-3β,12α,17α-triol (3) having the same structure as the aglycone of the glycoside (1). Consequently, compound (3) is the native aglycone of holothurin A.

Thus, the incubation of (1) with the combined glycosidases from the intestine of Far Eastern snail *Eulora maackii* [8, 9] for four days in aqueous solutions led to the aglycone (3) and to a program (4) which was identified by ^{13}C and ^{1}H NMR spectroscopy and from the results of acid hydrolysis as the β-D-xyloside of the aglycone (3).

We obtained compound (3) after the periodate oxidation of (4) with the subsequent Smith cleavage of the product.

In its mass spectrum 22,25-holost-9(11)-ene-3β,12α,17α-triol (3), mp 274-275°C, had characteristic peaks with m/z 502 (M^+), 484 ($M^+ - H_2O$), 451 ($M^+ - 2H_2O - CH_3$), 283 and 99 (cleavage of the C-20–C-22 bond). Its PMR spectrum confirmed the presence of a 12α-hydroxy-9(11)-ene fragments. In actual fact, in addition to the signals of the protons of seven methyl groups and of the protons at C-3 (3.25 ppm, m) and at C-22 (4.2 ppm, t), the spectrum of (3) has a doublet signal of C-12 H at 4.57 ppm and the signal of the C-11 vinyl proton at 5.38 ppm. The spin-spin coupling constants of the protons $J_{11,12}$ = 5.75 Hz showed the α-OH configuration at C-12.

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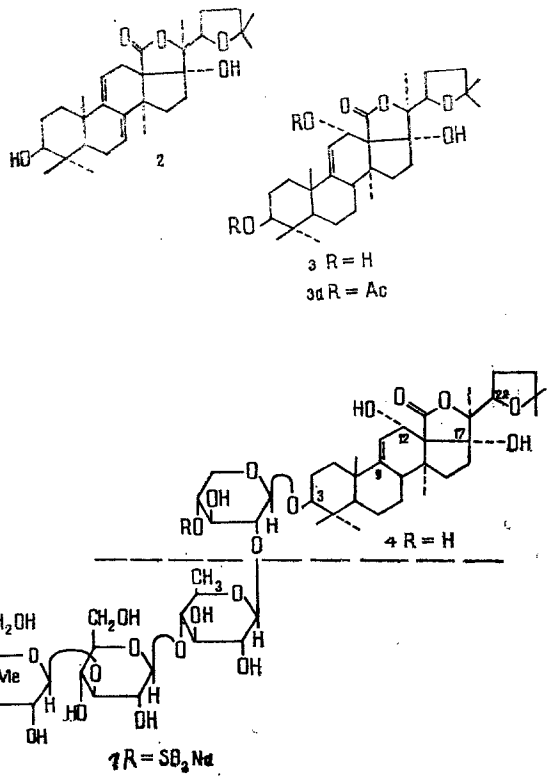

The acetylation of (3) under the usual conditions led to a diacetate (3a) in the IR spectrum of which the weak absorption of a tertiary hydroxy group (3525 cm^{-1}), obviously relating to the C-17—OH fragment, was observed. The mass spectrum of (3a) had signals with m/z 526 and 367 showing the successive elimination of two acetic acid molecules and the side chain in the fragmentation of the molecular ion.

EXPERIMENTAL

Spectral Analyses. Mass spectra were taken on a LKB-9000 chromatometer with direct introduction of the sample. The ionization energy was 20 eV. The ^{13}C and ^{1}H NMR spectra were taken on a Bruker HX-90E instrument.

Isolation of Holothurin A. The holothurians were collected on the Great Barrier Reef (Australia) during the voyage of the Scientific-Research Ship "Professor Bogorov" in 1980.

The comminuted animals were extracted with ethanol. The extract was evaporated, the residue was dissolved in water, and the glycosides were extracted with n-butanol, after which the butanol was evaporated off. The combined holothurians (500 mg) were separated on Polikhrom-1 (0.25-0.5 mm) in a LKB 2137 chromatographic column (30 × 650 mm). Holothurin A (62 mg) was isolated in the water—ethanol (100:25) system: mp 230-231°C, $[\alpha]_D^{20}$ −14° (c 0.2; H_2O); UV — transparent above 210 nm.

^{13}C NMR spectrum (C_5D_5N, δ, ppm): 35.5 (C-1); 27.4 (C-2); 88.7 (C-3); 40.0 (C-4); 52.9 (C-5); 20.2 (C-6); 28.2 (C-7); 41.0 (C-9); 153.5 (C-9); 39.7 (C-10); 115.5 (C-11); 71.9 (C-12); 59.0 (C-13); 46.0 (C-14); 38.6 (C-15); 27.2 (C-16); 89.9 (C-17); 174 (C-18); 19.0 (C-19); 86.5 (C-20); 22.5 (C-21); 80.7 (C-22); 36.6 (C-23); 28.7 (C-24); 81.3 (C-25); 28.2 (C-27); 24.5 (C-28); 27.4 (C-29); 16.7 (C-30); 105.7 (C'-1); 83.1 (C'-2); 76.7 (C'-3); 74.9 (C'-4); 63.8 (C'-5); 105.1 (C"-1); 76.0 (C"-2); 76.7 (C"-3); 86.6 (C"-4); 71.78 (C"-5); 18.2 (C"-6); 105.0 (C'"-1); 73.8 (C'"-2); 88.0 (C'"-3); 70.8 (C'"-4); 77.5 (C'"-5); 62.2 (C'"-6); 104.5 (C""-1); 74.9 (C""-2); 87.7 (C""-3); 69.8 (C""-4); 78.0 (C""-5); 61.8 (C""-6); 60.5 (C""-OMe).

Acid Hydrolysis of Holothurin A. A mixture of 20 mg of (1) and 2 ml of 12% HCl was boiled for 8 h. The precipitate of aglycone (2) was filtered off, washed with water, and dried. It gave 8 mg of (2) with mp 303-305°C. MS, m/z: 484, 451, 397, 99. The hydrolysate was neutralized with Dowex (HCO_3^-), and the filtrate was evaporated. This gave the combined

monosaccharides, $[\alpha]_D^{18}$ +33°. Glucose, 3-O-methylglucose, quinovose, and xylose (1:1:1:1) were identified in the form of the acetates of the corresponding aldononitriles by GLC-MS.

Partial Hydrolysis of (1) with Snail Glycosidases. Preparation of (3) and (4). A solution of 100 mg of holothurin A in water was treated with 5 mg of the intestinal contents of the snail *Eulota maackii*, and the mixture was incubated in a thermostat at 38°C for 24 h. Analysis by TLC in the chloroform–methanol–water (75:25:1) system showed the presence of four progenins. Another 1 ml of the enzyme preparation was added daily for 4 days. Then the reaction mixture was diluted with water and extracted with n-butanol, the extract was filtered through a layer of silica gel, and the filtrate was evaporated. This yielded 70 mg of the combined products, which were separated by column chromatography on silica gel, giving 5 mg of genin (3) and 30 mg of progenin (4). The genin (3) (3 mg) was acetylated in the usual way, and 3.5 g of the diacetate (3a) was obtained.

Acid Hydrolysis of (4). Progenin (4) (10 mg) was hydrolyzed with 12% HCl for 8 h. The precipitate of aglycone (2) was filtered off, the acid was neutralized with anion-exchange resin, the water was evaporated off, and TLC analysis showed the presence of D-xylose.

Smith Degradation of (4). A solution of 20 mg of (4) in ethanol was treated with 200 mg of $NaIO_4$ in water (20 ml), and the mixture was left at room temperature for 48 h. Then ethanol was added until a precipitate had formed. This was filtered off, and the aqueous ethanolic solution was evaporated. The residue was dissolved in 50% ethanol, 20 mg of $NaBH_4$ was added, and the mixture was left for 2 h. The excess of Na ions was eliminated with a cation-exchange resin, the boric acid was distilled off with methanol, and the solution was concentrated in vacuum. The residue was treated with 0.5% HCl and the mixture was left to stand until a precipitate had deposited. This was separated off on a filter and dried. In this way, 10 mg of combined aglycones was obtained. Column chromatography isolated the predominating aglycones (2) (3 mg) and (3) (5 mg).

Aglycone (3), mp 274-275°C, $[\alpha]_D^{20}$ +1.5° (c 0.5; $CHCl_3$). IR ($CHCl_3$, cm^{-1}): 3640 (br., OH); 1755 (γ-lactone); 1640 (C=C). MS, m/z: 502 (M^+), 484, 451, 283, 99 (100%). PMR ($CDCl_3$, ppm): 0.84; 1.05; 1.16; 1.25; 1.27; 1.29; 1.33 (c, 7CH_3); 2.75 (C-8, m, 1H); 3.25 (C-3, m, 1 H); 4.28 (C-22, t, 1 H); 4.62 (C-12, d, 1 H); 5.38 (C-1, q, 1 H, $J_{11,12}$ = 5.75 Hz. The diacetate (3a), mp 218-220°C, $[\alpha]_D^{20}$ +48° (c 0.25; $CHCl_3$). MS, m/z: 526 ($M^+ - 60$); 466 ($M^+ - 2CH_3COOH$); 367 ($M^+ - 120 - 99$); 341 ($M^+ - 2CH_3COOH - CH_3 - 99 - 71$), 281, 99 (100%). PMR ($CDCl_3$, δ, ppm): 0.82, 0.86; 1.05; 1.16; 1.18; 1.22; 1.33; (s, 7CH_3); 2.00; 2.01 ($OCOCH_3$, 2s); 4.12 (C = 22, t, 1 H); 4.25 (C-3, m, 1 H); 5.24 (C-12, d, 1 H), 5.72 (C-11, q, 1 H).

SUMMARY

It has been shown that the main glycoside of the Pacific Ocean holothurian *Holothuria squamifera* is holothurin A. By an enzymatic method the native aglycone of holothurin A has been obtained, and this has the structure of 22,25-epoxyholst-9(11)-ene-3β,12α,17α-triol.

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