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5α-CHOLESTANE-3β,6β,15α,16β,26-PENTOL: A POLYHYDROXYLATED STEROL FROM THE STARFISH *HACELIA ATTENUATA*+

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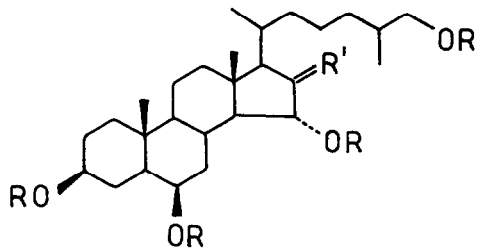
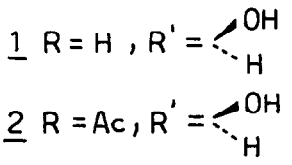
Abstract.- A pentahydroxylated sterol isolated from a starfish, Hacelia attenuata, has been shown by spectral data and chemical transformations to be 5α-cholestane-3β,6β,15α,16β,26-pentol.

Polyhydroxysterols are not uncommon to marine species and they have been observed especially in soft corals and gorgonians[1]. Although starfish contain a number of polyhydroxysterols as aglycone constituents of saponins[2], free hydroxylated sterols have never been encountered before in this class of marine animals. In our continuing search for active saponins in starfish we have now isolated and characterized a novel polyhydroxylated sterol, 5α-cholestane-3β,6β,15α,16β,26-pentol (*1*), from the mediterranean starfish *Hacelia attenuata*. The material was obtained in 0.004% yield (dry weight basis) from the chloroform-metanol 9.1 extract of the liophylized "*starfish*" by preparative h.p.l.c (LC/system 500 Waters; column: prep. Pak 500 SiO_2;solvent. chloroform-methanol from 9:1 to 8:2) followed by chromatography on Sephadex LH-20 in MeOH and eventually reversed phase h.p l.c. (C_{18} μ-bondapak, MeOH-H_2O, 65·35).

The sterol (*1*), m p. 197-199°C, $[\alpha]_D$ ± 0°, had molecular formula $C_{27}H_{48}O_5$ as deduced from the mass spectrum (M^+/e 452) and single-frequency off-resonance decoupled ^{13}C-n.m.r Acetylation with acetic anhydride and pyridine at room temeprature led to the introduction of four acetyl groups *2*, strong deacylated peaks at m/e 560 ($M^+-CH_3CO_2H$), 500 ($M^+-2\ CH_3CO_2H$), 440 ($M^+-3CH_3CO_2H$) and 380 ($M^+-4\ CH_3CO_2H$), 4 $\underline{CH_3}$-C=O at δ 2.02, 2.05, 2.06 and 2.08 . Oxidation of





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the tetraacetate *2* using Jones reagent gave the ketone *3*, M^+/e 618. The single-frequency off-resonance decoupled ^{13}C-n.m.r. spectrum of *1* showed three doublets at 84.7, 82.2 and 71.3 (in the spectrum of the tratraacetate *2* the latter signal was splitted in two doublets at 72.8 and 73.3 ppm) and one triplet at 67 4 ppm establishing the presence of four secondary and one primary hydroxyls in *1*. The ^1H-n.m.r. spectrum of *1* (Table I) showed only two methyl doublets, centered at δ 0.93 and 0 99, which were transformed into singlets on irradiation at δ 1.60 and 1.96, respectively, thus locating the primary hydroxyl function at C-26. The 7-lines multiplet ($W_{\frac{1}{2}}$ = 20 Hz) at δ 3.57 in the ^1H-n.m.r. spectrum of *1* is typical of the 3α-proton of an A/B *trans* steroid, while the quite narrow nature of the signal at δ 3.76 (in the spectrum of *1* this signal is superimposed on the signal for H-15, but in the spectrum of the tetraacetate *2* it is observed as well separated broad singlet at δ 4.98 with $W_{\frac{1}{2}}$ = 7 Hz) was suggestive of the equatorial proton associated with the 6β-hydroxyl group[3]. The 3β,6β-dihydroxy oxidation pattern in *1* was supported from the ^{13}C-n.m.r. chemical shifts of the carbons 1-12 in *1* (Table II) which are in close agreement with those of the corresponding atoms in 5α-cholestane-3β,6β-diol[4] Several features of the ^1H-n.m.r. spectrum of *1* suggested that the two remaining secondary hydroxyls were located on the ring D. The 270-Mz n.m.r. displayed two doublets of doublets at δ 4.00 (H-16 J_1= 8.5 Hz, J_2 = 3 Hz) and 3 80 (H-15 J_1= 10.5 Hz, J_2= 3 Hz) coupled each to other (3Hz) as confirmed by decoupling. The magnitude of the coupling constant between H_{15} and H_{16} is only compatible with a *trans* relationship and the whole pattern is consistent with a 15β,16α-protons system on a 17α-H C/D *trans*-steroid bearing 15α- and 16β-hydroxy groups. Further evidence was provided by several spectral features of the ketone *3* : a) the mass spectrum[5] displayed two diagnostically important ions at m/e 448 (*a*) and 433 (*b*), namely side chain loss with migration of one hydrogen from the side chain to the nucleus and 18-methyl fission, typical of 16-ketosteroids[6]; b) the ^1H-n.m.r. displayed a doublet (J = 14 Hz) at δ 4.91 which had to be associated with the 15β-proton of a 14α-steroid; c) the transformation of the tetraacetate *2* into the ketone *3* was accompained by change in the resonance frequency of the CH_3-18 protons (δ 1.01 ⟶ 0.96) which is only compatible with a 16β-oriented hydroxy group in *2*[9]

The 3β,6β-hydroxylation pattern has been encountered before among polar marine steroids and recently in steroidal aglycones of cyclic saponins from two starfishes[7], the functionalization of the CH_3-26 is reminiscent of the bile alcohols, myximol and deoxymyximol, isolated from hayfish[8], but the 15,16-diol structure is an unprecedent feature among sterols reported earlier

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TABLE I - 270 MHz ^{1}H-n.m.r. data in δ (Hz)

Compound	CH_3-18[a]	CH_3-19[a]	CH_3-21	CH_3-27	H-3	H-6	H-15	H-16	H_2-26
1(CD_3OD)	0.95	1.07	0.99	0.93	3.57	3.76[b]	3.80[c]	4.00	3.46[d]
			(d 7)	(d 7)	(7-lines $W_{\frac{1}{2}}$ 20)		(d 10.5,3)	(dd 8.5,3)	(dd 12,6)
2($CDCl_3$)	1.01	1.04	0.93	0.91	4.72	4.98	4.45	3.92[e]	3.94-3.82
			(d 7)	(d 7)	(7-lines $W_{\frac{1}{2}}$ 20)	(bs,$W_{\frac{1}{2}}$ 7)	(dd10.5,2.5)		(dd 12,7) (dd 12,8)
3($CDCl_3$)	0.96	1.05	1.01	0.92	4.72	5.01	4.91	-	3 95-3 84
			(d 7)	(d 7)	(7-lines $W_{\frac{1}{2}}$ 20)	(bs,$W_{\frac{1}{2}}$ 7)	(d 14)		(dd 12,7) (dd,12,8)

a.- Calculated according to Zurcher[9] : *1*, CH_3-18.0.97, CH_3-19 1.05, *2*, CH_3-18:1.02, CH_3-19:1.06, *3*, CH_3-18:0.94, CH_3-19:1.08.

b.- Partially overlapped with the H-15 signal.

c.- The 10.5 Hz coupling between H_{15} and H_{14} was proved by decoupling.

d.- The highfield part of the AB portion of the HC-CH_2-OH ABX system is submerged under methanol signal

e.- Overlapped with the H-26 signal.

TABLE II - Carbon-13 chemical shifts (ppm, TMS=0)[a]

Compound	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1($py-d_5$)	39.2	32.5	71.3	37.0	48.3	71.3	41.1[b]	30.9	55.0	36.1	21.3	41.2[b]	43.9	61.0
1(CD_3OD)	39.8	32.2	72.5	36.4	c	75.2	41.9	31.2	55.8	36.6	21.9	40.6	44.7	61.1
2($CDCl_3$)	38.0	27.2	73.3	30.9	46.0	72 8	36.0	30.3	53.6	35.4	20.6	40.1	43.3	56.2

Compound	15	16	17	18	19	20	21	22	23	24	25	26	27
1(py)	84 7	82.2	59.4	15.0	16.2	30.2	18.4	36.8	24.3	34.4	36.6	67.4	17.3
1(CD_3OD)	85.0	82 9	59.9	15.0	16.3	30.9	18.6	37.4	24.8	34.9	37 0	68.4	17.3
2($CDCl_3$)	88.4	79 3	58.7	14.5	15.2	29.4	17.9	35.8	23.6	33.7	32 4	69.5	16.9

a.- The carbon signal were assigned by means of single-frequency off-resonance decoupling techniques, by using the hydroxy substituent effects found in simpler steroids[10] and the expected deviations from additivity for proximate diols[11], from comparison with the published data on the 5α-cholestane-3β,6β-diol resonances and from comparison of the spectrum of the pentol *1* with that of its tetraacetate *2*

b - Assignment may be reserved.

c.- Signal under solvent signal.

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5.- m s. of compound *3*: m/e (%) 618 (M^+< 1%), 558 (5), 543 (10), 498 (8), 483 (12), 448 (*a*, 2), 438 (20), 433 (*b*, 34), 423 (20), 388 (*a* $-CH_3CO_2H$, 12), 373 (*b* $-CH_3CO_2H$, 34), 328 (*a* $-2CH_3CO_2H$, 70), 313 (*b* $-2CH_3CO_2H$, 50), 295 (18), 268 (*a* $-3CH_3CO_2H$, 100), 253 (*b* $-3CH_3CO_2H$, 70).

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