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STARFISH SAPONINS I. 3β-HYDROXY-5α-CHOLESTA-8,14-DIEN-23-ONE, THE MAJOR GENIN FROM THE STARFISH ECHINASTER SEPOSITUS+

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The occurrence of toxic saponins in starfish has been known since some years ago[1]. They are cytotoxic, hemolytic, antiviral[2] and also induce escape reaction in molluscs[3]. A number of steroidal aglycones produced by acid hydrolysis of starfish saponins (asterosaponins) have been characterized; all of them possess a 3β,6α-dihydroxy oxidation pattern and the biosynthetically unusual 9(11)-double bond is present in all but two of the aglycones so far identified[4]. In this paper we report the isolation of the major genin from the starfish Echinaster sepositus and show that it lacks both the 6α-hydroxyl group and the 9(11)-double bond.

Starfishes, collected in November 1977 in the bay of Naples, were extracted by chopping and soaking them in water and the saponins were removed by Amberlite XAD-2 resin using the improved procedure of Gilgan et al.[5]. The crude saponins were chromatographed on silica gel to give, on elution with methanol, a white solid (0.06% on fresh basis) which was separated by chromatography on Sephadex G-25 into two main fractions. Both showed the same u.v. spectrum with absorption maximum at 250 nm ($E^{1\%}_{1cm}$ 32 and 59, respectively) and, upon acid hydrolysis (HCl in benzene-

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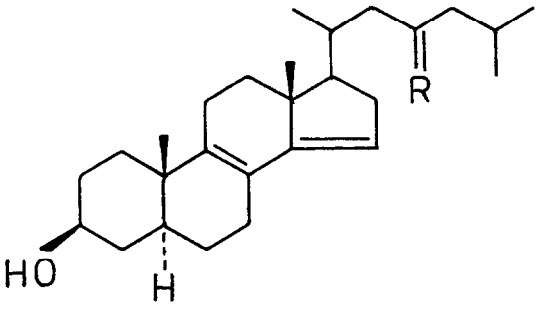
ethanol-water at reflux for 16 h[6]), gave the same mixture of aglycones. Silica gel chromatography of the aglycones mixture resulted in the isolation of one major compound, homogeneous in g.l.c. and h.p.l.c., and one minor product which was shown by reverse phase h.p.l.c. (C-18 microbondapak) to be a mixture of three components.

The major aglycone 1 crystallized from methanol, m.p. 96-98°, $[\alpha]_D$-2.3° (c, 1 in $CHCl_3$), M^+ 398.3186 (100%), $C_{27}H_{42}O_2$ (required 398.3184), λ_{max} (MeOH) 248 nm (ε, 18,000), ν_{max} ($CHCl_3$) 3400 and 1705 cm^{-1}. Its mass spectrum contained a prominent peak at m/e 298 (M-100,55%) indicative for a 23-oxo function and interpreted as derived trough a McLafferty rearrangement (cleavage of 20,22-bond and 1H transfer from C-17), and also major peaks at m/e 269-271 (100% and 95%, loss of the side-chain with and without 2H transfer), suggestive for two nuclear double bonds. Its ^{1}H-n.m.r. spectrum (100 MHz, $CDCl_3$) depicted two quaternary methyls at 0.88 and 0.99 ppm, a carbinol methine at 3.62 ppm (bm, $W_{\frac{1}{2}}$ = 20 Hz) and an olefinic proton at 5.34 ppm (bs, $W_{\frac{1}{2}}$ = 6 Hz). These data are closely comparable with those of 5α-cholesta-8,14-dien-3β-olo[7] [2; u.v. 248 nm (log 4.26); n.m.r. 13-Me 0.84, 10-Me 0.99, H-3 3.62, H-15 5.35 ppm] and suggested for the new sapogenin the 3β-hydroxy-5α-cholesta-8,14-dien-23-one structure (1). Most significant was the comparison of the ^{13}C-n.m.r. spectra of 1 and 2 (Table), which definitively confirmed



1 R=O
2 R=H$_2$

the location of the oxo function at C-23. The assignments were based on the multiplicities observed in the off-resonance spectra, published data on steroids[8] and general considerations[9]. Wolff-Kishner reduction of 1 furnished the known 2 (identical g.l.c., n.m.r., m.p. and rotation with synthetic 5α-cholesta-8,14-dien-3β-olo[7]). Genin 1 appears to be the first steroidal asterosapogenin lacking the 6α-hydroxyl group; the 8,14-diene system is also a quite rare feature, being discovered until now only in two phytosterols derived from Vernonia anthelmintica seeds[10]

and the green alga Chlorella ellipsoidea, when inhibited by the Δ^7-reductase trans-1,4-bis (2-chlorobenzylaminomethyl)cyclohexane[11].

Work on the minor genins of E. sepositus is currently in progress.

T A B L E

Carbon Shifts (ppm, TMS = 0) of 1 and 2

	1	2
C-1	36.8	37.0
C-2	31.6	31.7
C-3	70.8	70.9
C-4	38.2	38.3
C-5	40.9	41.0
C-6	25.3[a]	25.4[a]
C-7	35.3[b]	35.4[b]
C-8 C-9 C-14	122.9, 141.0, 151.0	123.1, 140.8, 151.1
C-10	36.6	36.6
C-11	21.8	21.9
C-12	36.0[b]	36.0[b]
C-13	45.1	45.1
C-15	116.7	117.4
C-16	26.6[a]	26.6[a]
C-18 C-19 C-21	15.8, 18.4, 20.0	15.8, 18.4, 19.0
C-20	30.8	34.1
C-22	50.3	36.2
C-23	210.0	23.8
C-24	52.6	39.6
C-25	24.5	28.1
C-26 C-27	22.6[c]	22.7 , 22.8

Spectra were determined in $CDCl_3$ at 25.20 Hz; a-b Signals within a column may be reversed; c in C_6D_6 C-26 and C-27 resonated as separate signals (22.8 and 22.7 ppm).

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