GLYCOSIDES OF MARINE INVERTEBRATES.

XVII. NEW GENINS OF GLYCOSIDES FROM THE CARIBBEAN HOLOTHURIAN *Parathyona* sp. (HOLOTHURIOIDAE, CUCUMARIIDAE)

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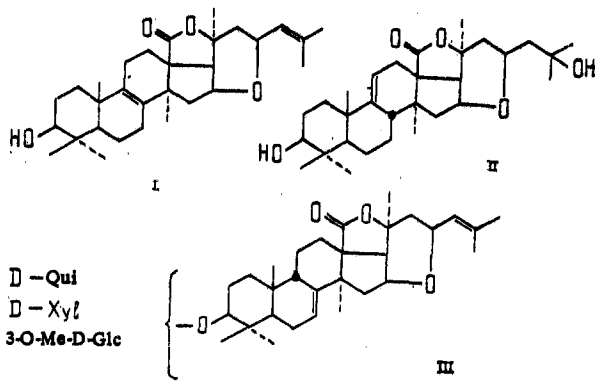
New triterpene glycosides — parathyosides R and T — have been isolated from a methanolic extract of a holothurian, *Parathyona* sp. It has been shown that the native aglycone of glycoside R is 16,23-epoxyholosta-7,24-dien-3β-ol (parathyonogenin). The acid hydrolysis of parathyonoside R gave two artifactual genins: 16,23-epoxyholosta-8(9),24-dien-3β-ol (parathyonogenin I) and 16,23-epoxyholost-9(11)-ene-3β,25-diol (parathyonogenin II).

In recent years the structures of several physiologically active triterpene glycosides from holothurians belonging to the families Holothuriidae and Stichopodidae have been determined [1-3]. At the same time, glycosides produced by animals of the Cucumariidae family have remained little studied. The aim of the present work was to establish the structures of the aglycone moiety of glycosides of the Caribbean holothurian *Parathyona* sp.

New triterpene oligosides — parathyonosides R and T — have been isolated from an ethanolic extract of the holothurian. Preliminary information on the structures of the aglycone of glycoside R (III) was obtained in an analysis of its spectral characteristics and, in particular its ^{13}C NMR spectra. Such spectra have recently been described for a whole series of glycosides of holothurians and their aglycones [4-6].



The ^{13}C NMR spectrum of the aglycone moiety of parathyonoside R (III) was close to that of the native genin from *Cucumaria fraudatrix* — 16β-acetoxyholosta-7,24-dien-3β-ol [4]. In both spectra there were characteristic signals for a γ-lactone grouping in a holostane nucleus at 179-180 ppm (C-18) and 78-79 ppm (C-20). Both compounds had a double bond in the 7(8) position giving signals in the spectra at 120-121 ppm (C-7) and 145-146 ppm (C-8). Hydrolysis of the glycoside (III) gave two artifactual genins — parathyonogenins I and II (scheme). Parathyonogenin I has two double bonds — one in the 24(25) and the other in the 8(9) position. The first of them is readily identified in the ^1H NMR spectrum by singlet signals of the protons of two methyl groups of the olefinic type (1.67 and 1.71 ppm) and by the signal of an olefinic proton (C-24H) at 5.12 ppm (d, J = 8.0 Hz) [7]. The hydrogenation of aglycone (I) formed a dihydro derivative in the spectrum of which the signal at 5.12 ppm

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TABLE 1. Characteristics of the ^{13}C NMR Spectra of Holothurinogenins from *Parathyona* sp.

Compound	Solvent	CH_3 group					3-H	16-H	24-H	11-H	23-H
		30/31	19	32	21	26/27					
I	$CDCl_3$	0,83	1,05	1,11	1,41	1,72/1,65	3,25	4,70	5,12	—	4,23
I*	$CDCl_3$	0,83	1,17	1,10	1,40	0,86	3,22	4,73	—	5,21	3,54
I†	$CDCl_3$	0,84	1,20	1,12	1,40	0,86	4,52	4,73	—	5,19	3,52
II	$CDCl_3$	0,84 (0,91)	1,18	0,99	1,40	1,22/1,19	3,30	4,70	—	5,22	3,91

*Hydrogenated aglycone I.
†Acetate of hydrogenated aglycone I.

had disappeared, while methyl groups appeared in the form of a doublet with its center at 0.86 ppm (J = 6.0 Hz). The second double bond is tetrasubstituted and does not appear in the ^1H NMR spectrum. It is formed by the migration of the 7(8) double bond in the native aglycone during the process of acid hydrolysis. The migration of a double bond from the 7(8) into the 8(9) and then into the 9(11) position has been reported previously in a study of the transformations of the native aglycones of several holothurian glycosides in an acid medium [4, 5].

Parathyonogenin II has only one double bond, which occupies the 9(11) position. The signal of the olefinic proton in its ^1H NMR spectrum is observed in the form of a multiplet at 5.22 ppm, like the corresponding signals in the spectrum of other holost-9(11)-ene derivatives [5]. As compared with the genin (I), parathyonogenin II has an additional hydroxy group, as is shown by the mass spectra of the two aglycones (M^+ 468 and M^+ 486 m/z, respectively). The position of this OH group was shown by a consideration of the ^1H and ^{13}C NMR spectra of the aglycone (II). The protons of two methyl groups at C-25 appear in the form of singlets at 1.22 and 1.25 ppm, which is characteristic for 25-hydroxyholostanols [8]. In addition to this, the C-25 signal in the ^{13}C NMR spectrum of genin (II) is shifted downfield as compared with the same signal for holothurinogenins unsubstituted in the side chain [9].

In their ^1H NMR spectra, parathyonogenins I and II each have three signals in the weak field corresponding to CH_3-O groupings (Table 1). One of them is easily identified as relating to the 3βH proton, since, as in the case of other holostan-3β-ol genins, in the spectra of compounds (I) and (II) the signal is observed at 3.25-3.30 ppm [6].

Two other signals in the spectra of the parathyonogenins corresponded to protons attached to carbon atoms linked with one another by an ether bond (in the IR spectrum of the genin (I), cm^{-1}: 1020 and 1220). At the same time, on acetylation, genin (I) gave a monoacetate (M^+ 510 m/z), the IR spectrum of which contained no absorption bands of hydroxy groups. Parathyonogenin I gave a positive reaction with boron trifluoride etherate. The position of the C—O—C fragment was revealed with the aid of experiments using selective suppression of spin–spin coupling by double resonance. Thus, when the signal at 4.23 ppm in the spectrum of the genin (I) was irradiated, the doublet at 5.12 ppm (24-H) was converted into a singlet. It followed from this fact that one end of the ether ring was connected to C-23. When the signal at 4.60 ppm in the spectrum of parathyonogenin II was irradiated, the doublet at 2.27 ppm (17-H) was converted into a singlet, the quartet at 1.83 ppm (15-H) into a doublet, and the triplet at 1.60 ppm (15-H) with a broadened central line into a doublet. Consequently, the second end of the ether bridge is attached at C-16. The values of the spin-spin coupling constants $J_{16/17}$ = 9.8 Hz, $J_{16/15}$ = 6.6 Hz, 10.8 Hz, $J_{15/15}$ = −11.1 Hz changed insignificantly in comparison with the analogous constants for 3β,16-diacetoxyholost-8(9)-ene [4]. This is apparently connected only with the deformation of ring D and not with a change in the configuration of the substituent at C-16. The presence of an oxygen function in position 16 is shown by the shifts of the C-14 and C-15 signals in the ^{13}C NMR spectrum of parathyonogenin II upfield and downfield, respectively, (Table 2) as compared with the spectrum of the unsubstituted holostanol [5].

It has been established previously [10] that when the oxide ring between C-8 and C-13 is closed in sclareol the signals of the C-9, C-11, and C-12 carbon atoms shift upfield by 2.9, 2.5, and 9.8 ppm, respectively. In our case, we observed similar shifts of the C-17, C-20, and C-22 signals in the ^{13}C NMR spectra of parathyonogenin II (Table 2) as compared with their positions in the hypothetical holost-7-en-3β, 16β,23-triol in which these signals should be located at 51.0, 84.7, and 46.5 ppm, respectively. The values of 84.7 and 46.5 ppm

TABLE 2. Characteristics of the ^{13}C NMR Spectra of Parathyonogenin II ($CHCl_3$) and the Aglycone Moiety of Parathyonoside R (C_5D_5N, 25°C)

Atom	II	R	Atom	II	R	Atom	II	R	Atom	II	R
C_1	37,1	35.8	C_9	151,2	47.0	C_{16}	75.0	b	C_{24}	46.7	125.8
C_2	27,8	27 3	C_{10}	39.6	35.8	C_{17}	46.3	48,8	C_{25}	70 4	135,6
C_3	78,9	89 0	C_{11}	110,4	21.9	C_{18}	177,1	179,9	C_{26}	28,2[a]	25,5
C_4	39,2	39,6	C_{12}	32,5	29.5	C_{19}	21,7	24 3	C_{27}	27.8[a]	17.9
C_5	52.4	49,2	C_{13}	58.2	58,9	C_{20}	78,2	78,8	C_{30}	15,7	17,3
C_6	21.2	23,2	C_{14}	42,8	46,2	C_{21}	30 8	29,2[a]	C_{31}	28,2	28,7[a]
C_7	28.2	120.3	C_{15}	40,4	40,3	C_{22}	39 2	39,6	C_{32}	21,1	30,9
C_8	39,4	145,9				C_{23}	63,3	62,8			

a — assignment of the signals ambiguous;
b — overlaps with the signals of the sugar moiety.

were taken from the ^{13}C NMR spectrum of the artifactual glycoside holosta-7,25-diene-3β,23-diol obtained in the enzymatic hydrolysis of a glycoside from the holothurian *Astichopus multifidus*.

In the ^{13}C NMR spectrum of the aglycone moiety of parathyonoside R (Table 2), the C-14, C-15, C-17, C-20, and C-22 signals undergo shifts similar to those described above for parathyonogenin II. This shows the existence of an oxide ring between C-16 and C-23 in the glycoside R (III) itself.

Parathyonoside R (III) has a 24(25) double bond in the aglycone, as is shown by the C-24, C-25, C-26, and C-27 signals in its ^{13}C NMR spectrum (125.8, 135.6, 25.5, and 17.9 ppm, respectively). As compared with the C-24 and C-25 signals in the ^{13}C NMR spectrum of cucumariogenin (II), in glycoside R (III) these signals are shifted downfield, which confirms the presence of an additional oxygen substituent at C-23 in the side chain of the aglycone [12, 13].

The value of 24.3 ppm for the C-19 chemical shift in the ^{13}C NMR spectra of parathyonoside R (III) shows a 9β-H configuration [11].

On acid hydrolysis, parathyonoside R (III) alone and parathyonosides R and T combined gave a single set of holothurinogenins in which the aglycones (I) and (II) predominated, i.e., the glycosides apparently have the same native aglycone and differ in the structures of the carbohydrate moieties. Qualitative analyses of the monosaccharides were the same for both glycosides — D-xylose, D-quinovose, and 3-OMe-D-glucose.

The results of the determination of the structures of the cucumariogenins of the glycosides of *Parathyona* sp. show that several common features exist in the biosynthetic pathways of the glycosides in holothurians of the families Stichopodidae and Cucumariidae (the formation of a 7(8) double bond and the introduction of oxygen functions in the C-23 and C-16 positions of the holostane skeleton). However, a number of differences in the structures of the carbohydrate chains and in the structures of the aglycones connected with the positions of the double bonds in their side chains also exist.

EXPERIMENTAL

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

Isolation of Parathyonosides R and T. An ethanolic extract of the animals collected in the sublittoral of the island of Cuba in 1978 was separated into fractions of polar and nonpolar glycosides on Polikhrom-1 using a gradient system from water (100%) to water—ethanol (1 : 1).

The fraction of polar glycosides was chromatographed on a silica gel L 40/100 μ (Czechoslovakia) in the chloroform—methanol—water (60 : 30 : 2) system. For parathyonoside R,

$[\alpha]_D^{20} - 23.5°$ (c 0.55; water). The melting point was not determined, since the substance decomposed without melting. For parathyonoside T, $[\alpha]_D^{20} - 40.5°$ (c 0.6; water). Again the melting point was not determined because it decomposed without melting.

The acid hydrolysis of parathyonosides R and T was carried out with 2% H_2SO_4 at 95°C for 2 h. The precipitate that deposited was separated off on a Schott No. 4 filter, washed with water, dried, and extracted with the chloroform—methanol (1 : 1) system. The solution was evaporated, giving a mixture of aglycones and progenins. The monosaccharides were identified with the aid of thin-layer chromatography on silica gel in the chloroform—methanol (2 : 1) system and by gas—liquid chromatography in the form of the peracetates of the corresponding aldononitriles.

Isolation of Parathyonogenins I and II. The combined aglycones and progenins were separated with the aid of column chromatography on silica gel. Aglycone (I) was eluted by the hexane—ethyl acetate (5 : 3) system, and aglycone (II) by hexane—ethyl acetate (1 : 4).

Parathyonogenin I, $C_{30}H_{40}O_{44}$, mp 218°C, (from ethanol), $[\alpha]_D^{20} - 30°$ (c 0.3; $CHCl_3$). IR spectrum ($CHCl_3$, cm^{-1}): 3450 (OH group), 1752 (C=O), 1020, 1220 (C—O—C). Mass spectrum, m/z: 468 (M^+), 453 ($M^+ - 15$), 435 ($M^+ - 15 - 18$), 417 ($M^+ - 15 - 18 - 18$), 399 ($M^+ - 69$), 397 ($M^+ - 71$), 379 ($M^+ - 171 - 18$), 307, 151, 109 (side chain), 95, 83, 69.

Parathyonogenin II, $C_{30}H_{36}O_5$, mp 235°C (from ethanol), $[\alpha]^{20} - 28.6°$ (c 0.7; $CHCl_3$).

IR spectrum ($CHCl_3$, cm^{-1}): 3500 (OH group), 1747 (C=O); 1020, 1240 (C—O—C). Mass spectrum, m/z: 486 (M^+), 471 ($M^+ - 15$), 468 ($M^+ - 15$), 453 ($M^+ - 18 - 15$), 435 ($M^+ - 18 - 15 - 18$), 413, 397 ($M^+ - 71 - 18$), 379 ($M^+ - 71 - 18 - 18$), 272, 109 (side chain), 95, 83, 71, 69.

SUMMARY

Two new triterpene oligosides — parathyonosides R and T — have been isolated for the first time from the holothurian *Parathyona* sp., family Cucumariidae. It has been shown that the native aglycone of parathyonoside R is 16,23-epoxyholosta-7,24-dien-3β-ol. During acid hydrolysis, the 7(8) double bond of parathyonoside R migrates into the 8(9), and then into the 9(11), positions.

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