NEW GLYCOSIDES FROM THE HOLOTHURIAN

Cucumaria japonica

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Four new compounds have been isolated from the fraction of weakly polar triterpenoid glycosides of the holothurian Cucumaria japonica: cucumariosides A1-2 (I), A2-1 (II), A0-2 (III), and A5-3 (IV). The structures of these substances have been established by chemical and physical methods.

A fraction of weakly polar glycosides of the holothurian Cucumaria japonica was obtained in [1] by chromatography on silica gel and did not undergo further separation by the usual methods. The desulfation of this fraction with the subsequent separation of the desulfation products on silica gel and by HPLC has led to the isolation of the individual derivatives (V), (VI), (VII), and (VIII), the amounts of which were 35.4, 4.0, 7.6, and 15.2%, respectively, on the weight of the fraction of desulfated derivatives.

A comparison of the 13C NMR spectra of (V) and of the desulfated derivative of cucumarioside A4-2 (IX) [1] permitted the conclusion that in (V) an acetate group at C-6 and 20.6 ppm) was attached to the C-6 atom of the terminal glucose residue. In fact, in (V) the C-6 signal of one of the glucose residues was shifted from 62.5 to 64.5 ppm, and the C-5 signal from 78.1 to 75.1 ppm, which is explained by the acetylation effect [2]. The assignment of these signals to the terminal glucose residue was confirmed by taking a series of partially relaxed spectra, in which slower relaxation is observed for a terminal monosaccharide residue than for the other monosaccharide units [3].

The mass spectra of (V) confirmed the proposed structure. In the LSIMS(+) (matrix - glycerol + NaCl) and LSIMS(-) (matrix - glycerol) spectra the peaks of ions with m/z 1267 (M + Na)+ and 1243 (M - H)-, respectively, were observed. The alternative splitting out from the latter of fragments with 132 and 204 a.m.u. (ions with m/z 1111 and 1039) showed the presence of terminal pentose and acetylhexose units in the molecule. The subsequent detachment of sugar residues led to ions with m/z 877 (1039 - 162), 745 (877 - 132), 599 (745 - 146), and 467 (AgI0+).

The structure of (V) was confirmed by the fact that when it was treated with a deacetylating agent (solution of NH3 in 50% ethanol) it gave (IX) in quantitative yield, while the analogous treatment of the initial total mixture of glycosides did not change the amount of minor glycosides present in the initial fraction but converted the (I) into the considerably more polar cucumarioside A1-2 (X) [1]. Thus, the structure of the aglycon, the linkage sequence of the monosaccharide residues in the carbohydrate chain, and the position of the acetyl group were established by the comparative study of (V) and (IX). The position of the sulfate group became clear after (X) had been obtained as described above. It was also confirmed by the presence in the LSIMS(-) spectrum of (X) of a sulfur-containing anion with m/z 679 arising as the result of a single-stage or multistage elimination of four nonsulfated carbohydrate units from the (MNa - Na)− ion with m/z 1281. Thus, cucumarioside A1-2 (I) is 3β-O-(6-O-acetyl-β-D-glucopyranosyl-(1 → 3)-O-β-D-glucopyranosyl-(1 → 4)-[O-β-D-xylopyranosyl-(1 → 2)]-O-β-D-quinovosyl-(1 → 2)-4-O-(sodium sulfato)-β-D-xylopyranosyloxy)holosta-7,25-dien-16-one. A1-2 (I) is the first glycoside containing an acetyl group in the carbohydrate chain to have been isolated from a holothurian.

*Deceased.

The treatment of this fraction of glycosides with ammonia solution led to the separation of the glycosides containing an acetate group in the carbohydrate chain and, accordingly, to a considerable "simplification" of the residual mixture of substances, which permitted it to be separated by the HPLC method. This gave glycosides (III) and (IV) in the individual form, while (II) was obtained in the form of a fraction weighing 20 mg that, according to NMR, contained about 85% of the main substance. Analysis of spectra permitted the conclusion that the glycosides contaminating the (II) had the same carbohydrate chains and differed by the structures of the aglycons.

A comparison of (II), (III), (IV), the previously described frondoside A (XI) [4], and the desulfated derivatives (VI), (VII), and (VIII) showed that these glycosides had identical carbohydrate chains, consisting of xylose, quinovose, and 3-O-methylglucose in a ratio of 3:1:1 and differed only in the structures of their aglycons, as was confirmed by a comparison of the results of the monosaccharide analysis of the corresponding signals in the $^{13}$C NMR spectra of the carbohydrate moieties of these compounds and the LSIMS$^{(+)}$ spectra of the native samples of (III) and (IV), containing the peaks of carbohydrate fragments with m/z 741, 609, and 477 that are characteristic for frondoside A [4].

A comparison of the $^{13}$C NMR spectra of (III) and of frondoside A showed that (III) differed from (XI) only by the presence of an additional double bond in the side chain of the aglycon. In actual fact signals appeared at 145.9 and 110.9 ppm in the $^{13}$C NMR spectra and at 4.77 ppm (2H-26) and 1.68 ppm (CH$_3$-27) in the $^1$H NMR spectrum, which are characteristic for a terminal 25(26)-double bond. The mass number of the (M$_{Na} + Na$)$^+$ ion in the LSIMS$^{(+)}$ spectrum of (III) with m/z 1355 was two units less than for the analogous ion from frondoside A. The catalytic hydrogenation of (III) led to the dihydro derivative (XI), coinciding completely in its physicochemical characteristics and spectra with frondoside A.

Consequently, cucumarioside A$_0$-2 (III) is 16-acetoxy-3β-{O-[3-O-methyl-β-D-glucopyranosyl]-

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**Fig. 1**

**Fig. 2**