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A NOVEL STEROID, 3β,6α,15α,24ξ-TETRAHYDROXY-5α-CHOLESTANE FROM ASTEROSAPONINS

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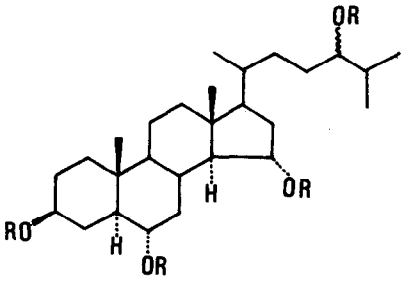
Asterosaponins A and B were first isolated from the Japanese starfish, Asterias amurensis, by Yasumoto et al.[1] who recognized that the both saponins contain the same aglycones being attached with four or five molecules of sugars and a molecule of sulfuric acid.[2-5] Subsequently, we isolated the same saponins as spawning inhibitors in the starfish[6] and established the structures of three constitutive aglycones as 3β,6α-dihydroxy-5α-pregn-9(11)-en-20-one (1),[7,8] 3β,6α,23ξ-trihydroxy-5α-cholest-9(11)-ene[9] and 3β,6α-dihydroxy-5α-cholesta-9(11),24-dien-23-one[9] which was first isolated by Smith et al.[10,11] from other starfish, Marthasterias glacialis. 1 was isolated independently from Acanthaster planci by Sheik et al.[12] and from Asterias forbesi by Shimizu.[13] In this paper we wish to describe the further isolation of a novel steroid, 3β,6α,15α,24ξ-tetrahydroxy-5α-cholestane (2) as one of minor aglycones in the asterosaponins.

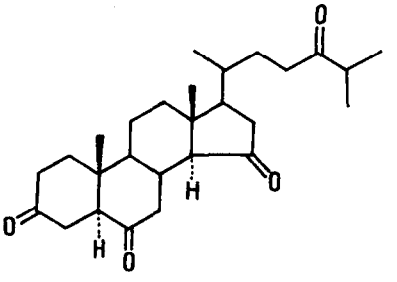
Hydrolysis of asterosaponins with 2N hydrochloric acid at 100°C for 2.5 hr furnished a mixture of aglycones which were separated and purified by silica gel column chromatography by use of chloroform-methanol as an eluant. Thin layer chromatography on silica gel PF_{254} with a solvent system of chloroform-methanol (6:1) revealed several spots. Extraction of the zone at Rf 0.32 with methanol followed by acetylation with acetic anhydride-pyridine gave a crystalline tetraacetate (3), mp 137-138.5°C (plate), $[\alpha]_D^{21}$ +66.2° (c=1.0, chloroform). The mass spectrum of 3 showed a strong deacetylated peaks at m/e 544 (M^+-60), together with peaks at m/e 484, 424, 373, 364, 313 and 253.

The pmr spectrum (100 MHz in $CDCl_3$) depicted five methyls (δ 0.73, s, 3H; δ 0.84, s, 3H; δ 0.90-1.00, broad 9H), four acetyls (δ 1.95-2.05, 4s, 12H) and four acetate methines (δ 4.40-4.90, m, 4H). The ir spectrum suggested the absence of hydroxy groups. Deacetylation of 3 with 5% potassium hydroxide-methanol gave crystalline 2, mp 197-199.5°C, $[\alpha]_D^{21}$ +45.7° (c=0.5, methanol). The mass spectrum of 2 showed peaks at m/e 418 (M^+-H_2O), 400, 357, 289, 271, 253 and 109. The pmr spectrum depicted five methyls (δ 0.70, s, 3H; δ 0.82, s, 3H; δ 0.88-0.94, d, 9H) and broad carbinol methines (δ 3.40, m, 4H). No carbonyl group was observed in the ir spectrum.



R = H 2

R = Ac 3

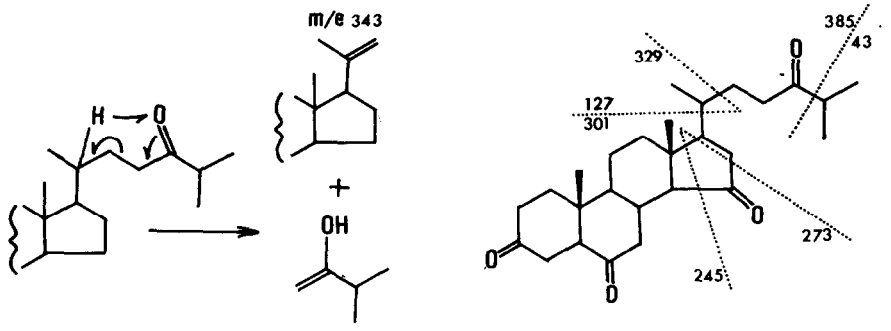


4

Oxidation of 2 with the chromium trioxide-pyridine complex gave a tetraketone (4), M^+ 428.2981 ($C_{27}H_{40}O_4$ required 428.2924), mp 194-195.5°C.. The pmr spectrum of 4 revealed five methyls (δ 0.79, s, 3H; δ 0.97, s, 3H; δ 1.00, d, J=5 Hz, 3H; δ 1.10, d, J=7.5 Hz, 6H). The ir spectrum showed a five-membered cyclic ketone at 1740cm^{-1} together with six-membered cyclic and/or aliphatic ketones at 1715cm^{-1}. The base peak in the mass spectrum was observed at m/e 43.0525 (C_3H_7). The peak at m/e 385.2370 ($M^+-C_3H_7$, 11%) and that of m/e 343.2297 ($M^+-C_5H_9O$, 45%) due to Mc-Lafferty-style clevage of the side chain between C-22 and C-23 suggested the presence of a ketonic group at C-24. This was further supported by the down-field shifts of methyls at C-25 in the pmr spectrum of 4 as compared with those of 2 and 3.

The Huang-Minlon reduction of 4 gave 5α-cholestane, which was identified by glc (OV-1 and SE-30). Treatment of 4 with isopropenyl acetate produced a

dienol diacetate-mixture with λ_{max}244 nm. This indicates the presence of the 3,6-dione system in 4.[11] The fragment ions of the mass spectrum of 4 at m/e 301.1774 (M^+-$C_8H_{15}O$, 29%), m/e 273.1485 (M^+-$C_{10}H_{19}O$, 10%) and m/e 245.1567 (M^+-$C_{11}H_{19}O_2$, 14%) established the position of the ketonic group in the five-membered ring at C-15. Thus the structure of 4 was proved to be 5α-cholesta-3,6,15,24-tetraone.



In the pmr spectrum of 3, signals at δ 4.40-4.90 due to acetate methines indicate that the acetoxy groups at C-3, C-6 and C-15 are equatorial. Consequently, the structure of 2 has been decided to be 3β,6α,15α,24ξ-tetrahydroxy-5α-cholestane. The chemical shifts of C-18 and C-19 methyls in 2, 3 and 4 are well consistent with calculated values tabulated by Zürucher.[14]

The configuration at C-24 is under investigation. The 3β,6α-dihydroxy-$\Delta^{9(11)}$ene system has been considered to be characteristic of aglycones constituing starfish saponins. 2 makes an exception in that it has no $\Delta^{9(11)}$-bond but contains a hydroxy group in the ring D.

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REFERENCES

1. T. Yasumoto, T. Watanabe and Y. Hashimoto, Bull. Jap. Soc. Sci. Fisheries, 30, 357 (1964).

2. T. Yasumoto and Y. Hashimoto, Agr. Biol. Chem., 29, 804 (1965).
3. T. Yasumoto and Y. Hashimoto, Agr. Biol. Chem., 31, 369 (1967).
4. S. Ikegami, Y. Hirose, Y. Kamiya and S. Tamura, Agr. Biol. Chem., 36, 2453 (1972).
5. S. Ikegami, Y. Kamiya and S. Tamura, Tetrahedron, 29, 1807 (1973).
6. S. Ikegami, Y. Kamiya and S. Tamura, Agr. Biol. Chem., 36, 2005 (1972)
7. S. Ikegami, Y. Kamiya and S. Tamura, Tetrahedron letters, 1601 (1972).
8. S. Ikegami, Y. Kamiya and S. Tamura, Agr. Biol. Chem., 36, 1777 (1972).
9. S. Ikegami, Y. Kamiya and S. Tamura, Tetrahedron letters, 3725 (1972).
10. A. B. Turner, D. S. H. Smith and A. M. Mackie, Nature, 233, 209 (1971).
11. D. S. H. Smith, A. B. Turner and A. M. Mackie, J. Chem. Soc. Perkin 1, 1745 (1973).
12. Y. M. Sheik, B. M. Tursch and C. Djerassi, J. Am. Chem. Soc., 94, 3278 (1972).
13. Y. Shimizu, J. Am. Chem. Soc., 94, 4051 (1972).
14. R. F. Zürucher, Helv. Chim. Acta., 44, 1380 (1961); 46, 2054 (1963).