CULCITOSIDE C_1 FROM THE STARFISH *Culcita novaeguineae* AND *Linckia guildingi*

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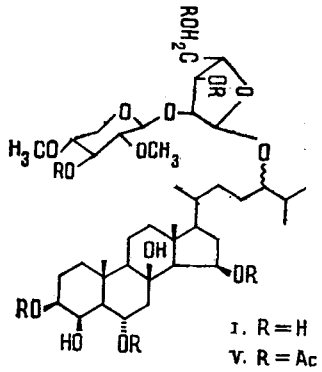
By repeated chromatography on Polikhrom-1, silica gel, and Florisil, ethanolic extracts of two species of starfish, *Culcita novaeguineae* and *Linckia guildingi*, have yielded the new steroid glycoside culcitoside C_1 (I): 5α-cholestan-3β,4β,6α,8,15β,24ξ-hexaol 24-O-[2,4-di-O-methyl-β-D-xylopyranosyl-(1 → 2)-α-L-arabinofuranoside], $C_{39}H_{68}O_{14}$, mp 245-248°C, $[\alpha]_D$ −35.8°, c 0.6; methanol). Its structure was shown by the results of acid hydrolysis, acetylation and methylation, and ^1H and ^{13}C NMR spectroscopy.

Investigating the glycoside composition of the tropical starfish *Culcita novaeguinae*, we have isolated the main component of the glycosidic mixture, culcitoside C_1 (I). Its structure has been established by chemical and physicochemical methods as 5α-cholestane-3β,4β,6α,15β,24ξ-hexaol 24-O-[O-(2,4-di-O-methyl-β-D-xylopyranosyl)-(1 → 2)-α-L-arabinofuranoside].



I. R = H
V. R = Ac

The arrangement and orientation of the hydroxy groups in the aglycone were determined by double-resonance experiments on protons and by a comparison of the ^1H NMR spectra of glycoside (I) (Table 1) with two model polyhydroxysteroids which we have investigated previously: 5α-cholestane-3β,4β,6α,7α,8,15α,16β,26-octaol (II) [1] and 5α-cholestane-3β,6α,8,15α,24ξ-pentaol 24-O-(3-O-methyl-α-L-arabinofuranoside) (III) [2]. The close values of the chemical shifts and spin-spin coupling constants of the protons of glycoside (I) and the corresponding values for the octaol (II) showed the presence of a 3β,4β,6α-tetrahydroxy fragment in (I). On comparing the ^1H NMR spectra of glycosides (I) and (III), we established that the carbohydrate chain in (I) was attached to C-24 and the extra hydroxy group of the aglycone to C-15. Its orientation was determined from the spin-spin coupling constant as β ($J_{14,15}$ = 5.6 Hz), while with the 15α orientation of the hydroxy group the values lies between 9.8 and 11 Hz [1, 2].

Recently, Italian authors have described a bioside similar to glycoside (I) from *Hacelia attenuata* [3] for which they gave only the ^{13}C NMR spectrum. The coincidence of the signals of carbon atoms C-1–C-27 in the ^{13}C NMR spectrum of (I) (Table 2) with the analogous signals of the bioside from *Hacelia attenuata* confirmed that the aglycone of (I) is 5α-cholestane-3β,4β,6α,8,15β, 24ξ-hexaol.

The acid hydrolysis of (I) gave two monosaccharides, which were identified as arabinose and 2,4-di-O-methylxylose (TLC, GLC, and chromato-mass spectrometry). From the specific rotations

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TABLE 1. ^{1}H NMR Spectrum of Glycoside (I) (C_5D_5N, δ, TMS = 0).

Proton	δ, ppm	Proton	δ, ppm
H-3	3 96 m	CH_3-26	1,02 d
H-4	5,24 t	CH_3-27	1,04 d
H-5	1 47 dd (J=2,3 and 10,6 Hz)	H-1′	5,70 d (J=1,0 Hz)
H-6	5,06 td (J=10.6 10,6 and 4,3 Hz)	H-2′	4,81 dd
H-7e	3,14dd (J=12,2 and 4,4 Hz)	H-3′	4,88 m
H-7a	1,84dd (J=12.2 and 10,7 Hz)	H-4′	4,75 m
H-14	1,10d (J=5,6 Hz)	H-5′	4,42 dd
H-15	4,74m	H-5′	4,29 dd
H-16	2,63dt	H-1″	4,92 d (J=7,5 Hz)
H-16′	1,74m	H-2″	3,40 dd
H-17	1,08td	H-3″	3,95 t
H-24	3,66m	H-4″	3,53 m
H-25	2,05 m	H-5″	4,20 dd
CH_3-18	1,62 s	H-5″	3,32 t
CH_3-19	1,85 s	OMe	3,54 s
CH_3-21	1,60 d	OMe	3,73 s

TABLE 2. ^{13}C NMR Spectra of Compounds (I), (IV), and (V) (C_5D_5N, δ, TMS = 0)

Atom	I	V	Atom	I	V	Atom	I	V	Atom	IV
C1	39,4	39 7	C14	61 8	60,7	C27	18,0	17,9	C1	105,5
C2	26,8	22,5	C15	70 0	74,8	C1′	107 2	107,2	C2	84,6
C3	73,0	76 4	C16	42,2	38,7	C2′	92 9	87,9	C3	76,2
C4	68,9	65,5	C17	57,2	56 6	C3′	77,6	78,9	C4	80,6
C5	57,2	53,5	C18	16,6	15,5	C4′	84,1	80,1	C5	63,9
C6	63,9	68,4	C19	17,3	17 0	C5′	62 3	64,3	OMe	60,6
C7	50,4	45,3	C20	35 6	35,2	C1″	104,8	103 6	OMe	58 7
C8	76 5	75,6	C21	18,8	18,6	C2″	84,8	81 6	OMe	50,4
C9	57,7	57,4*	C22	32 2	31,5	C3″	76,1	75,2		
C10	37,7	38 0	C23	27,9	27,7	C4″	80,5	77,8		
C11	18,5	18 4	C24	83,1	83,2	C5″	63,9	63,6		
C12	42,4	42 1	C25	30 6	30,6	OMe	60,7	60,3		
C13	43 7	44,1	C26	18,1	17,9	OMe	58 9	58,2		

*Assignment of the signals ambiguous.

of the monosaccharides, we assigned the arabinose to the L series and the 2,4-di-O-methylxylose to the D series.

To determine the sequence of the monosaccharides in the carbohydrate chain we evaluated the times of spin-lattice relaxation of the carbon atoms of the monosaccharides on the basis of the fact that the carbon atoms of a terminal monosaccharide relax more slowly. It follows from the results obtained that the set of values of the chemical shifts for four carbon atoms of 107.2, 92.9, 84.1, and 77.6 ppm relate to the monosaccharide residue attached directly to the aglycone. At the same time, the value of 107.2 ppm for the anomeric carbon atom can be assigned either to the β-D-xylopyranose or to the α-L-arabinofuranose unit [4]. However, the chemical shifts of the carbon atoms agree with the signals at 92.9, 84.1, and 77.6 ppm only for a 2-O-substituted α-L-arabinofuranose residue [3, 5]. Consequently, the 2,4-di-O-methyl-β-D-xylopyranose residue is terminal. In actual fact, the values of the chemical shifts of the carbon atoms for it agree well with the corresponding values for the model methyl 2,4-di-O-methyl-β-D-xylopyranoside (IV) (Table 2). Furthermore, in the products of the Hakomori methylation of glycoside (I) [6] followed by methanolysis and acetylation we detected methyl 2,3,4-tri-O-methyl-α- and -β-xylopyranosides, which were identified by GLC and by chromato-mass spectrometry. The results obtained definitively confirmed the terminal position of the 2,4-di-O-methylxylose residue.

The acetylation of glycoside (I) under the usual conditions led to the hexaacetate (V), in which the acetate groups were, as can be seen from Table 2, present at C-3, C-6, C-15, C-3', C-5', and C-3". The results additionally indicated the attachment of the terminal monosaccharide to C-2' of the α-L-arabinofuranose residue.

On the basis of the facts given, the structure of culcitoside C_1 was established as (I).

On completing the structural study of glucoside (I), we isolated the main component of the glycosidic fraction from another starfish Linckia guildingi. From its physical constants, ^1H and ^{13}C spectra, and the results of acid hydrolysis, this compound was identical with culcitoside C_1.

Thus, in two species of starfish, Culcita novaeguineae and Linckia guildingi, a new glysocide has been found – culcitoside C_1 – the structure of which differs from the bioside from Hacelia attenuata known previously by the terminal monosaccharide: in culcitoside C_1 this is 2,4-di-O-methylxylose, and in the previously known bioside it is 2-O-methylxylose.

EXPERIMENTAL

GLC, chromato-mass spectrometric analysis and the determination of the constants were carried out under the conditions described previously [1, 7]. C^{13} and ^1H NMR spectra were taken on a Bruker WM-250 spectrometer. The starfish Culcita novaeguineae and Linckia guildingi were collected in the north-western littoral of the island of Madagascar in February-March, 1983.

Culcitoside C_1 (I), $C_{39}H_{68}O_{14}$, mp 245-248°C, $[\alpha]_D$ – 35.8° (c 0.6; methanol) was isolated with yields of 0.024% (Culcita novaeguineae) and 0.5% (Linckia guildingi) from ethanolic extracts of the starfish as described previously [1].

The hexaacetate of bioside C_1 (II), amorphous after purification on silica gel in the hexane–ethyl acetate (1:1) system, had $[\alpha]_D$ –48.6° (c 1.5; ethanol); this was obtained on acetylation with a mixture of pyridine and acetic anhydride by the usual method.

The methylation of culcitoside C_1 was performed by Hakomori's method [6]. The methanolysis of the methylation products, and the acetylation and identification of the fully substituted α- and β-xylopyranosides was carried out as described in [7].

The acid hydrolysis of 30 mg of glycoside (I) was performed in 2 N HCl at 85-95°C for 2 h. The monosaccharides were separated preparatively on Whatman 3MM paper in the butanol–pyridine–water (10:3:3) system and were analyzed by TLC on silica gel impregnated with 0.2 M sodium dihydrogen phosphate in the butanol–acetone–water (4:5:1) system and by GLC-chromato-mass spectrometry in the form of peracetates of the corresponding aldonitriles. L-arabinose $[\alpha]_D$ +43° (c 0.2; water), and 2,4-di-O-methyl-D-xylose, $[\alpha]_D$ +21° (c 0.5; water), were identified. According to the literature [8]: for α-L-arabinose, $[\alpha]_D$ +47.65° (water); for 2,4-di-O-methyl-D-xylose, $[\alpha]_D$ +22° (water).

The sample of methyl 2,4-di-O-methyl-β-D-xylopyranoside was provided by E. V. Evtushenko.

SUMMARY

It has been shown that culcitoside C_1 from the starfish Culcite novaeguineae and Linckia guildingi is 5α-cholestane-3β,4β,6α,8,15β,24ξ-hexaol 24-O-[O-(2,4-di-O-methyl-β-D-xylopyranosyl)-(1 → 2)-α-L-arabinofuranoside].

LITERATURE CITED

1. A. A. Kicha, A. I. Kalinovskii, E. V. Levina, V. A. Stonik, and G. B. Elyakov, Bioorg. Khim., 9, No. 7, 975 (1983).
2. A. A. Kicha, A. I. Kalinovsky [Kalinovskii], E. V. Levina, V. A. Stonik, and G. B. Elyakov, Tetrahedron Lett., 24, 3893 (1983).
3. L. Minale, C. Pizza, R. Riccio, and F. Zollo, Pure Appl. Chem., 54, 1935 (1982).
4. R. G. S. Ritchie, N. Gyr, B. Korsch, H J. Koch, and A. S. Perlin, Can. J. Chem., 53, 1424 (1975).
5. P. A. J. Gorin and M. Mazurek, Carbohydr. Res., 48, 171 (1976).
6. S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).
7. V. I. Kalinin and V. A. Stonik, Khim. Prir. Soedin., 215 (1982).
8. F. Micheel and A. Klemer, Chemie der Zucker and Polysaccharide, Akademische Verlag Guest und Portig, K.-G., Leipzig (1956), p. 399.