STRUCTURE OF SYNAPTOGENIN B — AN ARTEFACTUAL AGLYCONE OF GLYCOSIDES FROM THE HOLOTHURIAN *Synapta maculata*

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The structure of the previously unknown synaptogenin B, the main product of the acid hydrolysis of the triterpene oligosides synaptosides S-2 and S-3 from the holothurian *Synapta maculata* has been established. To prove the structure, diketones were obtained from synaptogenin B and the known stichopogenin, and their identity was shown on the basis of ^{1}H NMR and mass spectra. Synaptogenin B is a 3β-hydroxyholost-9(11)-en-23-one. It has been shown that holost-9(11)-en-3β-ol is formed as minor component in the acid hydrolysis of the synaptosides.

The physiologically active triterpene glycosides of marine invertebrates — holothurians — form a unique group of natural glycosides which, up to the present, has been detected only in animals of this class. The polyfunctional triterpene aglycones of such glycosides are apparently formed in various oxidation reactions of lanosterol [1]. For the holothurians of one family the specific occurrence of the oxidative reaction leading to the formation of holothurinogenins [2-4], stichopogenins [5, 6] and cucumariogenins [7] that are biogenetically close to one another, is characteristic. The triterpene glycosides of holothurians of the family *Synaptidae* have not hitherto been studied.

Two individual glycosides — synaptosides S-2 and S-3 — have been isolated from a methanolic extract of the holothurian *Synapta maculata* (*Holothurioidea*, *Synaptidae*). Their physiochemical characteristics are given in the Experimental part. The ^{13}C and ^{1}H NMR spectra of the aglycone synaptogenin B (I) obtained on the acid hydrolysis of the synaptosides showed that S-2 and S-3 belonged to the triterpene oligosides with an aglycone of the holostane series. The ^{13}NMR spectra of synaptosides S-2 and S-3 contained signals characteristic for a Δ^7 double bond in the holostane skeleton (146.87 and 120.16 ppm for C-8 and C-7) [8] and for two carbon atoms linked to carbonyl groups (179.69 and 207.25).

We have established that the Δ^7 double bonds in the synaptosides, as in other holothurian glycosides [9], migrate on acid hydrolysis into the 9(11) position, as a result of which mixtures of artefactual aglycones are obtained. Synaptogenins B (I) and R (II) were isolated in the individual state. The physicochemical characteristics of the aglycones are given in the Experimental part. The ^{13}C and ^{1}H NMR spectra of synaptogenin B show the presence in the aglycone of $\Delta^{9(11)}$ double bond and of a carbonyl group in the side chain (Table 1). To prove the complete structure of synaptogenin B (I) we synthesized the keto derivative (Ia) and showed its identity with the diketone (IIIc) obtained from the known stichopogenin (III) [5].

It has been established previously that stichopogenin (IIIa) is formed from the native aglycone (III) on the acid hydrolysis of the stichoposides of the holothurian *Stichopus chloronotus* [9]. Structure (III) was established earlier on the basis of the results of x-ray structural analysis [10]. On the acid hydrolysis of the stichoposides from *Stichopus chloronotus* we obtained a mixture of artefactual aglycones — 23-acetoxyholost-8,25-dien-3β-ol, 23-acetoxyholost-9(11),25-dien-3β-ol, and (IIIa). The hydrogenation of this mixture over Adams catalysts led to (IIIa) containing a small amount of the 8(9)-isomer which we separated on silica gel impregnated with $AgNO_3$. The deacetylation of (IIIa) gave the diol (IIIb), which we subjected to oxidation with Sarett's reagent [11]. The diketone (IIIc) so obtained was, according to ^{1}H NMR and mass spectroscopy, identical with the diketone synthesized from synaptogenin B (Fig. 1).

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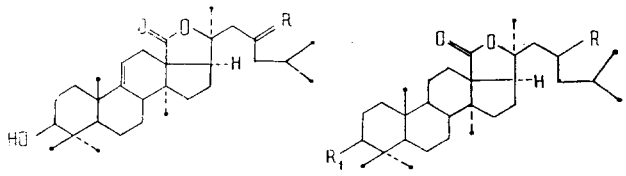
TABLE 1. Details of the ^{1}H NMR Spectra of Compounds (I), (IIIa), (IIIb), and (IIIc)

Compound	CH_3-19	CH_3-21	CH_3-32	CH_3-30	CH_3-31	CH_3-26,27	CH-8	CH-3	CH-11 / CH-23
I	1,157s	1,49s	0,833s	0,992s	0,865s	0,921 (3H, d, J=6,7) 0,928 (3H, d, J=6,7)		3,22	5,2 —
IIIa	1,15s	1,40s	0,83s	0,99s	0,87s	0,92 (6H, d, J=6,5)	3,0	3,22	5,2
IIIb	1,15s	1,52s	0,83s	0,99s	0,88s	0,92 (3H, d, J=6,5) 0,93 (3H, d, J=6,5)	3,0	3,23	5,2 3,98
IIIc	1,35s	1,5s	0,87s	1,08s	1,07s	0,92 (3H, d, J=6,5) 0,93 (3H, d, J=6,5)	3,05	—	5,26 —

Note. s — singlet; d — doublet.



I. R = O

II. R = H

III. R = OAc, R_1 = OH, Δ^7

IIIa. R = OAc, R_1 = OH, $\Delta^{9(11)}$

IIIb. R = OH, R_1 = OH, $\Delta^{9(11)}$

IIIc. R = O, R_1 = O, $\Delta^{9(11)}$

Fig. 1. Structures of compounds (I), (II), (III), (IIa), (IIIb), and (IIIc).

Thus, the structure of the artefactual glycone (I) obtained on the acid hydrolysis of the combined synaptosides S-2 and S-3 has been established. The ^{13}C NMR spectrum of (I) confirms the structure established:

C atom	ppm	C atom	ppm	C atom	ppm
C-1	36,1	C-11	110,5	C-21	27,7
C-2	27,7	C-12	33,0	C-22	52,4
C-3	78,8	C-13	57,0	C-23	207,3
C-4	39,05	C-14	47,2	C-24	52,05
C-5	52,4	C-15	35,7	C-25	25,6
C-6	21,1	C-16	24,5	C-26	22,4
C-7	27,7	C-17	51,5	C-27	22,4
C-8	39,6	C-18	177,2	C-30	15,5
C-9	151,3	C-19	21,8	C-31	28,6
C-10	39,6	C-20	82,2	C-32	19,7

The second algycone, synaptogenin R (II) is formed in minor amounts in the acid hydrolysis of synaptosides S-2 and S-3. The IR and mass spectra of the aglycone show the absence of a carbonyl group in the side chain. Its ^{1}H NMR spectrum is given in the Experimental part. The results obtained for synaptogenin R confirm the structure of (II) as holost-9(11)-en-3β-ol.

EXPERIMENTAL

Melting points were determined on a Boëtius stage. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker WH-250 spectrometer in $CDCl_3$. The signals are given relative to TMS as internal standard.

The holothurians *S. chloronotus* and *S. maculata* were collected during tropical voyages on the Scientific Research Ship "Professor Bogorov" on the Great Barrier Reef (January, 1980) and on the islands of the Socialist Republic of Vietnam (December, 1980), respectively. The total glycosides of *S. chloronotus* were given to us by the young scientific workers of the I. I. Mil'tsev Biosynthesis Laboratory of the Pacific Ocean Institute of Bioorganic Chemistry of the Far Eastern Scientific Center of the Academy of Sciences of the USSR.

Isolation of Synaptosides S-2 and S-3. An ethanolic extract of the holothurian *S. maculata* (28 g) was separated on a column of Polikhrom-1 in the water–50% aqueous ethanol system. The S-2 and S-3 fractions so obtained were purified on silica gel in the chloroform–methanol–water (60:30:2) system. Synaptoside S-2 (from chloroform–methanol–water (2:1:0.2) had mp 183°C, $[\alpha]_D^{20}$ −11.7° (*c* 0.28 ; pyridine. Synaptoside S-3 (from water-saturated butanol) had mp 201°C, $[\alpha]_D^{20}$ − 24.4° (*c* 0.635 ; aqueous methanol). The IR spectra (KBr) of both glycosides had absorption bands of carbonyl groups at 1761 and 1714 cm^{-1}.

Isolation of Synaptosides B (I) and R (II). The combined synaptosides S-2 and S-3 were hydrolyzed with 12% HCl at 90°C for 2 h. After the usual working up, the mixture of aglycones obtained was separated twice on silica gel in the hexane–ethyl acetate (3:1) system. Synaptogenin B (from aqueous methanol) had mp 234°C. IR spectrum ($CHCl_3$): 1712, 1756, 3624 cm^{-1}. Mass spectrum (m/z): 470 (M^+), 455 (M^+ −15), 473 (M^+ − 15 − 18).

Synaptogenin R (from methanol) had mp 253°C. IR spectrum ($CHCl_3$): 1744 and 3610 cm^{-1}. Mass spectrum (m/z): 456 (M^+), 441 (M^+ − 15), 423 (M^+ − 15 − 18). ^1H NMR spectrum (ppm): 0.833, 0.864, 0.891, 0.99, 1.18, 1.41, 3.22 (1 H), 5.22 (1 H).

Hydrogenation of the Combined Aglycones of the Glycosides of *S. chloronotus* and Isolation of the Aglycone (IIIa). The combined algycones (53 mg) were hydrolyzed in 3 ml of ethyl acetate over Adams catalyst at room temperature for 72 h. The reaction mixture was worked up in the usual way. The hydrogenation product was stirred in chloroform saturated with HCl at room temperature for 24 h. The resulting mixture of the aglycone (IIIa) and its $\Delta^{8(9)}$-isomer was separated on silica gel impregnated with $AgNO_3$. The aglycone (IIIa) had mp 241°C (from methanol). Its ^1H NMR spectrum, detaisl of which are given in Table 1, agreed with that published previously [5].

Deacetylation of the Aglycone (IIIa) and Production of the Diol (IIIb). The aglycone (IIIa) (21 mg) was deacetylated in a saturated solution of K_2CO_3 in methanol at room temperature for 24 h. The reaction product was purified on silica gel in the benzene–ethyl acetate (4:1) system. Compound (IIIb) had mp 235–238°C (benzene–hexane). Details of its ^1H NMR spectrum are given in Table 1.

Oxidation of (IIIb) and Production of the Diketone (IIIc). Compound (IIIb) (18 mg) was oxidized by Sarett's reagent, obtained as described in [11]. The reaction product was purified on silica gel in the benzene–ethyl acetate (4:1) system. The yield of the diketone (IIIc) was 8 mg. It had mp 190–193°C (methanol), $[\alpha]_D^{20}$ +1.28° (*c* 0.234; ; chloroform). Mass spectrum of (IIIc): 468 (M^+), 453 (M^+ − 15). Details of its ^1H NMR spectrum are given in Table 1.

The oxidation of synaptogenin B (I) with Sarett's reagent led to a diketone (M^+ 468, mp 118°C (from methanol)), which, accordings to its ^1H NMR spectra and its physiochemical constants, was identical with (IIIc).

SUMMARY

It has been shown that the holothurian *Synapta maculata* contains new triterpene glycosides not described previously – synaptosides S-2 and S-3. The structure of the main aglycone obtained on the acid hydrolysis of the synaptosides has been established as 3β-hydroxyholost-9(11)-en-23-one, and a minor aglycone has been characteristized as holost-9(11)-en-3β-ol.

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TRITERPENE SAPONINS FROM *Thalictrum minus*. V. STRUCTURE OF THALICOSIDE B

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The epigeal part of *Thalictrum minus* L. has yielded a new bidesmoside — thalicoside B — which has the structure of oleanolic acid 28-O-β-D-glucopyranoside 3-O-[O-α-L-rhamnopyranosyl-(1 → 2)-O-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranoside].

We have previously reported the structure of the predominating glycoside of the cycloartane series — thalicoside A — isolated from *Thalictrum minus* [1, 2]. In the present paper we consider the structure of a new triterpenoid glycoside — thalicoside B (I) — isolated from the same plant [1].

The IR spectrum of thalicoside B contains the absorption band of an ester grouping (1740 cm^{-1}). Its PMR spectrum shows the presence in the glycoside of eight methyl groups, one of which is secondary (1.55 ppm, d, J = 6.1 Hz), a proton at double bond (5.33 ppm, m), and four anomeric protons of carbohydrate residues (5.05, d, J = 6.1 Hz, 4.84, m, and 4.65 ppm, 2 H, m).

The acid hydrolysis of thalicoside B led to a genin containing, according to IR spectroscopy and ^1H and ^{13}C NMR spectroscopy, a free carboxy group (1700 cm^{-1}, 180.1 ppm), a trisubstituted double bond (5.42 ppm, m), and seven tertiary methyl groups (0.82, 0.87, 0.93, and 0.94 (2 CH_3) 1.18, and 1.19 ppm). The positions of the ^{13}C NMR signals of the carbon atoms at the double bond (122.5, 144.8 ppm) enabled a choice to be made between the α- and β-amyrin series [3] in favor of the latter. From its physicochemical constants, molecular formula ($C_{30}H_{48}O_3$), and the chemical shifts (CSs) of the carbon atoms in the ^{13}C NMR spectrum [4] (Table 1), the genin was identified as oleanolic acid (II).

Rhamnose, arabinose, and glucose were identified in the hydrophilic fraction of the products of the acid hydrolysis of thalicoside B. The quantitative GLC analysis of the carbohydrates in the form of aldonitrile acetates and alditol acetates showed that the rhamnose, glucose, and arabinose were present in a ratio of 1:2:1. Consequently, in the ^1H NMR spectrum of thalicoside B the doublet at 1.55 ppm belonged to the secondary methyl group of the rhamnose residue.

The alkaline hydrolysis of glycoside (I) led to a progenin with a free carboxy group (1696 cm^{-1}) containing the sugars mentioned in an equimolar ratio (1:1:1). No carbohydrates were detected in the hydrolysate. Thus, one of the two glucose residues is bound to the oleanolic acid through an ester group, and thalicoside B is a bisdesmosidic glycosidic. The position of attachment of the second carbohydrate chain was obvious and was confirmed by the downfield shift of the C-3 signal in the genin by 11.1 ppm on glycosylation (Table 1).

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