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A NEW STEROL FROM ASTEROSAPONINS A AND B

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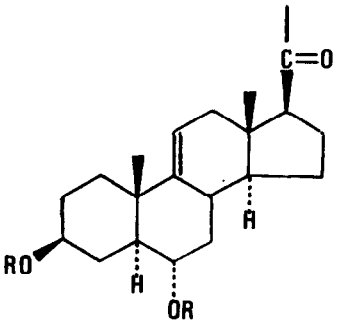
Asterosaponins A and B were isolated from the Japanese starfish, Asterias amurensis, by Yasumoto et al.(1). The both saponins were recognized to contain the same aglycones, to which four or five molecules of sugars and a molecule of sulfuric acid (as a sodium salt) are attached. Subsequently, the sugar components were identified as D-fucose, D-galactose, D-quinovose and D-xylose by the same authors (2,3). In succession to these studies, we subjected the saponins to acid hydrolysis to afford a mixture of aglycones being composed of 21-28 carbon and 2-3 oxygen atoms. This communication presents the evidence leading to assignment of the hitherto unknown structure, 3β,6α-dihydroxy-5α-pregn-9,(11)-ene-20-one (1), for the main aglycone.

Hydrolysis of asterosaponin A and/or B with p-toluenesulfonic acid in ethanol gave a mixture of at least six steroids, of which 1 was the major constituent. The mixture was treated with acetic anhydride in pyridine and was subjected to silica gel column chromatography by use of benzene-ethyl acetate as an eluant. The acetate of 1 thus obtained was saponified with 5% KOH in methanol at room temperature for 30 minutes to recover 1. Purity of 1 was confirmed to be 85% or more on the basis of the natural abundance ^{13}C resonance spectrum (fully proton-decoupled). $C_{21}H_{32}O_3$, M^+ 332.2363 (required 332.2350), mp 157-160°, $[\alpha]_D^{23}$ 65.2° (c 1.08% in chloroform), ν_{max} (nujol) 3240, 1700, 1040 and 825 cm^{-1}. 1 showed the mass spectral peaks at m/e 43, 95, 211-212, 229-230 (ring-D fission and dehydration), 263, 294, 299 and 314 ($M^+ - H_2O$), which are characteristic of a conventional steroidal nuclei with an acetyl group at C-17. 1 was negative for the Liebermann-Burchard reaction.

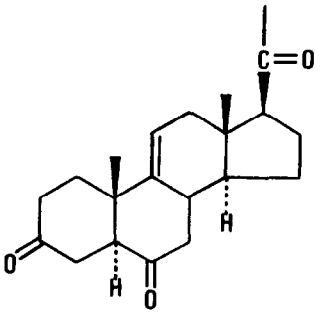
It was not precipitated with digitonin and was not oxidized with selenium oxide. The nmr spectrum (100 MHz) in deuterochloroform revealed the presence of two quatenary methyls (δ 0.59 and 0.97, s, 3H each), an acetyl (δ 2.17, s, 3H), two hydroxyls (δ 2.72, broad s, 2H), two secondary carbinol methines (δ 3.55, broad m, 2H) and an olefinic proton (δ 5.35, t, 1H). The ord curve of 1 showed positive Cotton effect, which suggests that configuration of the acetyl group at C-17 should be β. Treatment of 1 with acetic anhydride in pyridine furnished an oily diacetate 2, M^+ 416, $[\alpha]_D^{23}$ 60.5° (c 1.69% in chloroform).

Oxidation of 1 with chromium trioxide in pyridine gave a triketone 3,

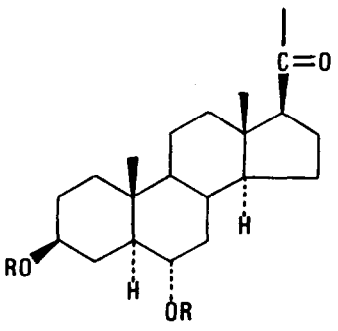


1 (R=H)

2 (R=Ac)



3



4 (R=Ac)

5 (R=H)

$C_{21}H_{28}O_3$, M^+ 328.2053 (required 328.2037), mp 194-196°, $[\alpha]_D^{25}$ 21.6° (c 1.20% in chloroform), ν_{max} (nujol) 1700 cm^{-1}. Transparence in the uv region suggests the absence of α,β-unsaturated ketone in 3. The nmr spectrum (100 MHz) of 3 in deuterochloroform exhibited two quatenary methyls (δ 0.62 and 1.11, s, 3H each), an acetyl (δ 2.15, s, 3H) and an olefinic proton (δ 5.63, t, J 4cps, 1H coupling to a doublet of A_2X type, 2H). No signals corresponding to allylic methine or methylene protons adjacent to carbonyl groups were recognized in 3. Accordingly, the trisubstituted double bond should be located solely at the 9:11 position, and hence 3 is assigned as 5α-pregn-9,(11)-ene-3,6,20-trione.

Table I. Chemical Shifts of the C-18 and C-19 Methyl Groups in 1 - 5

(δ, ppm Downfield from Internal TMS)

Compound	C-18 Methyl		C-19 Methyl	
	Obsd.	Calcd.	Obsd.	Calcd.
1	0.59	0.56	0.97	0.95
2	0.57	0.56	1.03	1.02
3	0.62	0.60	1.11	1.12
4	0.63	0.61	0.88	0.87
5	0.61	0.62	0.82	0.81

The signal at δ 3.55 corresponding to two carbinol methines in 1 is only understandable by assuming that configurations of C-3 and C-6 hydroxyls are equatorial, that is 3β and 6α (4). Thus, 1 must be 3β,6α-dihydroxy-5α-pregn-9,(11)-ene-20-one.

Hydrgenation of 2 in acetic acid over platinum dioxide, followed by oxidation with chromium trioxide in pyridine, gave an oily dihydro-diacetate 4, M^+ 418, $[\alpha]_D^{25}$ 46.1° (c 2.33% in chloroform). On hydrolysis, 4 afforded the corresponding diol 5, M^+ 334, mp 183-185°, $[\alpha]_D^{25}$ 50.7° (c 1.26% in chloroform). The mass spectrum of 5 revealed every peak which had been observed with 1, though mass-units were higher by two in the former. The nmr spectrum of 5 exhibited no olefinic proton signals suggesting disappearance of the 9:11 double bond.

Chemical shifts of two quatenary methyls at C-18 and C-19 of compounds 1 - 5 were in good agreement with the values calculated according to the method of Zürcher (5) (Table I).

Finally, 2,4-dinitrophenylhydrazone of 5 was synthesized starting with that of pregnenolone. 2,4-Dinitrophenylhydrazone of pregnenolone was treated with diborane to afford a dialkylborane, which was subsequently oxidized with basic hydrogen peroxide. Since the Brown hydration of Δ^5 steroids occurs in a cis manner from the less hindered side of the double bond (6), the product should be

3β,6α-dihydroxy-5α-pregnane-20-one hydrazone. Expectedly, its mp, glc, tlc, mass, nmr and ir data were in complete identity with the 2,4-dinitrophenylhydrazone, M^+ 514, mp 260-261.5° (decomp.), derived from natural 5. Accordingly, the structure of 1 has been unambiguously established.

1 was probably originated in metabolism of progesterone. This assumption might be supported by the recent discovery of progesterone in the ovary of Asterias amurensis (7).

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