Tetrahedron Letters No. 11, pp 959 - 962. 0040-4039/79/0308-0959$02.00/0

STARFISH SAPONINS III. A NOVEL STEROIDAL SAPOGENIN, 17β-METHYL-3β,6α-DIHYDROXY-18-NOR-5α-CHOLESTA-9(11),13-DIEN-23-ONE, FROM THE STARFISH *Astropecten aurantiacus*+

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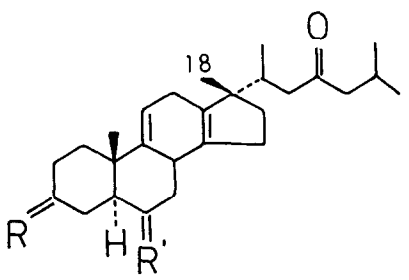
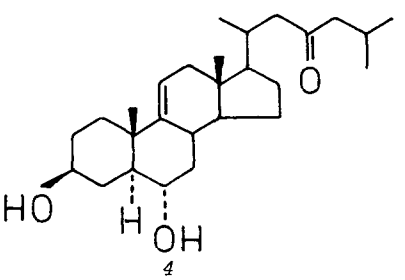
In continuation of our work[1] on starfish saponins, we report the isolation of the hitherto unknown 17β-methyl-3β,6α-dihydroxy-18-nor-5α-cholesta-9(11)-13-dien-23-one (*1*) from the sapogenin portion of *Astropecten aurantiacus*, and show that it is an artefact produced during acid hydrolysis. Compound *1* appears to be identical with the partially characterized calcarigenin, produced by acid hydrolysis of the calcarsaponin isolated from *Patiriella calcar*[2]. Mass and i.r spectra of calcarigenin diacetate, reported by the authors in graphic form[2], were superimposable with the corresponding spectra of *2*[3].



1; R =  ; R' = 

2; R =  ; R' = 

3; R = R' = O



4

5; $\Delta^{24(25)}$

6; $\Delta^{17(20)}$

7; $\Delta^{20(22)}$

8; 20-OH

+ This contribution is part of the progetto finalizzato "Oceanografia e Fondi Marini", C.N.R., Roma.

Saponins were extracted by using the procedure of Gilgan *et al.*[4] and purified by silica gel chromatography to afford in the 1 : 1 $CHCl_3$ -CH_3OH fractions a saponin mixture (*ca.* 0.07% from the dried whole body). This material was hydrolyzed (2N-HCl in benzene-EtOH-H_2O at reflux for 16 h[5]) and the aglycone mixture was resolved into four fractions by chromatography on silica gel using chloroform and increasing amount of ethyl acetate. The less polar fraction (*ca.* 15% of the total sapogenin mixture) further purified by reverse phaese h.p.l.c. (C-18 microbondapack, CH_3CN : H_2O, 4 : 1) was homogeneous in g.l.c. (2.5% OV-1, 250°, as TMS ether) and had a retention time of 0.96 (1.0 = cholesterol TMS ether) much shorter than those of the "normal" C_{27}-3β,6α-dihydroxy-23-oxosteroids TMS ethers (e.g. *4*, rt = 2.07; *5*, rt = 2.38; *6*, rt = 1.87). While the mass spectrum of the genin *1*, oil $[\alpha]_D$ - 2.8°, did not show the molecular ion at m/e 414, the composition $C_{27}H_{42}O_3$ could be assigned to *1* by a) the very small molecular ion at m/e 498 observed in the spectrum of the diacetate *2*[3], consistent with the composition $C_{31}H_{46}O_5$; b) proton resonance decoupled and single frequency off-resonance decoupled ^{13}C-n.m.r. spectra (Table I); and c) high resolution measurements on the intense ions at m/e 285 (73%) and 287 (65%) ($C_{19}H_{25}O_2$ and $C_{19}H_{27}O_2$, calculated 285.1854, 287.2010; observed 285.1868, 287.2031) in the spectrum of *1* and attributed to M^+ - side chain with and without 2H transfer. Consecutive losses of two molecules of water from the $C_{19}H_{27}O_2$ fragment suggested the presence of two hydroxyl groups in a tetracyclic nucleus containing two double bonds [fragments at m/e 269 (100%) and 251 (20%)]. The ketonic nature of the remaining oxygen atom was demonstrated by an i.r. band at 1705 cm^{-1} and the ^{13}C-n.m.r. signal at 211.4 ppm; the intense fragment at m/e 85 (50%, $>\!\!-C\equiv\overset{+}{O}$), already observed in the mass spectrum of dihydromarthasterone *4*[6], suggested the oxo group to be located at C-23. In agreement with this assignment are ^{13}C-n.m.r. frequencies for C-22 and C-24 (52.4 & 52.6, t) in excellent analogy with corresponding signals at 50.6 and 52.6 in *4* (Table 1).

The 270 MHz ^1H-n.m.r. spectrum of the genin *1* depicted two tertiary methyl (1.00 and 1.04 ppm, s, 10-Me and 17-Me) three secondary methyl (0.84, 0.88 and 0.89, d, J = 6Hz), two carbinol methine [3.62 (c, 2H) shifted to 4.69 ($W_{\frac{1}{2}}$ = 20Hz; H-3) and 4.89 (ddd, J = 10.5, 10.5, 5.4Hz; H-6) in the diacetate *2*] and one olefinic methine (5.47, $W_{\frac{1}{2}}$ = 6Hz). These data, along with the ^{13}C-n.m.r. spectrum (one HC= at 114.4 and three C= respectively at 135.1 s, 136.5 s and 144.3 s) indicated the presence of one trisubstituted and one tetrasubstituted double bond. The conspicuous absence in the ^1H-n.m.r. spectrum of the absorption in the range of 0.6-0.8 ppm, typical of the 13-Me protons in a steroidal nucleus, together with the presence of a 3H singlet at 1.04 ppm strongly suggested the presence of a perturbation in the immediate sourronding of C-13. Apart this unusual feature the general appearance of the ^1H-n.m.r. spectrum suggested a $\Delta^{9(11)}$ 3β,6α-dihydroxysteroidal partial structure, common for many asterosapogenins[7]. Oxidation of *1* with Jones reagent gave a triketone (*3*), M^+/e 410, which was transparent in u.v. and showed a strong i.r. band at 1705 cm^{-1}; this transformation was accompained by changes in the resonance

TABLE I. - Carbon-13 Chemical Shifts (ppm, TMS=0) of *1*, *4* and *4*-diAc

Compound	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	35.5	30.9	71.0	32.7	50.6	69.5	42.5	34.3	144.3	38.1	114.4	30.9[b]	135.1[c]	136.5[c]
4[a]	35.7	30.9	70.9	32.7	49.8	69.1	42.2	35.3	145.5	38.2	116.6	41.7	41.2	53.5
4-diAc[a]	35.5	27.3	73.1	28.9	46.8	72.2	38.4	35.7	144.7	38.4	117.5	41.8	41.3	53.6

	15	16	17	18	19	20	21	22	23	24	25	26	27
1	24.0	31.5[b]	46.5	24.5	18.3	34.7	15.2	52.4[d]	211.4	52.6[d]	24.6	22.6	22.6
4[a]	25.3	28.5	56.3	11.7	19.3	32.3	19.5	50.4	210.9	52.6	24.5	22.6	22.7
4-diAc[a]	25.2	28.5	56.3	11.7	19.1	32.4	19.5	50.5	211.0	52.7	24.6	22.6	22.7

a. - ^{13}C signals in *4*, isolated from *Marthasterias glacialis*[6], were assigned by means of single-frequency off-resonance decoupling techniques, from comparison with the published data on the 5α-cholestane-3β,6α-diol resonances[11], and from comparison of the spectrum of *4* with that of its diacetate (*4*-diAc), which allowed differentiation between C-5 and C-14 and between C-7 and C-12.

b,c,d.- Assignments may be reversed.

frequencies of the 10-Me protons (1.00 ⟶ 1.16 ppm) and the olefinic proton (5.47 ⟶ 5.70 ppm) which closely paralleled those reported for *4*[6]. An intense fragment at m/e 95 in the m.s. of *1* (38%; one hydroxyl in ring A and the other at position 6[8]), and the comparison of the ^{13}C-n.m.r. data of *1* with those of *4* (Table I) strongly supported this assignment. The comparison of the ^{13}C-n.m.r. spectra also provided convincing evidence for the presence in *1* of a 23-oxosubstituted "conventional" C_8 side chain. All above evidence required a rearranged steroidal structure and *1* seemed the most reasonable.

Formation of 17β-methyl-Δ^{13}-olefins from reactions that were initiated by the ionization of a 17α- or 17β-linkage or that would create C-17 cation by the protonation of a 17,20 double bond has been reported[9]. So we treated the 3β,6α-dihydroxy-5α-cholesta-9(11),17(20)-dien-23-one *6*, one of the major genin from the same starfish *A. aurantiacus*[10], with aqueous 2$\underline{\underline{N}}$-HCl under reflux and obtained *1* without recover of the starting *6*.

On the basis of the above accumulated evidence, the structure of the novel genin is thus established to be 17β-methyl-3β,6α-dihydroxy-18-nor-5α-cholesta-9(11),13-dien-23-one (*1*). Formation of both *1* and *6* during acid hydrolysis of the saponin mixture is due to the reaction conditions (HCl in benzene-EtOH-H_2O at reflux), when the saponins were hydrolyzed by aq. 2$\underline{\underline{N}}$-HCl at reflux for 2 h very little of *6* was formed and *1* became the major genin. The genuiness of the many reported asterosapogenins is a matter of discussion. It has recently been reported[7] that acid treatment of *8*, obtained by enzymatic hydrolysis of the total saponin mixture of *Acanthaster planci*, produces *7*, previously isolated from *A. planci*[8], and the 3β,6α-dihydroxy-5α-pregna-9(11)-en-20-one (*9*), hitherto isolated non only from *A. planci* but also from other species of Asteroidea[7]. The authors[7] therefore consider that 20-hydroxy steroids are the true sapogenols whilst aglycons such *7* and *9* may be artefacts produced during acid hydrolysis. We suggest that in addition to *1*

the genin *6* may also be an artefact originating from a 17-hydroxyprecursor during acid hydrolysis.

ACKNOWLEDGEMENTS.- We wish to thank Professor Y. Shimizu (University of Rhode Island, U.S.A.) for advice and Professor R. H. Thomson (University of Aberdeen, U.K.) for providing high resolution mass spectra.

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(Received in UK 5 December 1978)