GLYCOSIDES OF MARINE INVERTEBRATES.

XIII. NEW HOLOTHURINOGENINS OF HOLOTHURIN B_1

FROM *Holothuria floridana*

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We have isolated for the first time the native aglycone of a triterpene glycoside of the holoturin series — holothurin B_1 — and have established its structure as holost-9-ene-3β,12α,17α-triol. The structures of two new holostane derivatives have been established — holosta-8,11-diene-3β,17α-diol and 3β,17α-dihydroxyholost-9-en-12-one. A scheme of transformation of the native genin of holothurin B_1 under the conditions of the acid splitting of the glycoside has been put forward.

Continuing investigations on the structures of glycosides of marine invertebrates [1], we have isolated a new triterpene bioside from an ethanolic extract of the holothurian *Holothuria floridana*. Like the holothurin B from *H. leucospilota* [2] and *H. atra* [1] that has been described in detail previously, holothurin B_1 (1) contains D-quinovose and D-xylose residues and a sulfate group. However, these glycosides differ in the structure of their aglycones.

In the present paper we consider the structure of the native aglycone (2) of holothurin B_1 (1), which we have called floridanogenin I, and the holothurinogenins (3-5) obtained in the transformation of genin 2 under the conditions of the acid cleavage of the glycoside (Fig. 1).

The acid hydrolysis of (1) led to a mixture of holothurinogenins (2-5), which were separated by column chromatography on silica gel. Of the aglycones isolated, only (4) was known previously, having been identified by Habermehl [3] in hydrolysates of the glycoside fraction from *H. polii*.

The aglycone (4) that we obtained coincided in its constants and its UV, mass, and ^1H and ^{13}C NMR spectra (Tables 1 and 2) with holosta-7,9-diene-3β,17α-diol [3-5]. As compared with the genin (4), holothurinogenin (2) contains an additional secondary hydroxy group (^1H NMR spectrum: 4.61 ppm, doublet, 1 H), and only one double bond (^1H NMR spectrum: 5.62 ppm, doublet, 1 H, pyridine). Such a position of the signal of a vinyl proton in a ^1H NMR spectrum is characteristic for a 9(11)-enol fragment in holostane derivatives having a hydroxy group at C-12 [2]. There is a similar signal in the ^1H NMR spectrum of the glycoside (1), and its ^{13}C NMR spectrum confirms the presence of a 12-hydroxy group and a 9(11)-double bond (^{13}C NMR spectrum: C_9 153.9 ppm; C_{11} 115.5 ppm; C_{12} 71.5 ppm [6], see Tables 1 and 2). The spin—spin coupling constant determined in the ^1H NMR spectrum of floridanogenin I showed the α orientation of the hydroxy group in position 12 ($J_{11,12}$ = 5.5 Hz) [7]. Earlier, compounds with a 12α-hydroxy-9(11)-enol fragment were postulated as the native genins of the holothurins [8, 9], but it had not been possible to isolate them from hydrolysates of the glycosides because of their small amount in the mixture and the ease with which they undergo transformation into 12β-hydroxy derivatives or 7,9(11)-dienes. The holothurinogenin which we have described is the first native genin of the holothurins that it has been possible to obtain in the individual state. When the aglycone (2) was treated with chloroform saturated with hydrogen chloride, the aglycone (4) was formed.

The UV spectrum of the holothurinogenin (3) has three absorption maxima, at 234, 244, and 309 nm. The ^{13}C NMR spectrum of the aglycone (see Table 1) contains four signals of

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TABLE 1. Features of ^{13}C NMR Spectra of Compounds (1), (4), and (3) (C_5D_5N, ppm, TMS — 0)

C atom	Compound			C atom	Compound		
	1	4	3		1	4	3
C-1	36,6	36,65	36,5	C-16	36,6[a]	34,1	36,5
C-2	27,0	28,0[b]	27,3[b]	C-17	89,8	86,1	83,8
C-3	88,6	78,2	78,1	C-18	174,9	175,85	175,8
C-4	40,0	39,4	39,4	C-19	22,5	23,2	21,25[a]
C-5	52,7	50,2	50,95	C-20	87,2	86,2	86,2
C-6	21,2	23,5	18,5	C-21	23,0	23,2	23,2
C-7	28,0	120,0	27,9[b]	C-22	38,9	39,1	38,5
C-8	40,9	142,2	137,8	C-23	22,3	22,4	22,7
C-9	153,9	147,9	136,5	C-24	39,7	39,7	39,7
C-10	39,7	38,1	38,5	C-25	28,0	28,7[b]	28,7[b]
C-11	115,5	112,7	124,5	C-26	22,6	22,7	22,7
C-12	71,5	29,1	129,2	C-27	22,6	22,7	22,7
C-13	58,6	57,8	63,0	C-30	16,7	16,7	16,2
C-14	46,4	49,1	49,6	C-31	28,0	28,9	28,7
C-15	36,0[a]	45,9[a]	36,5	C-32	20,1	25,3	21,71[a]

a, b — assignment of the signals ambiguous.

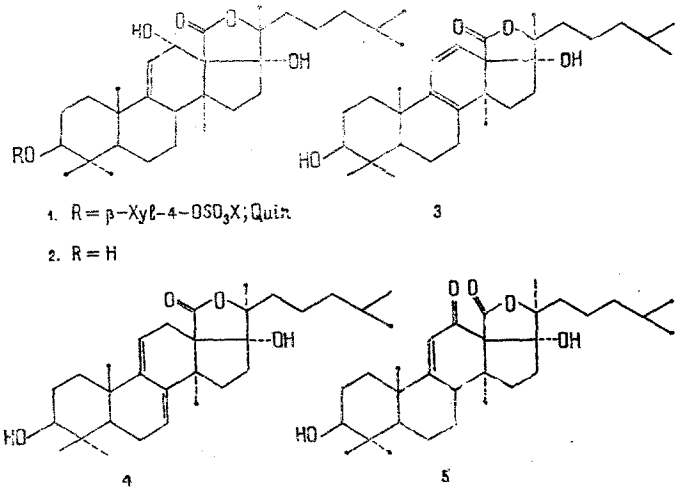

Fig. 1. Chemical formulas of the holothurinogenins of holothurin B_1 from *H. floridana*.

carbon atoms at double bonds (ppm): 137.8 (singlet), 136.5 (singlet), 129.3 (doublet), and 124.5 (doublet). In the ^1H NMR spectrum of compound (3) (see Table 2), a characteristic AB quartet of olefinic protons belonging to a single double bond [10] appears at δ = 6.04 ppm ($\delta_A - \delta_B$ = 0.36, J_{AB} = 9.8 Hz, 2 H). Consequently, the second double bond must be tetrasubstituted. After compound (3) had been treated with chloroform saturated with hydrogen chloride, the aglycone (4) was formed. This shows that the aglycone (3) has the same holostane skeleton with 3β- and 17α-hydroxy groups. For holostane derivatives, a diene system with one tetrasubstituted double bond can be located in the 8,11 or the 6,8 position. The formula with an 8,11-diene fragment is preferable for this holothurinogenin, since otherwise we should observe H_5-H_7 spin–spin coupling in the ^1H NMR spectrum of the aglycone. Moreover, in the ^{13}C NMR spectrum of (3), the C_6 signal is located at 18.5 ppm, which is characteristic for 8(9)-lanostene derivatives [11]. Such a signal would be absent in the case of the alternative $\Delta^{6,8}$ compound. The noncorrespondence between the maximum absorption in the UV spectrum of (3) with that calculated for $\Delta^{8,11}$-dienes (λ_{max} 275 nm) [4] is obviously the result of the hyperconjugation of the carbonyl group of the lactone with the diene chromophore in the aglycone. In actual fact, after the reduction of the holothurinogenin (3) with lithium tetrahydroaluminate [12] a compound was obtained which had the spectrum of an AB quartet of a —CH=CH— group in the ^1H NMR spectrum, but had λ_{max} 276 nm. Thus, the structure of hollosta-8,11-diene-3β,17α-diol has been established for this holothurinogenin. The isolation

TABLE 2. Features of the ^1H NMR Spectra of Compounds (1-5)

Compound	Solvent	CH_3 group					3-H	11-H	12-H
		4,4'	10	14	21	26,27			
1	C_5D_5N	1,28/1,13	1,39	1,68	1,77	0,86, doublet J=6,1 Hz	4,35 m	5,62, m	4,73, d
2	"	1,24/1,09	1,39	1,66	1,75	0,86, doublet J=6,1 Hz	3,37 m	5,62 m J_{11-12}=5,5 Hz	4,61, d
2	$CDCl_3$	1,01/0,85	1,16	1,31	1,53	0,88, d J=6,2 Hz	3,19 m	5,38 q J_{11-12}=5,5 Hz	
3	"	1,02/0,83	1,24	1,07	1,46	0,88, d J=6,2 Hz	3,26 m	5,85, d J_{11-12}=9,8 Hz	6,23 d
4	"	1,02/0,91	1,11	1,15	1,40	0,88, d J=6,0 Hz	3,22 m		5,53, m (7-H)
5	"	1,03/0,80	1,31	1,19	1,48	0,88 d J=6,2 Hz	3,25 m	5,75, d J_{11-12}=2,0 Hz	3,25 (8-H)

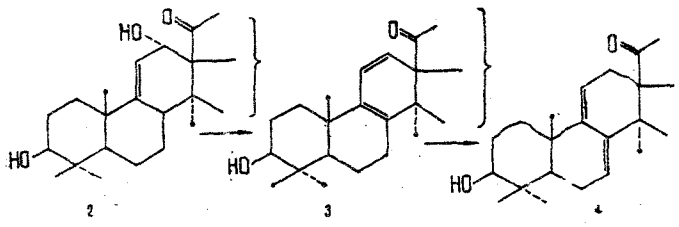

Fig. 2. Scheme of transformation of the native aglycone (2) under the conditions of acid hydrolysis.

of the holothurinogenin (3) having a homoannular diene system shows that under the conditions of acid hydrolysis a transformation of the native genin into aglycone (4) takes place by the scheme shown in Fig. 2. The ease with which the (3) → (4) transformation takes place on treatment with acid confirms this hypotheses.

The IR and UV spectra of the holothurinogenin (5) show the presence of an unsaturated ketone group in this compound ($\nu_{C=O}$ 1656 cm^{-1}, λ_{max} 231 and 254 nm). The ^1H NMR spectrum of the aglycone (see Table 2) showed the presence of seven methyl groups in the holostane skeleton and of a trisubstituted double bond in the 9(11) position. In the ^1H NMR spectrum of the aglycone, the vinyl proton of this double bond appeared in the form of a doublet at 5.75 ppm with $J_{11,8}$ = 2.0 Hz, which indicates the existence of an interaction between C_{11}–H and C_8–H [12]. The facts given show that (5) is 3β,17α-dihydroxyholost-9(11)-en-12-one, which is formed as the result of the autooxidation of the native genin or of the initial glycoside. The reduction of (5) with $NaBH_4$ led to a holothurinogenin identical with (2) according to ^1H NMR and UV spectra.

EXPERIMENTAL

Spectral Analyses. Mass spectra were recorded on LKB-9000 chromato-mass spectrometer, with direct introduction. The ionization energy was 20 eV. The ^{13}C and ^1H NMR and IR spectra were recorded on Bruker HX-90E and Specord 75 IR instruments, respectively, and the UV spectra on a Specord UV-VIS instrument.

Isolation of Holothurin B_1. An ethanolic extract of animals collected in May-June, 1978 in the sublittoral of the island of Cuba (Batabano Bay) was separated into fractions of polar and nonpolar glycosides on Polikhrom-1, using the gradient system water (100%) → water–ethanol (1:1). The fraction of nonpolar glycosides was chromatographed on silica gel in the chloroform–methanol–water (75:25:2) system. Holothurin B_1, $C_{41}H_{65}O_{11}$, mp 227-229°C (from aqueous ethanol), $[\alpha]_D^{20}$ −14.4° (c 0.19; ethanol).

The acid hydrolysis of holothurin B_1 was carried out with 12% HCl at 80°C for 4 h. The reaction mixture was worked up in the usual way. The monosaccharides were identified by paper chromatography in the butanol–pyridine–water (60:40:3) system and by gas–liquid chromatography in the form of the peracetates of the corresponding aldononitriles.

Isolation of the Mixture of Aglycones. The total aglycones were separated by repeated column chromatography in the hexane–ethyl acetate (6:1) system.

The holothurinogenin (2), $C_{30}H_{48}O_5$, mp 238-239°C (from methanol). The UV spectrum showed no absorption above 210 nm. IR spectrum ($CHCl_3$, cm^{-1}); 3455, 3598 (OH group), 1764 (C=O). Mass spectrum, m/z: 488 (M^+), 473 (M^+ −15), 470 (100%, M^+ −18), 455 (M^+ −33), 426 (M^+–CO_2 −18), 411 (M^+–CO_2 −33).

The holothurinogenin (3), $C_{30}H_{46}O_4$, mp 202-203°C (from methanol), $[\alpha]_D^{20}$ −117.9°C (c 0.28; ethanol). UV spectrum, λ_{max}, nm: 234 (ε 9790); 244 (ε 9222); 309 (ε 5770). Mass spectrum, m/z: 470 (M^+), 452 (M^+ −18), 437 (M^+ −33), 411 (M^+–CO_2 −15), 393 (M^+–CO_2 −33).

The holothurinogenin (4), $C_{30}H_{46}O_4$, mp 277-278°C (from methanol), $[\alpha]_D^{20}$ −14.8° (c 0.485; ethanol). UV spectrum, λ_{max}, nm: 237 (ε 13,300), 244 (ε 14,300); 252 (ε 10,250). Mass spectrum, m/z: 470 (M^+), 455 (M^+ −15), 452 (M^+ −18), 437 (M^+ −33), 393 (M^+–CO_2 −33).

The holothurinogenin (5), $C_{30}H_{46}O_5$, mp 229-230°C (from methanol). IR spectrum (CCl_4, cm^{-1}): 3622, 3484 (OH group), 1756 (C=O in a γ-lactone); 1656 (conjugated ketone). UV spectrum, λ_{max}, nm: 231, 254. Mass spectrum, m/z: 486 (M^+), 471 (M^+ −15), 468 (M^+ −18), 453 (M^+ −33), 427 (M^+–CO_2 −15), 409 (M^+–CO_2 −33). The reduction of the holothurinogenin (5)

by sodium tetrahydroborate, performed by the method of Chanley et al. [5], led to a compound identical with the holothurinogenin (2).

SUMMARY

A new triterpene oligoside, holothurin B_1, has been isolated from the holothurin *H. floridana*. It has been shown that the native aglycone of holothurin B_1 has the structure of holosta-9(11)-ene)3β,12α,17α-triol (2). Two new holostane derivatives have been isolated and characterized — holosta-8,11-diene-3β,17α-diol, and 3β,17α-dihydroxyholost-9(11)-en-12-one. A scheme of the transformation of the holothurinogenin (2) under the conditions of acid hydrolysis has been put forward.

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CHEMICAL INVESTIGATION OF *Hippophaë rhamnoides*.

I. THE MAIN COMPONENTS OF THE UNSAPONIFIABLE PART OF AN EXTRACT OF THE FRUIT PULP

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It has been established that the sterol fraction of the unsaponifiable part of a pentane extract of the fruit pulp of common sea buckthorn contains not only sterol but also triterpene alcohols and higher fatty alcohols. β-Sitosterol, 24-methylenecycloartanol, citrostadienol, and uvaol have been isolated from it. β- and α-Amyrins, 24-ethylcholest-7-en-3β-ol and erythrodiol have also been identified in it by chromato-mass spectrometry.

The high value of common sea buckthorn as a natural polyvitamin concentrate and the source of an oil possessing a broad pharmacological action spectrum [1] has served as the basis for a far-ranging chemical investigation of this plant with the aim of finding the biologically active components in it.

In a chemical and pharmacological investigation of common sea buckthorn it was observed that its biological activity is determined by the unsaponifiable fraction, the most active part of which is the "sterols." The main component of the "sterol" fraction proved to be β-sitosterol [2]. A preliminary attempt to investigate the "sterol" fraction of pharmacopoeial sea-buckthorn oil recently made with the aid of GLC and TLC led to the identification of one more component — stigmasterol [3].

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