TRISULFATED GLYCOSIDES FROM THE HOLOTHURIAN *Cucumaria japonica*

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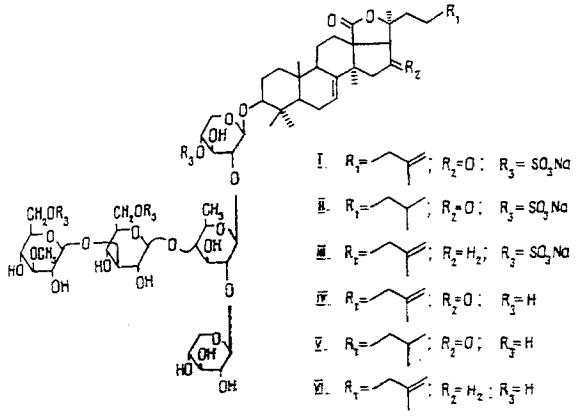
Three new triterpene glycosides have been isolated from an alcoholic extract of the Pacific Ocean commercial holothurian Cucumaria japonica: cucumariosides A_7-1 (I), A_7-2 (II), and A_7-3 (III). The structures of these glycosides have been established by chemical and physical methods.

A fraction of polar glycosides from the holothurian *Cucumaria japonica* was obtained by chromatographing the total glycosides on silica gel and consisted of a mixture of substances that gave a single spot in TLC. The separation of this fraction by reversed-phase HPLC led to the isolation of the main components. Cucumariosides A_7-1 (I) and A_7-3 (III) were isolated in the individual form, while cucumarioside A_7-2 (II) was obtained with a purity of about 80% (according to NMR).

The solvolytic desulfation of these compounds (followed by HPLC purification) led to the desulfated derivatives (IV), (V), and (VI), respectively. Desulfation of the initial glycoside fraction followed by separation of the derivatives obtained enabled the ratio in the mixture to be determined. The proportions of (IV), (V), and (VI) amounted to about 53.6, 6.4, and 21.3%, respectively, on the weight of the whole fraction of desulfated derivatives, while the other 18.7% consisted of a difficultly separable mixture of minor components.

A comparative study of the desulfated derivatives (IV), (V), and (VI) showed that these substances had identical carbohydrate chains, consisting of xylose, quinovose, glucose, and 3-O-methylglucose in a ratio of 2:1:1:1, and differed only by the structure of the aglycons. This was confirmed by a comparison of the results of monosaccharide analysis, which corresponded to the signals in the ^{13}C NMR spectra of these compounds (Table 1) and to the results of mass spectroscopy. The LSIMS$^{(+)}$ spectra of the desulfated samples contained the peaks of the $(M + H)^+$ ions with *m/z* 1217 (IV), 1219 (V), and 1203 (VI), and also the peaks of the $AglOH_2^+$ and Agl^+ ions with *m/z* 469 and 451 (IV), 471 and 453 (V), and 455 and 437 (VI), respectively. Fragments of other types were not characteristic for these spectra, and only in the spectrum of (IV) were the peaks of the ions of the carbohydrate moiety of the molecule observed, with *m/z* 749 and 617. At the same time, the fragmentation of the carbohydrate chains was detected satisfactorily in the LSIMS$^{(-)}$ spectra of the desulfated samples and confirmed the identity of the structures of the oligosaccharide chains in the (IV)-(VI) molecules (Scheme 1).



*Deceased.

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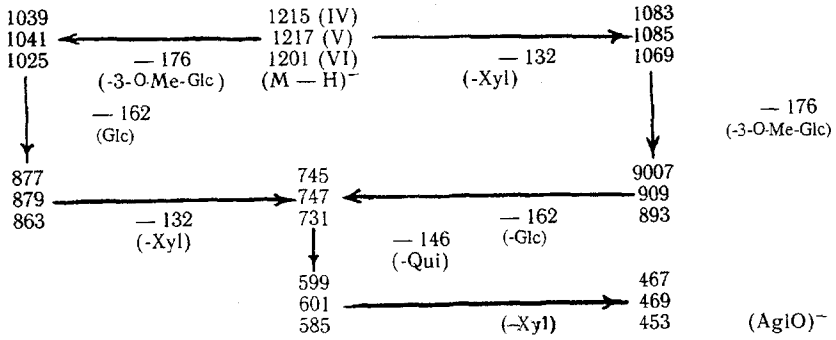
Furthermore, the physicochemical characteristics of derivatives (IV), (V), and (VI) coincided completely with those of the desulfated derivatives of cucumariosides A_2-2, A_2-3, and A_2-4, which were obtained from the corresponding glycosides of the holothurian *Cucumaria japonica* previously [1].



Consequently, compounds (I)-(III) differed from cucumariosides A_2-2, A_2-3, and A_2-4, respectively, by the number of sulfate groups. In actual fact, a comparison of the ^{13}C NMR spectra of the desulfated derivative (IV) with that of cucumarioside A_7-1 (I) showed that (I) had three sulfated hydroxy groups in the carbohydrate moiety of the molecule. Thus, the C-4 signal of the first xylose residue (70.7 ppm) in (IV) was located in a stronger field by 5.4 ppm, and the C-3 and C-5 signals in a weaker field by 1.6 and 1.9 ppm, respectively. Furthermore, the signals of C-6 of the 3-O-methylglucose residue (68.3 ppm) and of C-6 of the glucose residue (68.5 ppm) of glycoside (I) were shifted downfield in comparison with the corresponding signals in the spectrum of derivative (IV) (62.7 and 62.8 ppm). The C-5 signals of the 3-O-methylglucose and glucose residues (77.2 and 76.1 ppm, respectively) were shifted upfield in the spectrum of (I) relative to the analogous signals in (IV) (78.3 and 77.9 ppm). Such shifts are characteristic for the α- and β-effects of sulfate groups [2] and indicate that in (I) sulfate groups were attached to C-4 of the first xylose residue and to the C-6 atoms of the 3-O-methylglucose and glucose residues.

This was confirmed by the results of mass-spectrometry. the $LSIMS^{(-)}$ spectrum of cucumarioside A_7-1 (I) contained the peak of an ion with *m/z* 1499 $(M_{3Na} - Na)^-$. Its successive breakdown at the glycosidic bonds led to anions with *m/z* 1221 (1499 — $[NaSO_3O$-3-O-Me-Glc]) and 957 (1221 — $[NaSO_3O$ — Glc]). Also characteristic of the spectrum under consideration were processes of breakdown at the C_1—C_2 and C_5—O bonds of the methylglucose, glucose, and quinovose residues (Scheme 2).

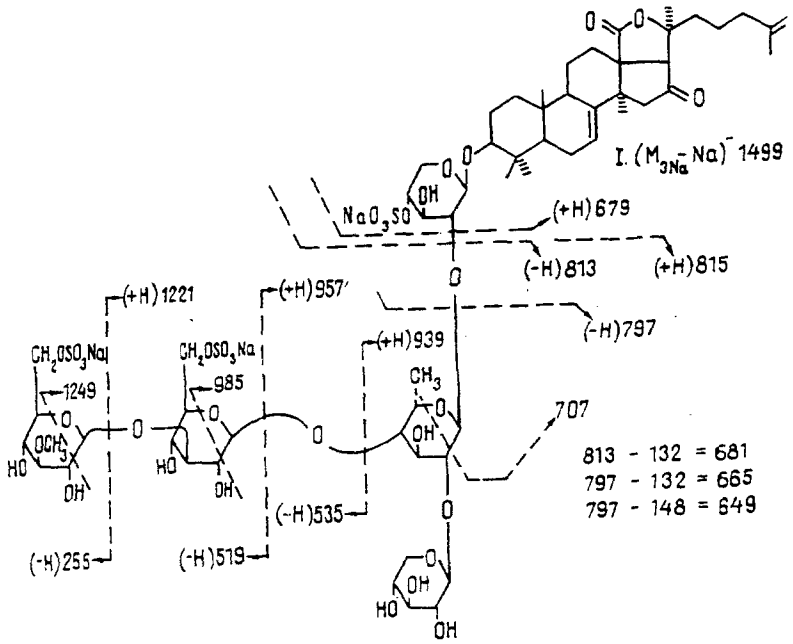


TABLE 1. ^{13}C NMR Spectra of the Aglycon Moieties of the Glycosides of the A_7 Group ($\delta_{TMS} = 0$)

Atom	I*	IV*	IV**	V*	III*	VI**
C-1	36.8	36.8	36.1	36.1	37.1	36.7
C-2	27.7	27.8	27.1	27.2	27.7	27.6
C-3	90.0	90.1	89.1	89.1	90.1	89.5
C-4	40.4	40.4	39.7	39.7	40.4	40.0
C-5	49.5	49.6	48.7	48.7	49.2	48.7
C-6	24.2	24.2	23.4	23.4	24.2	23.8
C-7	122.7	122.7	121.7	121.8	120.8	120.2
C-8	144.8	144.8	144.0	144.0	147.6	147.0
C-9	48.2	48.1	47.3	47.2	48.4	47.9
C-10	36.7	36.7	35.9	35.9	36.5	36.1
C-11	23.3	23.4	22.6	22.6	23.8	23.4
C-12	30.7	30.7	29.9	29.9	31.4	30.9
C-13	57.9	57.9	56.7	56.8	59.8	59.1
C-14	46.7	46.7	45.7	45.7	52.3	51.8
C-15	53.0	52.9	52.0	52.1	35.3	34.8
C-16	215.3	215.0	212.4	212.4	25.4	25.0
C-17	64.6	64.6	63.7	63.7	54.2	53.8
C-18	180.3	180.0	178.2	178.3	181.9	180.1
C-19	25.0	24.9	24.1	24.1	24.9	24.5
C-20	84.9	84.8	83.2	83.3	85.9	84.3
C-21	27.2	27.2	26.3	26.3	27.1	26.6
C-22	39.2	39.1	38.5	39.1	39.6	39.3
C-23	23.2	23.2	22.6	22.2	23.1	22.7
C-24	38.8	38.8	38.1	39.4	38.8	38.4
C-25	146.5	146.4	145.4	28.0	146.4	145.5
C-26	111.4	111.3	110.3	22.6	111.6	111.0
C-27	23.2	23.1	22.3	22.6	23.0	22.6
C-28	18.4	18.4	17.5	17.5	18.4	17.9
C-29	29.8	29.8	28.9	28.9	29.8	29.3
C-30	32.8	32.8	31.9	31.9	31.8	31.3

*The spectrum taken in C_5D_5N—D_2O (4:1).

**The spectrum taken in C_5D_5N.

Girard et al. [3] have established that the nonterminal sulfated xylose residue of frondoside A is characterized in the $FAB^{(-)}$ spectrum by ions with *m/z* 195, 211, and 227. The peaks of these ions and also of ions of sulfated glucose formed by an analogous method with *m/z* 225, 241, and 257 were present in the $LSIMS^{(-)}$ spectrum of (I). The terminal 3-O-methylglucose residue was characterized by ions with *m/z* 255, 271, and 273. The cleavage of the bond between the sulfated glucose residue and the quinovose residue gave ions with *m/z* 519, 535, and 537, while cleavage between the quinovose and sulfated xylose residues gave ions with *m/z* 797, 813, and 815. Different methods of loss by the ions with *m/z* 797 and 813 of sulfated xylose units led to fragments with *m/z* 649, 665, and 681. The spectrum also contained a weak peak of the (M_{3Na} — Na-Agl)$^-$ ion with *m/z* 1047. Thus, the results of mass spectrometry agreed completely with those of NMR.

On the basis of what has been said above, it follows that cucumarioside A_7-1 (I) is 3*β*-{O-(3-O-methyl-6-O-(sodium sulfato)-*β*-*D*-glucopyranosyl)-(1 →3)-O-(6-O-(sodiumsulfato)-*β*-*D*-glucopyranosyl)-(1 →4)-[O-*β*-*D*-xylopyranosyl)-(1 →2)]-O-*β*-*D*-quinovopyranosyl-(1 → 2)-(4-O-(sodium sulfato)-*β*-xylopyranosyloxy)}holosta-7.25-dien-16-one.

A study of the ^{13}C NMR spectra of A_7-3 (III) and A_7-1 (I) and of their desulfated derivatives (VI) and (IV) (Table 1) showed that these compounds differed only by details of the structure of the aglycon: compound (III) lacked the keto group at the C-3 carbon atom that was present in (I). The practically complete coincidence of the signals corresponding to the carbohydrate moieties of glycosides (I) and (III) permitted the conclusion that their carbohydrate chains were identical. These facts are in convincing agreement with the results of mass spectrometry. In the $LSIMS^{(-)}$ spectrum of (III) there were the peaks of anions consisting of the aglycon and part of the carbohydrate units analogous to the corresponding ions in the spectrum of (I) but differing by 14 units (1207, 943, 925, 665). The fragmentary ions formed from the carbohydrate chain had agreeing mass numbers in the spectra of (I) and (III).

Thus, cucumarioside A_7-3 (III) is 3*β*-{O-(3-O-methyl-6-O-(sodiumsulfato)-*β*-*D*-glucopyranosyl)-(1 →3)-O-(6-O-(sodium sulfato)-*β*-*D*-glucopyranosyl)-(1 →4)-[O-*β*-*D*-xylopyranosyl-(1 →2)]-O-*β*-*D*-quinovopyranosyl-(1 →2)-(4-O-(sodiumsulfato)-*β*-*D*-xylopyranosyloxy)}holosta-7.25-diene.

The formation of one and the same derivative (V) from cucumariosides A_7-2 (II) and A_2-3 unambiguously showed that the aglycon of glycoside (II) was 3*β*-hydroxyholost-7-en-16-one. Since the carbohydrate chains of all three glycosides were

TABLE 2. ^{13}C NMR of the Carbohydrate Moieties of the Glycosides of the A_7 Group ($\delta_{TMS} = 0$)

Atom	I*	IV*	IV**	II*	V**	III*	VI**
C^1-1	105.2	105.3	105.1	105.2	105.1	105.2	105.4
C^1-2	83.0	83.1	82.8	83.0	82.8	83.0	83.1
C^1-3	75.5[a]	77.3	77.7	75.6[a]	77.7	75.6[a]	78.1
C^1-4	76.1[d]	70.7	70.3	76.1[d]	70.3	76.1[d]	70.7
C^1-5	64.6	66.5	66.5	64.7	66.5	64.7	66.9
C^2-1	103.3	103.4	103.0	103.3	103.0	103.2	103.4
C^2-2	83.4	83.6	83.2	83.5	83.1	83.4	83.5
C^2-3	75.5[a]	76.2	75.8	75.6[a]	75.8	75.6[a]	76.1
C^2-4	87.1[b]	86.6	86.6	87.2[b]	86.6	87.1	87.0
C^2-5	71.9	72.1	71.1	72.0	71.1	71.9	71.5
C^2-6	18.8	18.8	18.1	18.8	18.1	18.8	18.5
C^3-1	104.7	104.7	104.6	104.7	104.6	104.7	105.0
C^3-2	74.4	74.5	73.5	74.5	73.6	74.4	74.0
C^3-3	87.3[b]	87.8[a]	88.2	87.4[b]	88.2	87.4[b]	88.5
C^3-4	70.0	70.3	69.8	70.0	69.8	70.1	70.1
C^3-5	76.1[d]	77.9[b]	77.7	76.1[d]	77.8	76.1[d]	78.1
C^3-6	68.5	62.8	62.3	68.6	62.1	68.5	62.5
C^4-1	105.3	105.3	105.5	105.3	105.5	105.4	105.9
C^4-2	75.0	75.2	74.9	75.1	74.9	75.1	75.3
C^4-3	87.1[b]	87.7[a]	87.8	87.2[b]	87.8	87.2[b]	88.2
C^4-4	70.9[c]	71.1[c]	70.7[a]	70.9[c]	70.7[a]	70.9[c]	71.1[a]
C^4-5	77.2[d]	78.3	78.2	77.3[d]	78.2	77.3[d]	78.6
C^4-6	68.3	62.7	62.1	68.4	62.3	68.3	62.7
O — CH_3	61.3	61.4	60.5	61.3	60.5	61.3	61.0
C^5-1	105.9	106.1	105.9	105.9	105.9	105.9	106.2
C^5-2	75.7[a]	75.8	75.5	75.7[a]	75.5	75.7[a]	75.9
C^5-3	77.2	77.4[b]	77.0	77.3	77.0	77.3	77.4
C^5-4	70.7[c]	70.9[c]	70.5[a]	70.7[c]	70.5[a]	70.7[c]	70.9[a]
C^5-5	67.3	67.3	66.9	67.3	66.9	67.3	67.3

*Spectrum taken in C_5D_5N—D_2O (4:1).

**Spectrum taken in C_5D_5N.

[a,b,c,d]Ambiguous assignment of the signals.

identical, as follows from the results of mass spectroscopy and monosaccharide analysis and the practically complete coincidence of the carbohydrate parts of the ^{13}C NMR spectra of cucumariosides (I)-(III) and the corresponding signals in the spectra of their desulfated derivatives (Table 2), it may be concluded that the native cucumarioside A_7-2 is 3β-{O-(3-O-methyl-6-O-(sodiumsulfato)-β-*D*-glucypyranosyl)-(1 → 3)-O-(6-O-(sodiumsulfato)-β-*D*-glucopyranosyl)-(1 → 4)-[O-β-*D*-xylopyranosyl-(1 → 2)]-O-β-*D*-quinovopyranosyl-(1 → 2)-(4-O-(sodium sulfato)-β-*D*-xylopyranosyloxy)}holost-7-en-16-one.

EXPERIMENTAL

Physical constants were determined and NMR and mass spectra and GLC—MS analyses were obtained under the conditions described in [4]. HPLC was conducted on a Du Pont 8800 chromatograph with a Silasorb C-18 column, 10 × 150 mm.

The animals were gathered in September, 1981, by a commercial bottom trawl from a depth of 30-130 m in Pos'et Gulf (Peter the Great Bay).

Isolation of the Glycoside Fraction. The total glycosides were obtained as described in [4]. They were then chromatographed repeatedly in the chloroform—methanol—water (650:300:54) system with the collection of the corresponding fractions.

Desulfation of the Total Glycosides. The desulfation of 1200 mg of the initial glycoside fraction was carried out as described in [4]. The residue was filtered through silica gel in the chloroform—methanol—water (650:150:22) system. This gave 560 mg of a fraction of desulfated derivatives [containing (IV)-(VI)]. Then the mixture obtained was separated by the HPLC method with the mobile phase 80% methanol, 2.8 ml/min. This gave 295 mg of (IV), mp 288-290°C, $[\alpha]_{578}$ −89° (*c* 0.1; pyridine); 35 mg of (V), mp 293-295°C, $[\alpha]_{578}$ −80° (*c* 0.1; pyridine); and 117 mg of (VI), mp 288-290°C, $[\alpha]_{578}$ −52°C (*c* 0.1; pyridine).

Desulfation of (I)-(III). Each glycoside (25 mg) was desulfated by the procedure described in [4]. The residue was filtered through silica gel in the chloroform—methanol—water (650:150:22) system. This gave derivative (IV), mp 289-291°C, $[\alpha]_{578}$ —90°C (*c* 0.1; pyridine); derivative (V), mp 292-294°C, $[\alpha]_{578}$ —80°C (*c* 0.1; pyridine); and derivative (VI), mp 290-292°C, $[\alpha]_{578}$ —53°C (*c* 0.1; pyridine).

Isolation of Individual Glycosides. The initial fraction (450 mg) was separated by HPLC using 40% methanol as the mobile phase at 2.8 ml/min. This gave A_7-1 (I), mp 223-225°C (decomp.), $[\alpha]_{578}$ —83° [*c* 0.1, pyridine—water (1:1)]; and a fraction containing about 80% of A_7-2 (II) and A_7-3 (III), mp 238-240°C (decomp.), $[\alpha]_{578}$ —43° [*c* 0.1, pyridine—water (1:1)].

Acid Hydrolysis of (IV)-(VI). Each desulfated derivative (10 mg) was dissolved in 1.5 ml of 2 N HCl and was hydrolyzed as described in [4]. The reaction mixture was evaporated, filtered through silica gel in chloroform, and analyzed by GLC-MS.

REFERENCES

1. S. A. Avilov, V. A. Stonik, and A. I. Kalinovskii, *Khim. Prir. Soedin.*, No. 6, 787 (1990).
2. S. Honda, H. Yuki, and K. Takiura, *Carbohydr. Res.*, **28,** 150 (1973).
3. M. Girard, J. Belanger, J. W. Apsimon, F.-X. Garneau, C. Harvey, and J.-R. Brisson, *Can. J. Chem.*, **68,** No. 11 (1990).
4. O. A. Drozdova, S. A. Avilov, A. I. Kalinovskii, V. A. Stonik, Yu. M. Mil'grom, and Ya. V. Rashkes, *Khim. Prir. Soedin.*, No. 2, 242 (1993).