CONCLUSION

The structures of two new triterpenehexaosides have been established: astichoposide C from the holothurian *A. multifidus* as 23(S)-acetoxy-3β-{4'-O-[O-(3-O-methyl-β-D-glucopyranosyl)-(1→3)-β-D-glucopyranosyl]-2'-O-[O-(3-O-methyl-β-D-glucopyranosyl)-(1→3)-O-β-D-xylopyranosyl-(1→4)-β-D-quinovopyranosyl]-β-D-xylopyranosyloxy}holosta-4,25-diene (I) and stichoposide C from the holothurian *S. chloronotus* as 23(S)-acetoxy-3β-{4'-O-[O-(3-O-methyl-β-D-glucopyranosyl)-(1→3)-β-D-glucopyranosyl]-2'-O-[O-(3-O-methyl-β-D-glucopyranosyl)-(1→3)-O-β-D-xylopyranosyl-(1→4)-β-D-quinovopyranosyl]-β-D-xylopyranosyloxy}holost-7-ene (VIII).

It has been shown that glycosides of these holothurians collected in various regions of the world oceans have identical carbohydrate chains and differ from one another only by details of the structures of the side chains in the aglycone.

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GLYCOSIDES OF MARINE INVERTEBRATES.

XII. STRUCTURE OF A NEW TRITERPENE OLIGOGLYCOSIDE FROM HOLOTHURIANS OF FAMILY *Stichopodidae*

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UDC 547.996:593.96

From an ethanolic extract of the holothurians *Stichopus chloronotus* by column chromatography on silica gel a new triterpene oligoside has been isolated the structure of which has been established as 23(S)-acetoxy-3β-{4'-O-[O-(3-O-methyl-β-D-glucopyranosyl)-(1→3)-β-D-glucopyranosyl]-2'-O-[O-(3-O-methyl-β-D-glucopyranosyl(-(1→3)-O-β-D-xylopyranosyl-(1→4)-β-D-glucopyranosyl]-β-D-xylopyranosyloxy}holost-7-ene. A hypothesis has been put forward concerning the biosynthesis of the carbohydrate chains in the glycosides of holothurians of the order *Aspidochirota* from bioside blocks.

Continuing an investigation of the structure of glycosides from holothurians [1], we have isolated a new triterpene glycoside, stichoposide D (I) from ethanolic extracts of the holothurians *Stichopus chloronotus* and *S. variegatus* and have made a structural study of it.

We have shown previously that the native genin of the stichoposides from *S. chloronotus* is 23(S)-acetoxyholost-7-en-3β-ol (II) [2]. The acid hydrolysis of glycoside (I) gave, in addition to (II) and artefactual sapogenins, D-glucose, D-xylose, and 3-O-methyl-D-glucose in a ratio of 2:2:2.

A comparison of the ^{13}C NMR spectra of (I) and the genin (II) [3] showed that the carbohydrate chain in the glycoside was attached to the C-3 atom of the native genin. In ac-

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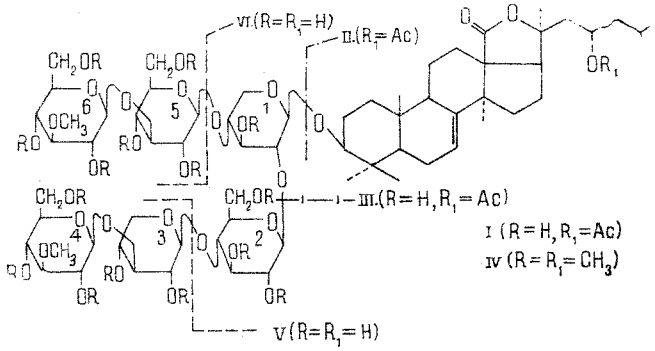
Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 200-204, March-April, 1982. Original article submitted July 31, 1981.

0009-3130/82/1802-0182$07.50

tual fact, the C-3 signal is shifted downfield in the spectrum of (I) by 0.2 ppm, as compared with the spectrum of (II), as the result of glycosylation [4].

The periodate oxidation of (I) led to the destruction of only one of the glucose residues, which permitted the assumption that 1→3 bonds between the monosaccharides were present.

As a result of the cleavage of the glycoside by Smith's method [5], the progenin (III), known previously [1], was obtained; it was shown to be identical with a standard sample by comparing their ^{13}C NMR spectra and by a mixed melting point. After this, the problem of determining the structure of glycoside (I) reduced to determining the structure and position of grafting on of a trisaccharide fragment consisting of glucose, xylose, and 3-O-methylglucose. The monosaccharide attached to one of the carbon atoms in the carbohydrate chain of the progenin (III) must be glucose, since it is precisely that one which underwent periodate oxidation in the process of Smith cleavage.



The methylation of (I) by Hakomori's method [6] led to the per-O-methyl derivative (IV), the IR spectrum of which shows that it contained no hydroxy groups. The methanolysis of (IV), followed by acetylation, gave a mixture of methyl 2,3,4,6-tetra-O-methyl-α- and -β-glucopyranosides, methyl 3-O-acetyl-2,4,6-tri-O-methyl-α- and -β-glucopyranosides, methyl-3-O-acetyl-2,4-di-O-methyl-α-xylopyranoside, methyl 2,4-di-O-acetyl-3-O-methyl-α-xylopyranoside, and methyl 4-O-acetyl-2,3,6-tri-O-methyl-α- and -β-glucopyranosides, which were identified by GLC and GLC-MS methods. The results of methylation indicated the presence of branching in the carbohydrate chain of the glycoside at a xylose residue attached to the aglycone and permitted formula (I) to put forward for stichoposide D.

Structure (I) agreed well with spectral characteristics. In actual fact, the ^{13}C NMR spectrum of (I) can be obtained by replacing the signals of carbon atoms of the quinovose residue in the spectrum of the related stichoposide C by the signals of a similarly bound glucose residue calculated, as described by Usui [7]. The spectrum obtained theoretically in this way agreed with the experimental spectrum of the glycoside (I) (Table 1).

The final proof of the correctness of formula (I) for stichoposide D was obtained by studying the enzymatic cleavage of this glycoside by cellulase. This gave the progenins (V) and (VI).

The minor progenin (V), according to the results of acid hydrolysis, contained xylose, glucose, and 3-O-methylglucose residues in its carbohydrate chain (1:2:1).

The acid hydrolysis of the progenin (VI) gave glucose, 3-O-methylglucose, and xylose in a ratio of 1:1:2. When (VI) was subjected to periodate oxidation and subsequent acid hydrolysis, only xylose and 3-O-methylglucose (1:1) were identified. The methylation of (VI), the methanolysis of the per-O-methyl derivative, and the acetylation of the products so obtained led to the formation of methyl 2,3,4,6-tetra-O-methyl-α- and -β-glucopyranosides, methyl 4-O-acetyl-2,3,6-tri-O-methyl-α- and -β-glucopyranosides, methyl 2-O-acetyl-3,4-di-O-methyl-α-xylopyranoside, and methyl 3-O-acetyl-2,4-di-O-methyl-α-xylopyranoside. The signals of the anomeric carbon atoms in the ^{13}C spectrum of progenin (VI) indicated the β configurations of all the glycosidic bonds [4] (see Table 1).

An ethanolic extract of *S. variegatus* contained a substance coinciding in chromatographic behavior with stichoposide D. After its isolation by column chromatography on silica gel and a study of ^{13}C NMR spectra, we found that the product obtained was a mixture of (I) and its 25(26)-dihydro derivative. In actual fact, the spectrum of this substance contained, in addition to signals at 141.6 ppm (s) and 114.3 ppm (t), which are characteristic for the

TABLE 1. ^{13}C NMR Spectra of the Carbohydrate Moieties of Glycoside (I) and of the Progenins (III) and (IV) (C_5D_5N, 60°C, TMS – 0)

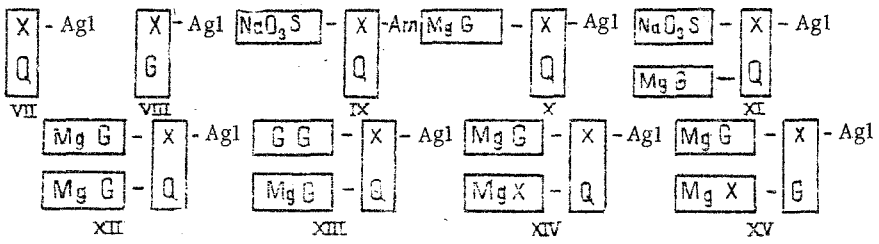
Atom	I	IV	III	Atom	I	IV
C_1^1	105,3	105,3	106,8	C_1^2	105.3	105.3
C_2^1	88,8	83,5	75.0	C_2^2	75.6	75,8
C_3^1	75,6	77,7	76,0	C_3^2	76,3	76.4
C_4^1	78,05	70,9	78,3[a]	C_4^2	80,9	80,9
C_5^1	64.0	66,45	64,3	C_5^2	76,3	76,4
C_1^5	102,9	—	103,0	C_6^2	62.4	61,6
C_2^5	73,2	—	73,2	C_1^3	105,3	104.9
C_3^5	88,1	—	87.7	C_2^3	73,2	73,4
C_4^5	69,9	—	69,9	C_3^3	87,7	87,8
C_5^5	78,05	—	78,1[a]	C_4^3	69,1	69,05
C_6^5	62,4	—	62,45	C_5^3	66,4	66 45
C_1^6	105,3	—	105,4	C_1^4	105,3	105,3
C_2^6	75,0	—	75,0	C_2^4	75,0	75,0
C_3^6	87,7	—	88,1	C_3^4	87,7	87,8
C_4^6	70,8	—	70,8	C_4^4	70,8	70,9
C_5^6	78,05	—	78,3[a]	C_5^4	78,05	78,15
C_6^6	62,4	—	62,45	C_6^4	62,4	62 4
OCH_3	60,6	—	60,5	OCH_3	60 4	60,7

Note: a – ambiguous assignment of the signals.

carbon atoms of a 25(26)-double bond in the side chain of the aglycone, signals at 23.4 and 23.1 ppm (doublet and quartet, respectively, with incomplete suppression of proton coupling), which showed the saturated nature of the bond between C-25 and C-26. After hydrogenation on Adams catalyst of the mixture that we had isolated, we obtained pure stichoposide D (mixed melting point with a standard sample, complete coincidence of the ^{13}C NMR spectra).

After the completion of the determination of the structures of stichoposides C and D, the number of glycosides with known structures from holothurians of the order *Aspidochirota* exceeded ten. A comparison of their carbohydrate chains showed that all these compounds had even numbers of monosaccharides and a series of bioside fragments which were repeated in many structures. Thus, the fragment quinovose-(1→2)-xylose (QX) is attached to the aglycone in all known glycosides of this series apart from (I) and stichoposide B [8]. In the last two cases, it is replaced by the analogous fragment glucose-(1→2)-xylose (GX). The biosynthesis of the oligosides of the holostane series in this group of holothurians probably takes place predominantly through intermediate biosides of the QX or GX type (for holothurians of the genera *Holothuria* and *Actinopyga*, through sulfated derivatives of these biosides). The further growth of the chain takes place by the addition of new bioside blocks, such as 3-O-methylglucose—glucose (MgG), glucose—glucose (GG), and 3-O-methylglucose—xylose (MgX). In the added bioside blocks, the monosaccharides are linked with one another by 1→3 bonds while 1→4 bonds predominate between the blocks.

Our hypothesis on the block type of structure of the carbohydrate chains in glycosides of *Aspidochirota* holothurians is in harmony with the structure of all known compounds of this series, without exception. Thus, the carbohydrate chains of stichoposide A [8] and of bivittoside A [9] can be represented by the block formula (VII), that of stichoposide B by (VIII), those of holothurin B and of echinoside B [10, 11] by formula (IX), that of bivittoside B [9] by (X), those of holothurin A and of echinoside A [11, 12] by (XI), those of holotoxin B [13] and of bivittosides C and D [9] by (XII), that of holotoxin A [13] by (XIII), those of stichoposide C and of astichoposide C [1] by (XIV), and that of stichoposide D by the block formula (XV).



Our hypothesis on the biosynthesis of the carbohydrate chains of glucosides from bioside fragments contradicts opinions expressed previously in a number of publications [13, 14] of the random nature of their structure, depending on the living conditions of the producing animals and, in particular, on their feeding spectrum. According to our results, ecological factors have more influence on the relative amounts of the particular glycosidic components in the glycoside fraction than on the structure of the carbohydrate chains of the components themselves.

EXPERIMENTAL

All the spectral characteristics were determined and the physical constants were obtained under conditions given in the preceding paper [1].

The animals were collected on the Great Barrier Reef (Australia) in January 1980 during a voyage of the scientific research vessel "Professor Bogorov."

Isolation of Stichoposide D (I). The ground holothurians were twice extracted with 70% ethanol, the extract was concentrated in vacuum to a volume corresponding to 1/20 of its initial volume, and the resulting precipitate was separated off by centrifugation, washed with ethyl acetate, and repeatedly chromatographed on a column containing Teflon powder in the water–50% ethanol system, and then on columns containing silica gel L (40–100 μ) in the $CHCl_3$–MeOH–H_2O (75:25:1) system. Stichoposide D, mp 263–265°C (from ethanol), $[\alpha]_D^{20}$ –41.3° (c 0.45; pyridine), yield about 0.05% of the dry weight of the holothurians, had the ^{13}C NMR spectrum given in Table 1).

The hydrolysis of glycoside (I) and of the progenins (III), (V), and (VI) was carried out with 12% HCl as described in the preceding paper [1]. The following sugars were identified in the form of the acetates of the corresponding aldononitriles by GLC and GLC-MS methods: in the hydrolysis of (I), glucose, xylose, and 3-O-methylglucose (2:2:2); in the hydrolysis of (III), glucose, xylose, and 3-O-methylglucose (1:1:1); in the hydrolysis of (V), xylose, glucose, and 3-O-methylglucose in a ratio of 1:2:1; and in the hydrolysis of (VI), glucose, 3-O-methylglucose, and xylose in a ratio of 1:1:2.

The periodate oxidation of stichoposide D (I) and its progenins was carried out as described previously [1]. After the periodate oxidation of (I), glucose, 3-O-methylglucose, and xylose were identified in a ratio of 1:2:2; after the oxidation and subsequent hydrolysis of the progenin (III) glucose and 3-O-methylglucose (1:1); and after the oxidation and corresponding treatment of the progenin (VI), xylose and 3-O-methylglucose (1:1).

The degradation of glucoside (I) by Smith's method was carried out in the same way as in the case of the related glycoside stichoposide C [1]. This gave the progenin (III), mp 248–250°C (from ethanol), $[\alpha]_D^{20}$ –46.2° (c 0.5; pyridine), yield 55%.

The enzymatic hydrolysis of stichoposide D (I) and the separation of the products were carried out by the procedure described in the preceding paper [1]. This gave two progenins: (V), with mp 246–248°C (from ethanol), $[\alpha]_D^{20}$ –40.0° (c 0.45; pyridine), yield 2%; and (VI) mp 260–262°C (from ethanol), $[\alpha]_D^{20}$ –44.7° (c 0.45; pyridine), yield 11%.

The methylation of stichoposide D (I) was carried out as described previously [1], and subsequent methanolysis and acetylation led to the identification by GLC and GLC-MS methods under the conditions given previously [1] of the following monosaccharide derivatives: Methyl 2,3,4,6-tetra-O-methyl-α- and -β-glucopyranosides, methyl 3-O-acetyl-2,4,6-tri-O-methyl-α- and -β-glucopyranosides, methyl 4-O-acetyl-2,3,6-tri-O-methyl-α- and -β-glucopyranosides, methyl 3-O-acetyl-2,4-di-O-methyl-α-xylopyranoside, and methyl 2,4-di-O-acetyl-3-O-methyl-α-xylopyranoside. Similarly, the following derivatives were obtained from the progenin (VI) on the same treatment and were identified: methyl 2,3,4,6-tetra-O-methyl-α- and -β-glucopyranosides, methyl 4-O-acetyl-2,3,6-tri-O-methyl-α- and -β-glucopyranosides, methyl 2-O-

acetyl-3,4-di-O-methyl-α-xylopyranoside, and methyl 3-O-acetyl-2,4-di-O-methyl-α-xylopyranoside.

CONCLUSION

1. The complete structure of a new triterpene hexaoside — stichoposide D — from the holothurian *Stichopus chloronotus* has been established. It is 23(S)-acetoxy-3β-{4'-O-[O-(3-O-methyl-β-D-glucopyranosyl)-(1→3)-β-D-glucopyranosyl]-2'-O-[O-(3-O-methyl-β-D-glucopyranosyl)-(1→3)-O-β-D-xylopyranosyl-(1→4)-β-D-glucopyranosyl]-β-D-xylopyranosoloxy}holost-7-ene.

2. A hypothesis of the biosynthesis of the carbohydrate chains of the glycoside holothurians from bioside blocks has been performed.

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TRITERPENE GLYCOSIDES OF *Astragalus* AND THEIR GENINS.

IV. CYCLOSIEVERSIOSIDE E — A NEW DIGLYCOSIDE FROM *Astragalus sieversianus*

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M. B. Gorovits, and N. K. Abubakirov

UDC 547.587+581.192

A new glycoside of the cycloartane series has been isolated from the roots of the plant *Astragalus sieversianus* Pal.; it is cyclosieversigenin 3,6-di-O-β-xyloside.

We have previously [1] reported the isolation from the roots of the *Astragalus sieversianus* Pal. of cyclosieversigenin (III) — an isoprenoid of the cycloartane series. Continuing a study of the methylsteroids of this plant, we have isolated from a methanolic extract of the roots eight substances having a glycosidic nature. In order of increasing polarity they have been called compounds A, B, C, D, E, F, G, and H. In this paper we consider the determination of the structure of compound E, which we have called cyclosieversioside E.

It was shown with the aid of GLC [2] that cyclosieversioside E (I) contains two D-xylose residues. The presence in the PMR spectrum of glycoside (I) of a one-proton signal in the

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Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 204-208, March-April, 1982. Original article submitted July 2, 1981.

0009-3130/82/1802-0186$07.50