STEROID SULFATES FROM THE STARFISH Lethasterias nanimensis chelifera

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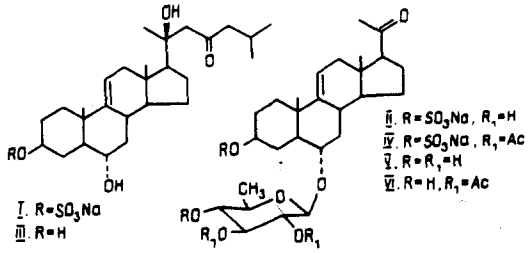
UDC 547.925:593.793:593.93

The sodium salt of 3β-sulfooxythornasterol A and a new glycoside – the disodium salt of 3β-sulfooxy-6α-[β-D-4-sulfooxyquinovopyranosyloxy]-5α-pregn-9(11)-en-20-one (cheliferoside LI) has been isolated from the starfish Lethasterias nanimensis chelifera. The structures of the compounds have been established by spectral methods.

We have isolated the steroid sulfate (I) and the new sulfated glycoside cheliferoside LI (II) from an ethanolic extract of the starfish Lethasterias nanimensis chelifera by column chromatography on Sephadexes XAD-2 and LH-20 and on silica gel. The structures of compounds (I) and (II) have been determined on the basis of their NMA spectra. Sodium ions were detected by flame-emission spectrophotometry.



The ^{13}C NMR spectrum of compound (I) was practically identical with that of thornasterol A 3-O-sulfate, which was obtained previously by the enzymatic hydrolysis of versicoside A from the starfish Asterias amurensis [1]. Assignment of the SCs of the protons in the PMR spectrum of (I) was made by comparison with the spectrum of thornasterol A (III) [2]. It must be mentioned that in [1] an inadequately complete spectrum of this compound was given. Sovolytic desulfation of compound (I) that we had obtained gave (III), which was identified from its PMR spectrum. The CSs of the carbon atoms in the side chain of (I) coincided with the corresponding values for synthetic (20S)-thornasterol A [3], showing the (S)-configuration of C-20 in compound (I).

Thus, compound (I) was identified as the sodium salt of (20S)-3β-sulfooxythornasterol A. This compound was detected long ago as a natural product in the starfish Asterias rubens and Asterias forbesi [2, 4]. The presence of (I) in starfish in the free form and in the form of the native aglycon of the asterosaponins shows that in the biosynthesis of the latter stage of the sulfation of the aglycon at the 3β-hydroxy group precedes the introduction of the carbohydrate chain.

In its ^{13}C NMR spectrum, compound (II) had the signal of an anomeric carbon atom at 104.84 ppm, showing the presence of a single monosaccharide residue. Its PMR spectrum contained the signals of the following protons: CH_3-18 (0.56 ppm, s), CH_3-19 (0.86 ppm, s), CH_3-21 (2.10 ppm, s), and H-11 (5.20 ppm, m), and its ^{13}C NMR spectrum the signals of the following carbon atoms: C-9 (146.19 ppm), C-11 (116.26 ppm), and C-20 (208.38 ppm). These facts permit the assumption that the aglycon of cheliferoside LI was the known asterone (3β,6α-dihydroxy-5α-pregn-9(11)-en-20-one) [5, 6].

The resolution of some of the signals in the NMR spectra of cheliferoside LI was unsatisfactory because of the association of the molecules in the solutions, and therefore some of its derivatives were obtained.

Pacific Ocean Institute of Biochemistry, Far Eastern Branch, Academy of Sciences of the USSR, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 520-523, July-August, 1991. Original article submitted December 10, 1990.

 0009-3130/91/2704-0452$12.50

TABLE 1. ^{13}C Chemical Shifts of the Cheliferoside LI Diacetate (IV)* (C_5D_5N, δ, ppm, TMS = 0)

Atom	ppm	Atom	ppm	Atom	ppm
C-1	35,9	C-11	116,1	C-21	31,0
C-2	29,2	C-12	40,8	C-1′	102,2
C-3	79,3	C-13	42,6	C-2′	73,0
C-4	30,5	C-14	53,8	C-3′	74,3
C-5	48,7	C-15	23,4	C-4′	77,6
C-6	79,8	C-16	25,6	C-5′	71,5
C-7	41,2	C-17	63,4	C-6′	18,5
C-8	35,6	C-18	13,1	OAc:	
C-9	145,7	C-19	19,1	C=O	170,8; 170,5
C-10	38,4	C-20	207,7	CH_3	21,3

*The assignment of the $>C-O-$ signals was made by selective decoupling from protons.

TABLE 2. Chemical Shifts and Multiplicities* of the Signals of the Protons of the Compounds (IV), (V), and (VI) (C_5D_5N; TMS = 0; δ, ppm; J, Hz)

proton	IV	V	VI
H-3	4,73 m	3,99 m	3,80 m
H-6	3,67 td (4,0; 10,5)	3,96 td (4,0; 10,0)	3,80 m
H-11	5,21 m	5,39 m	5,36 m
CH_3-18	0,56 s	0,62 s	0,62 s
CH_3-19	0,91 s	1,05 s	1,05 s
CH_3-21	2,10 s	2,10 s	2,10 s
H-1′	4,84 d (8,0)	4,96 d (7,5)	4,96 (8,0)
H-2′	5,50 dd (8,0;9,0)	4,09 t (7,5)	5,48 dd (8,0;9,0)
H-3′	5,80 t (9,0)	4,18 t (8,0)	5,69 t (9,0)
H-4′	4,87 t (9,0)	3,78 t (7,6)	3,76 t (9,0)
H-5′	3,85 m	3,83 m	3,84 m
H-6′	1,79 d (6,0)	1,70 d (5,5)	1,63 d (5,5)
$COCH_3$	2,20 s		1,98 s
$COCH_3$	2,53 s		2,30 s

*The assignment of the signals of the protons of the monosaccharide residues was made by spin-decoupling experiments.

The acetylation of (II) gave the diacetate (IV). The NMR spectra of (IV) are given in Tables 1 and 2. A comparison of the ^{13}C NMR spectrum of (IV) with that of pectinoside D from the starfish Patiria pectinifera [7] showed the identity of the aglycons and, consequently, the attachment of the sulfooxy group to C-3. The spectrum also showed that the monosaccharide residue in (IV) was attached to C-6 of the aglycon. The localization of the sulfooxy group at C-3 also followed from the downfield shift of the H-3 signal (4.73 ppm) in the PMR spectrum of (IV) as compared with the corresponding signals in the spectrum of asterone (3.70 ppm) [8].

The PMR spectrum of (IV) contained the doublet of a CH_3 group at 1.79 ppm, which is characteristic for a deoxyhexose residue, and the signals of the protons of two acetate groups, showing that one hydroxy group of the monosaccharide residue was sulfated.

The desulfation of cheliferoside LI led to compound (V). The CSs and SSCCs of the protons of the monosaccharide residue in the PMR spectrum of (V) (see Table 2) proved to be identical with the corresponding values for a β-D-quinovopyranose residue [4].

Desulfation of the diacetate (IV) gave the diacetate (VI). In the PMR spectrum of (VI) (see Table 2), the H-3 and H-4′ signals (3.80 and 3.76 ppm, respectively) were shifted upfield as compared with the analogous signals in the initial diacetate (IV) (4.73 and 4.87 ppm). Consequently, the sulfooxide groups were present at C-3 of the aglycon and C-4′ of the monosaccharide residue.

Thus, the structure of cheliferoside LI has been established as the disodium salt of 3β-sulfooxy-6α-[O-β-D-4-sulfooxyquinovopyranosyloxy]-5α-pregn-9(11)-en-20-one. Cheliferoside LI has a structure similar to that of the forbesides E, E1, E2, and E3 recently detected in

the starfish A. forbesi [4, 8]. Its compounds represent a new structural variety of the asterosaponins, which usually have five or six monosaccharide residues in the carbohydrate chain [6, 7].

EXPERIMENTAL

For general observations, see [9]. The animals were collected in June, 1988 off the island of Syasukotan (Kurile Islands) from a depth of 100-150 m.

Sodium ions were determined in a Nippon Jarrel Ash AA-780 atomic absorption/flame emission spectrophotometer.

Isolation of Compounds (I) and (II). An ethanolic extract of the starfish 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, giving 12 g of the fraction of steroid compounds. The fraction obtained was chromatographed successively on columns containing Sephadex LH-20 in the chloroform–methanol (1:1) system, silica gel in the chloroform–ethanol–water (30:100:to saturation) system, and Florisil in the chloroform–ethanol–water (300:70:to saturation) system. This gave 47 mg of compound (I) and 49 mg of compound (II).

Sodium Salt of (20S)-3β-sulfooxythornasterol A (I), mp 153-156°C, $[\alpha]_{Hg}$ −6.7° (c 0.6; methanol). PMR spectrum (C_5D_5N, δ, ppm): 0.90 (d; J = 6 Hz; CH_3-26.27); 0.96 (s; CH_3-18); 1.05 (s; CH_3-19); 1.63 (s, CH_3-21); 2.40 (d.d; J_1 = 6.0 Hz; J_2 = 16.0 Hz; H-22); 2.48 (d.d; J_1 = 7.5 Hz; J_2 = 16.0 Hz; H-22'); 2.65 (d; J = 14.5 Hz; H-24); 2.86 (d; J = 14.5 Hz; H-24'); 3.87 (t.d; J_1 = 4 Hz; J_2 = 11 Hz; H-6); 5.0 (m; H-3); 5.28 (m; H-11).

Cheliferoside LI (II), mp 159-161°C, $[\alpha]_{Hg}$ +35.4° (c; methanol).

Cheliferoside LI Diacetate (IV). Compound (II) (23 mg) was treated with acetic anhydride in pyridine at room temperature for 16 h, and the products were chromatographed on silica gel in the chloroform–ethanol–water (300:210:to saturation) system. This yielded 20 mg of the diacetate (IV), mp 172-174°C, $[\alpha]_{Hg}$ + 17.5° (c 1.1; methanol).

Desulfated Cheliferoside LI (V). Compound (II) (6 mg) was heated in a 1:1 mixture of pyridine and dioxane at 100°C for 2 h, and the product was purified on Florisil in the chloroform–ethanol (9:1) system. This yielded 2 mg of product (V) with mp 133-136°C, $[\alpha]_{Hg}$ +61.5° (c 0.2; ethanol).

Desulfated Cheliferoside LI Acetate (VI). Compound (IV) (6 mg) was treated as described above for compound (V) and was purified on Florisil in the chloroform–ethyl acetate (15:1) system. This gave 3 mg of product (VI), amorphous, $[\alpha]_{Hg}$ +28.3° (c 0.3; ethanol).

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