POLYHYDROXYLATED STEROIDS FROM THE STARFISH *Patiria pectinifera*

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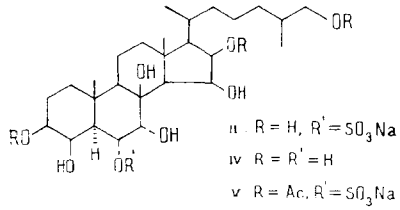
By chromatography on Polikhrom-1, silica gel, and Florisil, an ethanolic extract of the digestive organs of the starfish *Patiria pectinifera* has yielded the steroid polyols 5α-cholestan-3β,6α,8,15α,16β,26-hexaol and 5α-cholestan-3β,4β,6α,7α,8,15β,16β,26-octaol 6α-(sodium sulfate). The structures of the compounds have been shown by spectral characteristics and chemical transformations.

Having continued an investigation of steroidal polyols from the digestive organs of the Pacific Ocean starfish *Patiria pectinifera*, we have isolated steroids (I) and (II) with yields of 0.004 and 0.005%, respectively, on the weight of an ethanolic extract, and have determined the structures.

The cholestane skeleton of the compounds was confirmed by its fragmentation in mass spectra and by the Lieberman-Burchard reaction. According to the results of ^{13}C NMR spectroscopy (Table 1), each steroid has 27 carbon atoms. Six hydroxy groups were detected in compound (I) and eight in (II). The assignment of the signals in the ^{13}C NMR spectra of (I) and(II) was made by comparison with the spectra of polyhydroxysteroids that we had isolated previously from the same starfish [1, 2]. The mutual positions of the hydroxy groups were determined by high-resolution ^{1}H NMR spectroscopy with the aid of spin-decoupling experiments. The corresponding chemical shifts and spin–spin coupling constants are given in Table 2.

It followed from the results obtained that (I) was 5α-cholestane-3β,6α,8,15α,16β,26-hexaol. As a comparison of its spectral characteristics with those given in the literature [3] showed, this compound was identical with a hexaol recently found by Italian workers in the Pacific Ocean starfish *Protoreaster nodosus*.



III R = H, R' = SO_3Na
IV R = R' = H
V R = Ac, R' = SO_3Na

According to the results of elementary and atomic absorption analyses, compound (II), unlike steroidal polyols from starfish known previously, contained a sulfate ion and a Na^+ ion. The C-6 signal in the ^{13}C NMR spectrum of the steroid (II) (75.1 ppm) was shifted downfield by 9.4 ppm in comparison with the corresponding value of 65.7 ppm for 5α-cholestan-3β,4β,6α,7α,8,15α,16β,26-octaol (III) [1]. The signal of the proton at C-6 (6.12 ppm) was also shifted downfield by 0.97 in comparison with the 5.15 ppm for H-6 of the octoal (III). The chemical shifts given above agree well with the figures in the literature for the C-6 signals in the spectra of 2,3,6-trisulfate derivatives of steroidal polyols and their desulfated derivatives [4]. On this basis, we concluded that in (II) the hydroxy group at C-6 was sulfated.

An attempt at the mild desulfation of steroid (II) with pyridine–dioxane at 90°C was unsuccessful – apparently because of steric hindrance of the sulfate group. We obtained the desulfated derivative (IV) only when steroid (II) was treated with a 2 N solution of hydrochloric acid. In the ^{1}H spectrum of (IV) an upfield shift of the H-6 signal, in comparison that of the steroid (II) to 5.25 ppm was observed, which definitively confirmed the localization of the sulfated hydroxy group at C-6 of steroid (II).

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TABLE 1. ^{13}C NMR Spectra of Steroids (I) and (II) (pyridine-d_5, δ, ppm, TMS)

Atom	I	II	Atom	I	II	Atom	I	II
C1	39,0	39,0	C10	37,0	37,8	C19	14,2*	17,0*
C2	31,8	26,9	C11	18,8	18,2	C20	29,7	30,4
C3	71,8	71,7*	C12	42,5	42,4	C21	18,1*	18,5*
C4	32,9	69,4*	C13	44,6	43,8	C22	36,4	36,6
C5	53,5	46,3*	C14	64,3	54,3*	C23	24,1	24,5
C6	66,3	75,1*	C15	80,3	69,9*	C24	34,2	34,6
C7	50,7	75,0*	C16	82,3	72,0*	C25	36,4	36,7
C8	75,1	78,9	C17	60,0	62,5*	C26	67,3	67,6
C9	56,7	50,6	C18	17,0*	18,0*	C27	17,2*	17,6*

*The assignment of the signals was made by selective decoupling from protons.

The spin–spin coupling constants of H-14, H-15, and H-16 corresponded to a 15α,16α-orientation of the proton (Table 2) [1, 2]. In the ^{13}C spectrum of steroid (II), signals at 69.9 ppm for C-15 and at 72.0 ppm for C-16 were shifted upfield by approximately 10 ppm as compared with the octoal (III). This shift can be explained by the presence in compound (II) of a 15β,16β-epoxy fragment [5] or a 15β,16β-dihydroxy grouping. When steroid (II) was acetylated under the usual conditions, we obtained the triacetate (V) (OAc 1.97, 2.05, 2.23 ppm), in which as shown by spin-decoupling the acetate groups were present at C-3, C-16, and C-26. Consequently, ring D contained a 15β,16β-dihydroxy grouping, and the anomalous upfield shift of the C-15 and C-16 signals in the ^{13}C NMR spectrum of compound (II) was due to the disturbance of the additivity of the α,β-effects of the hydroxy groups in this ring because of their cis arrangement [6].

Additional confirmation of the configurations of the hydroxy groups at C-15 and C-6 was obtained with the aid of differential NOE spectrum for (II), which had no signals in the weak field when CH_3-18 was irradiated and contained the H-6 signal when CH_3-19 was irradiated.

On the basis of the facts given, steroid (II) was assigned the structure of 5α-cholestan-3β,4β,6α,7α,8,15β,16β,26-octaol 6α-(sodium sulfate).

EXPERIMENTAL

Patiria pectinifera starfish were collected in Posyet Bay, Sea of Japan, in August, 1981 at a depth of 1-3 m. Melting points were determined on a stage of the Boetius type. ^{13}C and ^1H NMR spectra were taken on a Bruker WM-250 spectrometer. Optical rotation were measured on a Perkin-Elmer 141 polarimeter. Direct-introduction mass spectra were obtained on LKB 9000 spectrometer at an ionizing energy of 70 eV. Metals were determined on a AA-780 atomic absorption spectrometer.

5α-Cholestan-3β,6α,8,15α,16β,26-hexaol (I), $C_{27}H_{48}O_6$, mp 284-287°C, $[\alpha]_D$ + 33.1° (c 0.83; methanol), was isolated with a yield of 0.004% from a methanolic extract of the digestive organs of *Patiria pectinifera* by a method described previously [1]. Mass spectrum, m/z (%): 450 (32), 432 (23), 417 (14), 414 (20), 399 (11), 381 (6), 363 (6), 352 (9), 331 (14), 321 (23), 303 (29), 285 (29), 267 (23), 249 (20), 225 (100), 207 (51).

5α-Cholestan-3β,4β,6α,7α,8,15β,16β,26-octaol 6α-(sodium sulfate) (II), $C_{27}H_{47}O_{11}SNa$, mp 205-208°C, $[\alpha]_D$ +27.7° (c 0.90; methanol) was isolated in a similar manner to (I) with a yield of 0.005%. Mass spectrum, m/z (%): 410 (18), 392 (45), 374 (81), 359 (32), 356 (45), 341 (18), 291 (45), 281 (100), 273 (73), 263 (81), 247 (64), 246 (56), 245 (56), 235 (45), 231 (36), 215 (36).

5α-Cholestan-3β,4β,6α,7α,8,15β,16β,26-octaol (IV). Compound (II) (14 mg) was hydrolyzed in 2 N HCl at 80-100°C for 1.5 h. The hydrolysate was neutralized with Dowex-1 × 10 (HCO_3^-), and the resin was separated off by filtration, and was washed with water and ethanol. The filtrate was concentrated in vacuum and was chromatographed twice on Florisil in the chloroform–ethanol–water (3:1:water to saturation) system. This gave 1.5 mg of the desulfated product (IV), amorphous after purification on Florisil; $[\alpha]_D$ +20.8, (c 0.19; methanol). ^1H NMR (C_5D_5N), δ: 1.08 (m, H-17); 1.10 (d, CH_3-21); 1.11 (d, CH_3-27); 1.68 (s, CH_3-18); 1.89 (s, CH_3-19); 2.02 (d, H-14); 2.25 (dd, H-5); 3.68 (Bdd, H-26'); 3.79 (Add, H-26); 3.93 (m, H-3); 4.49 (t, H-16); 4.78 (d, H-7); 5.08 (dd, H-4); 5.10 (t, H-15); 5.25 (dd, H-6).

TABLE 2. ^{1}H NMR Spectra of Steroids (I) and (II) (pyridine-d_5, TMS)

Proton	δ, ppm		Type of interaction	Spin-spin coupling constants, Hz	
	I	II		I	II*
H-3	4,04 m	3,90 dt	3,4		3,9
H-4e	3,17 dm	5,18 dd	4,5		2,1
H-5		2,53 dd	5,6		12,1
H-6	4,42 t	6,12 dd	6,7e	4,1*	2,4
H-7a	3,25 dd		6,7a	10,9*	
H-7e	3,53 dd	5,30 d	7a,7e	13,4*	
H-14	1,71 d	2,07 d	14,15	11,0	5,3
H-15	5,00 dd	5,06 t	15,16	2,4	6,9
H-16	4,77 dd	4,46 t	16,17	7,0	6,9
H-17	1,56 dd	1,05 dd	17,20	10,7	11,0
H-20	2,44 m	2,32 m	26',25	6,9	6,6
CH_3-18	1,785 s	1,643 s	26,25	5,6	5,4
CH_3-19	1,490 s	1,768 s	26,26'	10,3	10,4
CH_3-21	1,143 d	1,135 d	20,21	6,5	6,6
CH_3-27	1,085 d	1,075 d	25,27	6,6	6,5
H-26	3,74 Add	3,82 Add			
H-26'	3,63 Bdd	3,71 Bdd			
H-25		1,90 m			

*Constants measured in C_5D_5N—CD_3OD (4:1).

Triacetate of 5α-Cholestan-3β,4β,6α,7α,8,15β,16β,26-octaol 6α-(sodium sulfate) (V). Compound (II) (6 mg) was acetylated with acetic anhydride in pyridine at room temperature for 16 h, and the solvent was distilled off in vacuum. The dry residue was chromatographed twice on Florisil in the chloroform—ethanol—water (10:3:water to saturation) system. This gave 2 mg of the triacetate (V), amorphous after purification on Florisil; $[\alpha]_D$ +26.5, (c 0.08; methanol). ^{1}H NMR (C_5D_5N), δ: 0.93 (d, CH_3-27); 1.03 (d, CH_3-21); 1.18 (dd, H-17, measured with heating to 70°C); 1.73 (s, CH_3-18); 1.88 (s, CH_3-19); 2.05 (d, H-14); 1.97 (s, OAc); 2.05 (s, OAc); 2.23 (s, OAc); 2.54 (dd, H-5); 3.97 (Bdd, H-26'); 4.08 (Add, H-26); 5.05 (m, H-3); 5.17 (m, H-4); 5.25 (d, H-7); 5.29 (m, H-15); 5.38 (t, H-16); 6.08 (dd, H-6).

SUMMARY

The polyhydroxy steroids 5α-cholestan-3β,6α,8,15α,16α,26-hexaol and 5α-cholestan-3β,4β-6α,7α,8,15β,16β,26-octaol 6α-(sodium sulfate) have been isolated from the starfish *Patiria pectinifera* and characterized; this is the first time that the sulfate of a steroid polyol has been detected in starfish.

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