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NEW ASTEROSAPONINS FROM THE STARFISH *Dismolasterias nipon*

I. I. Kapustina, A. I. Kalinovskii,
S. G. Polonik and V. A. Stonik

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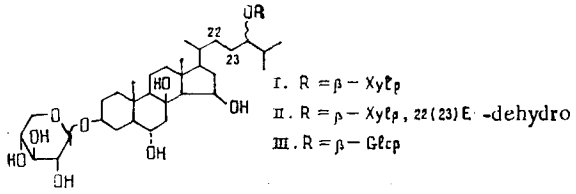
Three new glycosides, D_1, D_2, and D_3, have been isolated from the Far Eastern starfish *Distolasterias nipon*. They have been identified by chemical and physicochemical methods as 5α-cholestane-3β,6α,8β,15β,24ξ-pentaol 3,24-di-O-β-D-xylopyranoside, tα-cholest-22-ene-3β,6α,8β,15β,24ξ-pentaol 3,24-di-O-β-D-xylopyranoside (II), and 5α-cholestane-3β,6α,8β,15β,24ξ-pentaol 24-O-β-D-glucopyranoside 3-O-β-D-xylopyranoside (III).

Asterosaponins are a group of physiologically active steroid saponins present in extracts of starfish [1-3]. Recently, Italian chemists have reported the isolation of two asterosaponins of a new structural type having monosaccharide residues attached to C-3 and C-24 of the aglycon [4].

In studying physiologically active substances from mass species of Far Eastern marine invertebrates, we have isolated three new glycosides belonging to this group from extracts of the starfish *Distolasterias nipon*.

The structures of asterosaponins D_1 (I), D_2 (II), and D_3 (III) were determined by chemical and physicochemical methods.



I. R = β-Xylp
II. R = β-Xylp, 22(23)E-dehydro
III. R = β-Glcp

The structures of the aglycons of glycoside (I) and (III) were established with the aid of spin-decoupling experiments (high-resolution ^{1}H NMR), and also by comparing the ^{1}H and ^{13}C NMR spectra of glycosides D_1 and D_3 (Tables 1 and 2) with the spectra of model compounds: 5α-cholestane-3β,6α,8β,15α,24ξ-pentaol [3] and 5α-cholestane-3β,4β,6α,8β,15β,24ξ-hexaol [25].

The glycon of glycoside (II) was characterized by the presence of a 22(23)-double bond. Its position was confirmed with the aid of double resonance and difference ^{1}H NMR spectroscopy. The double bond had the E-configuration. In actual fact, signals at 39.9, 139.5, and 127.6 ppm for C-20, C-22, and C-23 (Table 1) were close to the corresponding signals in the spectra of 22E-cholestenes, while in the 22Z-isomers the C-20 signal resonates in a stronger field [6].

To confirm that (II) was a 22(23)-dehydro derivative of glycoside (I) we hydrogenated asterosaponin D_2 over Adams catalyst, and obtained D_1.

The carbohydrate chains of glycosides (I) and (II) included only residues of D-xylose, which was identified in hydrolysates with the aid of TLC, GLC, and chromato-mass spectrometry and by a determination of specific rotation. At the same time, the presence of ^{13}C NMR spectra of (I) and (II) (Table 1) of two signals of anomeric carbon atoms showed that each of

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TABLE 1. ^{13}C NMR Spectra of Glycosides (I), (II), and (III) (C_5D_5N, δ, TMS = 0)

Atom	δ, ppm			Atom	δ, ppm		
	I	II	III		I	II	III
C-1	39,0	39,0	38,9	C-20	35,5	39,9	35,4
C-2	29,7	29,8	29,6	C-21	18,4	20,7	18,5
C-3	78,3[a]	78,4[a]	78,6	C-22	32,1	139,5	32,1
C-4	29,0	29,0	29,0	C-23	28,3	127,6	28,5
C-5	53,5	53,6	53,5	C-24	84,6	86,7	84,9
C-6	66,5	66,5	66,4	C-25	31,3	33,1	31,4
C-7	49,7	49,9	49,8	C-26	19,0	19,0	18,9
C-8	76,5	76,6	76,5	C-27	18,3	18,2	18,1
C-9	57,0	56,8	56,9	C′-1	102,8	102,9	102,8
C-10	37,5	37,5	37,4	C″-1	104,8	104,5	104,5
C-11	19,3	19,2	19,2	C′-2	75,3[b]	75,3[d]	75,0
C-12	42,2	42,5	42,1	C″-2	74,9[b]	75,0[d]	75,6
C-13	43,7	43,7	43,7	C′-3	78,2[a]	78,2[a]	78,3[a]
C-14	61,7	61,9	61,7	C″-3	78,1[a]	78,3[a]	78,1
C-15	70,1	70,1	70,1	C′-4	71,2	71,1[b]	71,2
C-16	42,5	43,0	42,5	C″-4	71,2	71,2	72,2
C-17	57,0	56,9	56,7	C′-5	67,0[c]	67,0[c]	67,0[c]
C-18	16,5	16,7	16,6	C″-5	66,9[c]	67,1[c]	77,8
C-19	14,1	14,1	14,1	C″-6			63,3

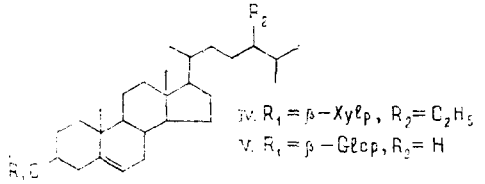
a, b, c, d — assignment of the signals ambiguous.

these compounds contained two xylose residues. Signals almost coinciding with one another, which were close to the corresponding signals in the ^{13}C NMR spectrum of methyl β-xylopyranoside [7], corresponded to the monosaccharide residues. Consequently, in glycosides (I) and (II) both xylopyranose fragments were attached directly to the aglycon by β-glycosidic bonds.

In contrast to these two glycosides, asterosaponin D_3 (III) contained D-xylose and D-glucose residues, the presence of which was shown by TLC, GLC, and GLC-MS analyses of hydrolysates. A comparison of the signals of the carbohydrate moiety in the ^{13}C NMR of (III) with the spectrum of a comparison sample of methyl β-glucopyranoside [7] showed that the glucose residue was present in the pyranose form and was attached to the aglycon by a β-glucosidic bond.

A comparison of the ^{13}C NMR spectra of compounds (I-III) with the spectrum of 5α-cholestane-3β,6α,8β,15α,24ξ-pentaol 24-O-(3-O-methyl-α-L-arabinofuranoside) [3] showed that there was good agreement of the C-23, C-24, and C-25 signals for these glycosides. Consequently, the glycosides from *Distolasterias nipon* each had one monosaccharide residue attached to C-24. The second monosaccharide in each of these glycosides was attached to C-3, since in the ^{13}C NMR spectra the C-3 signals were shifted downfield by 7 ppm, and the C-2 and C-4 signals upfield by 2.2 and 4 ppm as compared with the spectrum of an arabinoside unsubstituted at C-3, because of the glycosylation effect [8].

In order to determine to which of the atoms in glycoside (III) C-2 or C-24, the xylose residue was attached to which the glucose residue, we synthesized the two model compounds (IV) and (V) and compared their spectra with those of (III).



The ^{13}C NMR spectrum of (IV) had the signal of an anomeric carbon atom of a monosaccharide residue at 103.3 ppm, and in the spectrum of (V) the analogous signal was observed at 102.5 ppm.

In the spectrum of glycoside (III), two corresponding signals were observed at 102.8 and 104.5 ppm. In view of the fact that the double bond in ring B of the model compounds causes a downfield shift of the C-1 signal of about 0.5 ppm [8], it may be concluded that in compound

TABLE 2. ^{1}H NMR Spectra of Glycosides (I), (II), and (III) (C_5D_5N, δ, TMS = 0)

Atom	δ, ppm (J, Hz)		
	I	II	III
H-3	4,03m	4,00m	4,02m
H-4e	3,19dm(12,5)*	3,19dm(12,5)	3,19dm(12,5)
H-4a	1,61q(10,0; 12,0; 12,0)	1,51q	
H-5a	1,3 m(10,0; 12,5; 3,0)	1,35m(10,3; 12,8; 3,0)	1,39m
H-6a	4,23td (11,2; 4,2)	4,24td (11,0; 4,2)	4,27 m
H-7e	3,00dd(12,5; 4,25)	3,00dd(12,5; 4,25)	3,02dd(12,5; 4,5)
H-7a	1,69t(12,5; 11,2)	1,71t(12,5; 11,0)	1,72t
H-14a	1,03d(5,5)	1,07d(5,0)	1,06 d
H-15a	4,70m	4,67m	4,65m
H-16α	1,89m	1,75m	1,76 m
H-16β	2,54 dt	2,44dt	2,57m
H-17	1,07 q	1,05 q	
3H-18	1,60s	1,59s	1,58s
3H-19	1,25s	1,26s	1,27s
3H-21	1,06d(7,5)	1,12d(7,5)	1,05d(7,5)
H-24	3,67m	4,05t	3,70m
H-25	2,05m	2,03 m	2,10m
3H-26	1,00d(8,0)	1,04d(7,5)	1,12d(7,5)
3H-27	1,05d(8,0)	1,07d(7,5)	1,07d(7,5)
H_1'	4,95d(7,5)	4,95d(7,3)	4,96
H_1''	4,80 d(7,5)	4,82d(7,2)	4,91
H-22,23		5,6 m	

*Resolution of the signal 0.25 Hz/point.

(III) the xylose residue was attached to C-3. In actual fact, in this case the 102.8 ppm signal in its spectrum relates to C-1 of the xylose residue, which agrees with the calculated value obtained from the spectrum of the model compound β-sitosterol β-xylopyranoside. Furthermore, good agreement was observed of the signals of the protons at C-1 in the monosaccharide fragments in the ^{1}H NMR spectrum of glycoside D_3 (4.96 ppm) and the model β-xylopyranoside (4.94 ppm).

Thus, three new glycosides — asterosaponins D_1, D_2, and D_3 have been isolated from extracts of the Far Eastern starfish *Distolasterias nipon*. It has been shown that the first of them is 5α-cholestane-3β,6α,8β,15,24ξ-pentaol 3,24-di-O-β-D-xylopyranoside, the second is its 22(23)-dehydro derivative (II), and the third is 5α-cholestane-3β,6α,8β,15β,24ξ-pentaol 24-O-β-D-glucopyranoside 3-O-β-D-xylopyranoside (III).

EXPERIMENTAL

The starfish were gathered in May, 1984 in Peter the Great Bay near Vladivostok.

NMR spectra were obtained on Bruker HX-90E and WM-250 spectrometers (δ, ppm, TMS = 0).

GLC and GLC-MS analyses and the determination of the constants and IR spectra of the substances were performed as described in [9].

Liquid chromatography (LC) was carried out on a Du Pont 8800 chromatograph with a 21 × 250 mm Zorbax ODS 20 μ column at a rate of 4 ml/min.

For TLC, plates with a fixed layer of silica gel L 5/40 μ and, for preparative separation, columns of silica gel L 40/100 μ were used.

Glycosides D_1, D_2, and D_3 were isolated from an ethanolic extract of the animals as described previously [10]. Additional purification was performed on columns containing Sephadex G-10 (with water as the eluent) and Sephadex LH-20 (in the ethyl acetate—ethanol (3:1) system) and with the aid of LC on Zorbax ODS (with 50% aqueous ethanol as the eluent). The glycosides were recrystallized from 40% aqueous ethanol.

Compound (I): mp 285-288°C, $[\alpha]_D^{20}$ —17,9° (*c* 0,24; methanol); (II): mp 281-284°C, $[\alpha]_D^{20}$ —10,8° (c 0.27; methanol); (III): mp 295-298°C, $[\alpha]_D^{20}$ —13,1° *c* 0,15; methanol).

Hydrogenation of D_2 (II). A solution of 7 mg of (II) in methanol was stirred vigorously over Adams catalyst for 5 days at room temperature. The completeness of the occurrence of

the reaction was monitored with the aid of TLC. The reaction mixture was filtered and concentrated in vacuum.

This gave 10 mg of reduced product: mp 272-276°C, $[\alpha]_D^{20}$ −13,7° (*c* 0,3; methanol), which coincided with (I) in TLC and according to the results of ^{1}H spectroscopy.

The acid hydrolysis of D_1, D_2, and D_3 (I, II, and III) was carried out by the procedure described previously [10]. The monosaccharides were compared with standard samples by TLC in the isopropanol–acetic acid–diethyl ether–water (9:6:3:1) system, the spots being detected with aniline phthalate on heating. The GLC and chromato-mass spectrometric analyses of the peracetates of the corresponding aldononitriles were performed in parallel.

2,3,4-Tri-O-acetyl-1-α-bromo-D-xylopyranose (VI). This was synthesized as described in a handbook [12]. The syrup obtained was dissolved in dry diethyl ether, hexane was added to the solution in drops until a turbidity appeared and it was left for 1 h. The colorless crystals that had deposited were filtered off and were washed with cold diethyl ether. According to TLC in the hexane–benzene–acetone (2:1:1) system, the crystals contained about 70% of (VI).

β-Sitosterol 2,3,4-Tri-O-acetyl-β-D-xylopyranoside (VII) and β-Sitosterol 2,3,4-Tri-O-acetyl-α-D-xylopyranoside (VIII). The synthesis was carried out in a Soxhlet microextractor fitted with a magnetic stirrer and a reflux condenser containing a calcium chloride tube. Granulated dehydrated silica gel (7 g) was poured into the thimble of the extractor and its bottom part was charged with 1.05 g (2.54 mmole) of chromatographically pure β-sitosterol, 0.84 g (4.87 mmole) of cadmium carbonate and 25 ml of dry toluene, and the mixture was boiled for 15 min to eliminate moisture.

Then 300 mg of crystals of (VI) and, after 20 min, another 700 mg were added to the boiling reaction mixture, whereupon it acquired a coffee color. After the addition of 1 g of (VI), boiling was continued for 40 min. Then the cadmium salt was filtered off, the residue was washed with chloroform, and the filtrates were combined and evaporated to dryness. The solid residue, weighing 2.02 g, was triturated with absolute methanol to eliminate polar impurities.

This gave 0.78 g of a light yellow powder enriched with the desired reaction products, from which, by column chromatography on silica gel in the hexane–acetone (40:1→35:1) system, (VII) was isolated: 0.653 g (yield 38.6%), mp 192-194°C, $[\alpha]_D^{20}$ −30,4° (*c* 0,42; chloroform). ^{1}H NMR spectrum (ppm, chloroform): 3.48 m (3-H), 5.37 d, J = 5 Hz (6-H), 4.57 d, J = 7 Hz (1-H'), 4.9 dd, J = 7; 9 Hz (2-H'), 5.18 t, J = 8.5 Hz (3-H'), 4.96 m (4-H'), 4.11 dd, J = 5; 11 Hz (5-H'), 3.33 dd, J = 9; 11 Hz (5-H''), 2.07 s (OAc). IR spectrum (ν, cm^{-1}): 1754 (CH_3COO). A small amount of compound (VIII) was also obtained: 0.06 g (yield 3.5%), mp 113-124°C, $[\alpha]_D^{20}$ + 42.2°C (c 0.36; chloroform). IR spectrum (ν, cm^{-1}): 1748 (CH_3COO).

β-Sitosterol β-D-xylopyranoside (IV) and β-sitosterol α-D-xylopyranoside (IX) were obtained by saponifying (VII) and (VIII) in 0.1 N methanolic sodium methanolate at room temperature. The completeness of the reaction was followed by TLC in the chloroform–methanol (4:0.7) system. The saponification products were separated on silica gel columns in the chloroform–methanol (20:1) system.

The following products were obtained. (IV): $[\alpha]_D^{20}$ −41.6° (*c* 0,74; pyridine). ^{1}H NMR spectra (ppm, pyridine): 3.92 m (3-H), 5.40 d, J = 4.5 Hz (6-H), 0.68 s (18-H), 0.97 s (19-H), 1.01 d, J = 6 Hz (21-H), 0.90 dd, J = 5; 7 Hz (26,27-H), 0.91 s (29-H), 4.92 d, J = 7.5 Hz (1-H'). The structure of (IV) was confirmed by its ^{13}C NMR spectrum. (IX): $[\alpha]_D^0$ +39° (*c* 0,29; pyridine. ^{1}H NMR spectrum (ppm, pyridine): 5.38 m (6-H), 0.70 s (18-H), 0.96 s (19-H), 1.02 d, J = 6 Hz (21-H), 0.89 dd, J = 5; 6 Hz (26,27-H), 5.45 d, J = 4 Hz (1-H'). Cholesterol β-D-glucopyranoside (V) was obtained from cholesterol 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (mp 157-159°C, $[\alpha]_D^{20}$ −20° (*c* 0,4; chloroform)) by saponification and was purified by column chromatography on silica gel. Compound (V): mp 270-272°C $[\alpha]_D^{20}$ −52,3° (*c* 0,535; pyridine). Literature figures [11]: mp 262-264°C, $[\alpha]_D^{20}$ − 50 ± 8° (c 2.48; pyridine).

SUMMARY

It has been shown that the glycosides D_1, D_2, and D_3 isolated from the starfish *Distolasterias nipon* are, respectively, 5α-cholestane-3β,6α,8β,15β,24ξ-pentaol 3,24-di-O-β-D-xylopyranoside (I), 5α-cholest-22(23)-ene-3β,6α,8β,15β,24ξ-pentaol 24-O-β-D-glucopyranoside 3-O-β-D-xylopyranoside (III).

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CONFORMATIONAL CHANGES OF GOSSYPULIN IN SOLUTIONS AT VARIOUS pH VALUES

INFLUENCE OF SALTS

Z. S. Yunusova and G. P. Moiseeva

UDC 547.962

Work has continued on the study of the conformational transitions of gossypulin from the seeds of the cotton plant under various conditions. The denaturation of gossypulin as a function of the pH of the medium and the influence of salts (sodium chloride and sodium phytate) on the denaturation process have been studied with the aid of circular dichroism. The gossypulin from cotton plant seeds undergoes complex conformational changes in the pH interval from 2 to 13. Sodium phytate stabilizes the protein molecule at pH 2 and 3.

The conformational transitions of reserve proteins, especially the globulins from cotton plant seeds, have prime value in explaining many physicochemical properties. The change in conformation under the action of many factors is responsible for the biological role of these proteins in seeds [1], while since the globulins are one of the main components of food proteins, they also determine the functional properties of food proteins [2]. The conformational changes in the gossypulin of cotton plant seeds are particularly important since it lacks disulfide bridges, which imparts a high conformational mobility to its subunits [3].

In view of this, we have continued investigations of the conformational changes of gossypulin from cotton plant seeds under various conditions. We have studied the denaturation of gossypulin as a function of the pH of the medium, and the influence of salts (sodium chloride and sodium phytate) on the denaturation process. In the present work we used the method of circular dichroism (CD).

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