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# A NOVEL STEROID, 3β,6α,23ξ-TRIHYDROXY-5α-CHOLEST-9(11)-EN, FROM ASTEROSAPONINS

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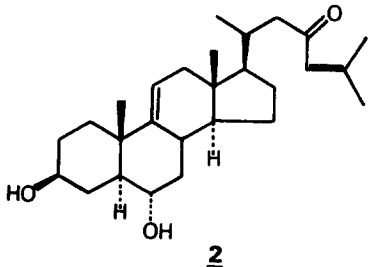
Asterosaponins A and B were isolated from the Japanese starfish, Asterias amurensis, by Yasumoto et al. (1). The both saponins were recognized to contain the same aglycones, to which four or five molecules of sugars and a molecule of sulfuric acid (as a sodium salt) are attached (2-4). Recently, we isolated the saponins as physiologically active substances which counteract the neural spawning-inducer in the same organism and suggested that they may play a part in inhibiting unfavourable spawning early in the breeding season (4). On acid hydrolysis, the saponins afforded a mixture of aglycones being composed of 21-28 carbon and 2-3 oxygen atoms. We elucidated the structure of the main aglycone in the hydrolyzate to be a novel steroid, 3β,6α-dihydroxy-5α-pregn-9(11)-en-20-one (1) (5). Quite recently, the occurrence of 1 in the starfish, Acanthaster planci, has been reported by Sheikh et al. (6).

On further close investigation, we succeeded in the isolation of two additional steroids in the hydrolyzate from the saponins. One was demonstrated to be identical to marthasterone isolated by Turner et al. (7) from the Marthasterias glacialis saponins which are different from asterosaponins (8). Another has been established to be a hitherto unknown steroid, 3β,6α,23ξ-trihydroxy-5α-cholest-9(11)-en. In this communication are reported our experimental results leading to these assignments for the aglycones.

Hydrolysis of crude asterosaponins with 2 N hydrochloric acid gave water-insoluble aglycones, which were separated and purified by chromatography on silica gel. Thin-layer chromatography (TLC) on silica gel with a solvent

system of benzene-acetone (3:2, v/v) revealed two spots at Rf 0.49 and 0.37. Extraction of the zone at Rf 0.49 with ethyl acetate followed by evaporation afforded a powder (2), $C_{27}H_{42}O_3$, $M^+$ 414, whose nmr, uv and mass spectra were identical to those reported for marthasterone, 3β,6α-dihydroxy-5α-cholesta-9(11),24-diene-23-one (9). Catalytic reduction of 2 with Pd-C gave the dihydro derivative (3), $C_{27}H_{44}O_3$, $M^+$ 416. Identity of 3 with dihydromarthasterone, kindly supplied by Dr. Turner, was verified through measurements of retention times on gas-liquid chromatography (GLC) (OV-1, 260°C, as trimethylsilyl derivative), Rf values on TLC and nmr, ir and mass spectra. Thus, 2 was

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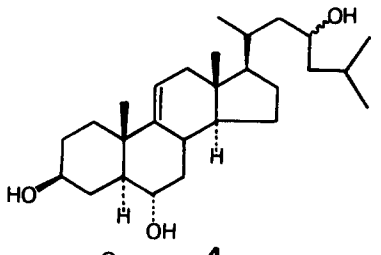


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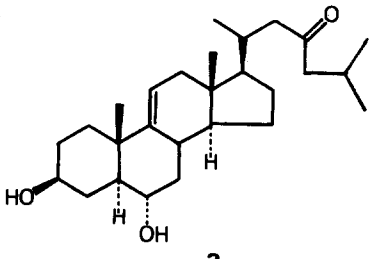


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assigned to be marthasterone.

The fraction with Rf 0.37 on TLC was extracted with acetone. The residue obtained after evaporation of the solvent was recrystallized from ethyl acetate to afford needles (4), $C_{27}H_{46}O_3$, $M^+$ 418, mp 240-243°C, $[\alpha]_D^{25}$ +41.5° (c=0.26, ethanol). The mass spectrum of 4 revealed characteristic peaks at m/e 287.2011 ($M^+$ - side chain; required for $C_{19}H_{27}O_2$ 287.2010) and m/e 211.1485 (ring D cleavage and dehydration; required for $C_{16}H_{19}$ 211.1485). Acetylation of 4 furnished the noncrystallized tri-acetate 5, $C_{33}H_{52}O_6$, $M^+$ 544, whose nmr spectrum (100 MHz, $CDCl_3$) depicted the presence of five methyls (δ 0.60, s, 3H; δ 0.85-

0.95, c, 9H; δ 1.02, s, 3H), three acetyls (δ 2.00, s, 9H), three broad acetate methines (δ 4.20-5.10, m, 3H) and an olefinic proton (δ 5.32, t, J=5 cps, 1H). These data suggested the presence of two hydroxyl groups and a trisubstituted double bond in ring A, B or C and of a hydroxyl group in the side chain in 4. The Zürcher's rules (10,11) supported the assumption that 4 would be a derivative of 3β,6α-dihydroxy-5α-cholestane containing a double bond at C-9(11). Then, 3 was treated with sodium borohydride to give tetrahydromarthasterone melting at 239-242°C, $[\alpha]_D^{25}$ +36.4° (c=0.25, ethanol). The synthetic compound possessed the ir, nmr and mass spectra and retention time on GLC (as trimethylsilyl derivative) identical to those of 4. Further, the melting point of 4 did not depress on admixture with the synthetic sample. Thus, 4 has been demonstrated to be 3β,6α,23ξ-trihydroxy-5α-cholest-9(11)-en.

4 is unique in that it possesses a hydroxyl at the position C-23 and the only one case hitherto encountered in nature is 23ξ-hydroxylanosterol which was isolated from the common fungus, Scleroderma aurantium (12). The absolute configuration at C-23 in 4 is under investigation.

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