NEW POLYHYDROXYSTEROIDS FROM THE FAR-EASTERN STARFISH Henricia sp.

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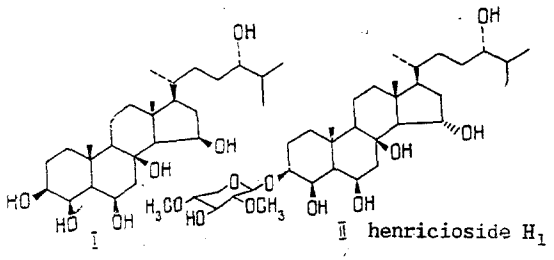
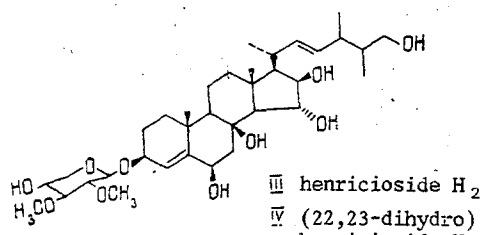
From the far-eastern starfish Henricia sp. we have isolated and characterized the new polyhydroxysteroid (24S)-5α-cholestane-3β,4β,6β,8,15β,24-hexaol and three new glycosides: (24S)-5α-cholestane-3β,4β,6β,8,15α,24-hexaol 3-O-(2,4-di-O-methyl-β-D-xylopyranoside) (henricioside H_1), 24-methyl-5α-cholesta-4,22E-diene-3β,6β,8,15α,16β,26-hexaol 3-O-(2,3-di-O-methyl-β-D-xylopyranoside) (henricioside H_2), and the 22,23-dihydro derivative of henricioside H_2 (henricioside H_3).

In an investigation of an ethanolic extract of the starfish Henricia sp., with the aid of column chromatography on the resin Amberlite XAD-2, on Sephadex LH-20, on silica gel, and on Florisil we have obtained the polyhydroxysteroid (I) and glycosylated polyhydroxysteroids – henricioside H_1 (II), H_2 (III), and H_3 (IV). The structures of compounds (I)-(IV) have been established by NMR spectroscopy. Spectral characteristics are given in Tables 1-3. In determining the positions and orientations of the oxygen substituents in the compounds studied, we made use of difference spin-decoupling experiments. To refine the CSs of the carbon atoms we recorded J-modulated ^{13}C NMR spectra.



I

II henricioside H_1



III henricioside H_2

IV (22,23-dihydro) henricioside H_3

The assignment of the CSs of the protons and of the carbon atoms in the NMR spectra of compound (I) (C_5H_5N) for rings A, B, and C of the steroid nucleus was made by a comparison with the spectra of crossasteroside P_2 (V) from the starfish Crossaster papposus [1]. The signals relating to ring D and the side chain agreed well, when glycosylation effects were taken into account, with the corresponding signals for culcitoside C_1 (VI) – a 24-O-glycosylated polyhydroxysteroid from the starfish Culcita novaeguineae [2, 3]. The CSs of the carbon atoms in the side chain of (I) (CD_3OD) coincided with analogous signals for (24S)-5α-cholestane-3β,4β,6α,8,15β,24-hexaol (VII) from the starfish Gomophi watsoni [4], on the basis of which we proposed the (S)-configuration at C-24 in the compound (I) that had been isolated.

Thus, the structure of compound (I) was established as (24S)-5α-cholestane-3β,4β,6β,8,15β,24-hexaol. 24-Hydroxysteroids are found mainly as the aglycons of starfish glycosides,

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TABLE 1. Chemical Shifts and Multiplicities of the Signals of the Protons of Compounds I and II (C_5D_5N; TMS = 0; δ, ppm; J, Hz)

Proton	I	II*
H-2	2,28 qd (3,5; 12,5)	2,35 qd (13,0; 3,0)
H-3	3,84ddd (3,5; 4,8; 12,0)	3,88 (3,67) (ddd 3,5; 5,0; 12,5)
H-4	4,47 m	4,50 (4,27) m
H-5	1,30t (2,0)	1,25 m
H-6	4,44 m	4,50 (4,27) m
H-7e	2,72 dd (2,7; 14,0)	3,15 (2,42) dd (3,0; 15,0)
H-7a	1,68 dd (3,0; 14,0)	2,04 (1,62) dd (3,1; 15,0)
H-14	0,95 d (5,5)	1,54d (10,0)
H-15	4,59 ddd (1,8; 5,5; 7,3)	4,84 (4,30) td (9,0; 4,0)
H-16	2,43 dt (14,4; 7,6)	2,15m
H-16′	1,75 m	2,10m
H-17	1,08 m	
H-24	3,58 m	3,56 m
H-25	1,85 m	1,83 m
CH_3-18	1,55 s	1,26 (0,96) s
CH_3-19	1,80 s	1,82 (1,45) s
CH_3-21	1,05 d (6,5)	1,05 (0,94) d (5,8)
CH_3-26	1,10 d (6,7)	1,10 (0,92) d (6,5)
CH_3-27	1,15 (6,7)	1,12 (0,90) d (6,5)
H-1′		4,80 (4,46) d (5,7)
H-2′		3,38 (2,93) dd (5,8; 9,0)
H-3′		4,00 (3,45) t (8,)
H-4′		3,56 (3,18) m
H-5′		4,28 (4,02) dd (5,0; 12,0)
H-5″		3,42 (3,16) t (11,0)
OMe		3,54 (3,46) s
OMe		3,78 (3,63) s

*In addition, the CSs of the protons in the spectrum taken in CD_3OD are given in parentheses.

a carbohydrate chain of one or two monosaccharide residues being attached to the C-24 side chain in such glycosides. Before our isolation of (I) only two free 24-hydroxysteroids had been found in starfish [4].

The acid hydrolysis of glycoside (II) gave a monosaccharide that was identified as 2,4-di-O-methyl-D-xylose (TLC, GLC, specific rotation). Analysis of the partially relaxed ^{13}C NMR spectrum of (II) revealed the signals of the carbon atoms of the monosaccharide residue. The spectral characteristics for the 2,4-di-O-methyl-D-xylose unit of compound (II) corresponded to the analogous characteristics for the 2,4-di-O-methyl-β-D-xylopyranose residue of culcitoside C_1 from the starfish C. novaeguineae [2].

The positions and orientations of the hydroxy groups in the aglycon of glycoside (II) were established by comparing its PMR spectra with the spectra of laeviuscoloside G (VIII) from the starfish Henricia laeviuscola [5]. We established that in glycoside (II), as in compound (VIII), hydroxy groups of the steroid nucleus were present in the 3β,4β,6β,8,15α-positions, the monosaccharide residue being attached at C-3 of the aglycon. On the irradiation of H-1' in the PMR spectrum of (II), a nuclear Overhauser effect was observed at the positions of the signals of protons H-3 and H-4. Selective decoupling from protons in the ^{13}C NMR spectrum of (II) showed that the CS of C-3 ws 79.0 ppm; i.e., the signal was shifted downfield in agreement with the α-effect of glycosylation as compared with the C-3 signal of steroid (I) (72.3 ppm) [3]. In the ^{13}C NMR spectrum of (II), in agreement with the β-effect of glycosylation, the C-2 signal (24.8 ppm) was shifted downfield as compared with C-2 in the spectrum of compound (I) (27.0 ppm) [3]. These results additionally confirmed the position of attachment of the monosaccharide residue in (II). The coincidence of the spectral characteristics of the side chains of compounds (I) and (II) showed their identity.

On the basis of what has been said above, the structure of henricioside H_1 was established as (24S)-5α-cholestane-3β,4β,6β,8,15α,24-hexaol 3-O-(2,4-di-O-methyl-β-D-xylopyranoside).

The acid hydrolysis of compounds (III) and (IV) gave one and the same monosaccharide, which was identified as 2,3-di-O-methyl-D-xylose (TLC, GLC, GLC-MS; specific rotation). As the result of an investigation of a number of partially relaxed ^{13}C NMR spectra of (III), we

TABLE 2. PMR Spectra of Compounds I, II, III, IV (δ, ppm; TMS = 0)

Atom	I (C_5D_5N)	I (CD_3OD)	II (C_5D_5N)	III (C_5D_5N)	III (CD_3OD)	IV (CD_3OD)
C-1	40,5	41,0	40,3	39,0	39,7	39,7
C-2	27,0	26,6	24,8	27,6	27,9	27,8
C-3	72,3	73,1	79,0	76,0	77,3	77,2
C-4	76,5	77,3	73,6	125,7	126,7	126,6
C-5	50,7	51,2	49,9	148,4	148,4	148,4
C-6	75,4	76,2	75,4	75,5	76,3	76,3
C-7	43,6	44,1	45,0	44,5	44,5	44,6
C-8	78,0	79,0	75,7	75,3	76,0	76,0
C-9	56,8	57,6	57,1	57,2	57,9	57,8
C-10	36,2	36,8	36,2	37,2	37,7	37,7
C-11	18,8	19,1	18,8	19,1	19,5	19,5
C-12	41,8	41,9	42,0	42,6	43,1	43,1
C-13	43,6	44,1	44,7	44,5	44,2	45,1
C-14	61,2	61,8	66,2	63,6	63,6	63,7
C-15	70,0	71,3	68,9	79,7	80,1	80,8
C-16	42,4	43,2	41,6	82,9	83,3	82,9
C-17	57,2	58,0	55,3	60,5	61,0	60,7
C-18	16,5	16,6	15,4	17,1	17,0	16,9
C-19	18,6	18,6	18,5	22,6	22,7	22,7
C-20	35,6	36,4	35,5	34,0	34,6	30,8
C-21	19,0	19,4	19,0	20,7	20,8	18,5
C-22	32,8	33,3	32,9	136,1	35,6	34,7
C-23	31,7	31,7	31,7	133,5	134,4	30,5
C-24	77,0	78,1	76,5	39,9	40,6	36,1
C-25	34,1	34,5	34,0	41,8	42,2	41,9
C-26	19,7	19,4	19,7	65,7	66,8*	66,6
C-27	17,5	17,5	17,5	17,6	17,7	17,5
C-28				14,6	14,5	14,0
C-1′			101,5	103,9	104,3	104,3
C-2′			84,6	84,3	84,8	84,7
C-3′			76,5	87,3	87,3	87,2
C-4′			80,6	70,3	70,9	70,8
C-5′			64,0	66,6	66,7*	66,6
OMe			60,6	60,1	60,8	60,7
OMe			58,7	60,5	60,9	60,7

*Assignment of the signals ambiguous.

determined the signals of the carbon atoms belonging to the monosaccharide residue, which enabled these signals to be determined in the spectrum of (IV).

The CSs and SSCCs of the protons in the PMR spectrum of (III) (CD_3OD) agreed well with the spectrum of the aglycon moiety of desulfated echinasteroside A (IX) from the starfish Echinaster sepositus [6]. A comparison of the signals of the carbon atoms of the aglycon of glycoside (III) with the corresponding signals of echinasteroid A (X) showed that all the CSs of the carbon atoms were close for the two structures, with the exception of the C-14, C-15, and C-16 signals, since in echinasteroid A the hydroxy group at C-15 is sulfated, while in (III) it is free. The CSs of C-14, C-15, and C-16 in the ^{13}C NMR spectrum of (III) proved to be identical with the corresponding signals in the spectrum of (25S)-5α-cholestane-3β,6α,8,15α,16β,26-hexaol (XI) from the starfish Poraster superbus [7].

It was shown by a comparison of the spectral characteristics of glycosides (III) and (IV) that, unlike glycoside (III), compound (IV) has no double bond in its side chain. The assignment of the signals of the carbon atoms in the side chain of (IV) was made on the basis of its J-modulated ^{13}C NMR spectrum in comparison with literature information for echinasteroside B (XII) from the starfish H. laeviuscola and for compound (XI) [5, 7].

As a result, the structure of henricioside H_2 was determined as 24-methyl-5α-cholesta-4,22E-diene-3β,6β,8,15α,16β,26-hexaol 3-O-(2,3-di-O-methyl-β-D-xylopyranoside). Henricioside H_3 is its 22,23-dihydro analogue. This is the first time that 2,3-di-O-methyl-D-xylose has been found in polyhydroxysteroid glycosides.

EXPERIMENTAL

For general information on the methods used, see [8]. The animals were collected from on board the SRTM-K [Medium Fishing Trawler-Freezer, K-type] Briz in July, 1990 in the Sea of Okhotsk near the island of Onekotan (Kurile Islands) from a depth of 20-100 m.

TABLE 3. Chemical Shifts and Multiplicities of the Signals of the Protons of Compounds III and IV (CD_3OD; TMS = 0; δ, ppm; J, Hz)

Proton	III*	IV
H-3	4,20 (4,40) m	4,20 m
H-4	5,65 (5,84) m	5,64 m
H-6	4,31 (4,65) t (3,[illegible])	4,31 t
H-7e	2,57 (3,2) dd (15,0; 3,2)	2,60 dd (15,0; 3,0)
H-7a	1,5) (1,97) dd (15,0; 3,5)	1,52 dd (15,0; 3,0)
H-14	(1,42) d (10,7)	
H-15	4,16 (4,87) dd (10,7; 2 6)	4,18 dd (10,7; 2,
H-16	3,90 (4,47) dd (7,2; 2,6)	3,99 dd (7,0; 2.5
H-22′	5,43 (5,66) m	
H-23	5,43 (5,66) m	
CH_3-18	1,16 (1,59) s	1,15 s
CH_3-19	1,37 (1,61) s	1,38 s
CH_3-21	1,02 (1,18) d (6,7)	0,93 d (6,5)
H-26	3,50 (3,74) dd (10,7; 6,0)	3,55 dd (10,8; 6,5)
H-26′	3,38 (3 65) dd (10,7; 6,2)	3,35 dd (11,0; 7,0)
CH_3-27	0 87 (0,98) d (7,5)	0,88 d (7,0)
CH_3-28	0,96 (1,01) d (7,5)	0,89 d (7,0)
H-1′	4,42 (4,71) d (7,5)	4,42 d (7,5)
H-2′	2,86 (3,28) dd (7,5; 9,0)	2,87 dd (7,5; 9,0)
H-3′	3,03 (3,40) t (8,8)	3,03 t (9,0)
H-4′	3,5) (3,98) m	3,50 m
H-5′	3,79 (4,15) dd (5,5; 11,0)	3,79 dd (5,5; 11,0)
H-5″	3,16 (3,53) t (11,0)	3,16 t (11,0)
OMe	3'55 (3,6) s	3,55 s
OMe	3'60 (3,77) s	3,60 s

*In addition, the CSs of the protons in the spectrum taken in C_5D_5N:CD_3OD (5:1) are given in parentheses.

Isolation of Compounds (I)-(IV). An ethanolic extract of the starfish (weight of the crude animals 13.5 kg) was concentrated in vacuum, diluted with water, and passed through a column of the resin Amberlite XAD-2. The column was washed with water and then with methanol. The methanolic eluate was evaporated. The total steroid fraction obtained was chromatographed successively on columns of Sephadex LH-20 in the chloroform–methanol (1:1) system, silica gel in the chloroform–methanol (from 10:1 to 1:1) system, and of Florisil in the chloroform–ethyl acetate (from 1:1 to 1:3) system. This gave 70 mg of steroid (I) (0.0005%), 113 mg of glycoside (II) (0.0008%), 265 mg of glycoside (III) (0.002%), and 42 mg of glycoside (IV) (0.0003%).

(24S)-5α-Cholestane-3β,4β,6β,8,15β,24-hexaol (I), $C_{27}H_{48}O_6$, mp 274-275°C, $[\alpha]_{Hg}$ −1.9° (c 0.80; methanol). Mass spectrum (m/z, %): 468 (4 M^+), 450(17), 432(37), 414(29), 412(28), 373(25), 371(8), 304(42), 296(54), 295(17), 265(100), 199(33).

Henricioside H_1 (II), $C_{34}H_{60}O_{10}$, amorphous, $[\alpha]_{Hg}$ −4.9° (c 0.93; methanol).

Henricioside H_2 (III), $C_{35}H_{56}O_{10}$, amorphous, $[\alpha]_{Hg}$ −24.7° (c 1.15; methanol).

Henricioside H_3 (IV), $C_{35}H_{58}O_{10}$, amorphous, $[\alpha]_{Hg}$ −23.8° (c 2.10; methanol).

Hydrolysis of Henriciosides H_1, H_2, and H_3. The acid hydrolysis of (II)-(IV) was carried out with 2 N HCl at 100°C for 2 h. 2,4-Di-O-methyl-D-xylose ws identified in the hydrolysate of (II), and 2,3-di-O-methyl-D-xylose in the hydrolysates of (III) and (IV) by TLC on silica gel and Silufol in the butanol–acetone–water (4:5:1) sytem and by GLC and GLC-MS in the form of aldononitile peracetates.

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ANALYSIS OF GLYCOSIDES OF Allochruza gypsophiloides IN THE PREPARATION "ALLOKHROZID" BY CHROMATOSPECTROPHOTOMETRY

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The possibility has been shown of using a readily available method for estimating the level of the main glycoside of the organs of the plant soapwort – acanthophylloside B – in commercial products used in the food and medical industries, and also in samples of the preparation Allokhrozid. The amount of the individual glycoside was calculated in relation to a standard sample of acanthophylloside B.

Medicinal forms based on plants containing triterpene glycosides are manufactured by the medical industry for the treatment and prevention of a number of diseases of man and animals. The most popular include medicinal forms obtained from ginseng, Manchurian aralia, and eleutherococcus – plants of the family Araliaceae. Many plants of the family Caryophillaceae form the raw material for obtaining soap substitutes and in the production of shampoos, effervescent beverages and halvas, and also in industry. The rising demand for these plants as a raw material for the industrial manufacture of such products requires the obligatory standardization of the initial raw material and the monitoring of the production steps and of the final product [1-4]. In the data bank on methods of monitoring the products on the basis of triterpene derivatives, in the main, two methods of preparing the material for analysis are known. The first is the hydrolysis of the glycosides, followed by the determination of the products so obtained – mainly aglycons and their degradation products. The second is the direct determination of the glycosides using some reactions or other.

The first method is not always suitable in view of the ambiguity (nonreproducibility) of the results of the analysis. This is due to a number of factors: the lability of the aglycon, the poor solubility of the initial preparation or of the products of its incomplete hydrolysis, and their inhomogeneity, which is connected with the complex structure of the carbohydrate chains of the glycosides.

When the second method is chosen, it is possible to determine both the total amount of glycosides and also the amounts of the individual components from the intensities of the coloration of the derivatization products of the glycosides. Their color reactions with H_2SO_4 must be included among the known reactions used for the quantitative estimation of triterpene glycosides. A method of photometric determination is based on this reaction. The reliability of the method depends largely on the degree to which the product being analyzed has been freed from impurities. Various forms and methods of chromatography are used to separate the glycosides to be determined from accompanying substances, including those close in structure.

The aim of the present work was to study the possibility of using the spectrophotometric method for analyzing the preparation Allokhrozid, obtained from the roots of Allochruza gypsophiloides (synonym – Acanthophyllum gypsophiloides). The method is based on the

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