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A NEW STEROIDAL SULFATE OBTAINED FROM A STARFISH SAPONIN, ASTEROSAPONIN A

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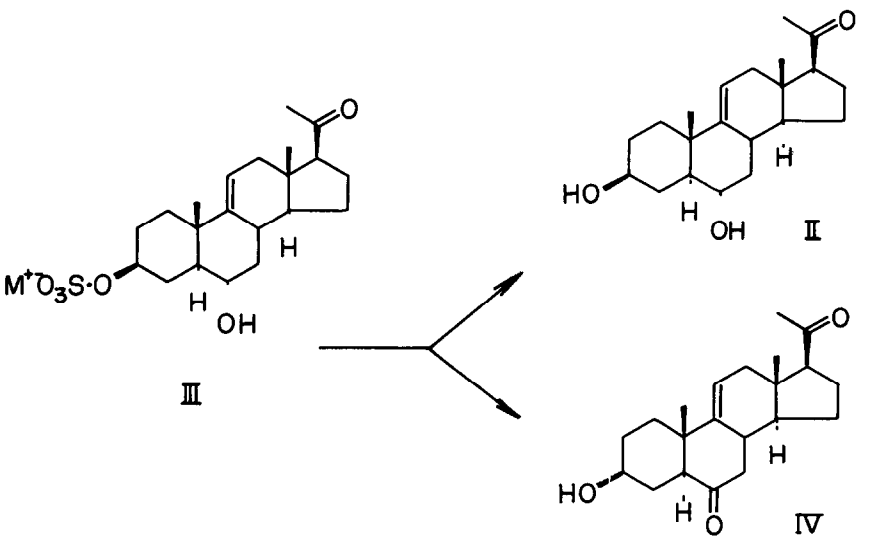
In 1965 Yasumoto et al (1) isolated a toxic saponin named asterosaponin A (I) from the starfish, Asterias amurensis and described that it contains a steroidal aglycone to which two moles each of 6-deoxy-D-galactose and 6-deoxy-D-glucose and a mole of sulfuric acid are attached Subsequently, we obtained I as a spawning inhibitor to the same organism (2,3) and established the structure of its main aglycone as 3β,6α-dihydroxy-5α-pregn-9(11)-en-20-one (II) (4,5). In this communication are reported the isolation from I and structure elucidation of a novel steroidal sulfate, 6α-hydroxy-3β-sulfo-oxy-5α-pregn-9(11)-en-20-one (III).

I was treated with 0 05 N hydrochloric acid for 5 hours at 100°C. After neutralization with aqueous sodium hydroxide, the hydrolyzate was subjected to partition between n-butanol and water The butanol layer was concentrated to dryness and applied to thin layer chromatography (tlc) on Silicagel GF_{254} plates using a solvent system of n-butanol-acetic acid-water (10 1 1, v/v) The zone at Rf 0.50 was extracted with methanol, which was then evaporated to dryness. The residue was dissolved in 0 6 M triethylammonium sulfate (pH 7 0), and the aqueous solution was extracted with chloroform After evaporation of the solvent the residual solid was recrystallized from ethyl acetate-methanol to afford triethylammonium salt of III as colorless needles $SO_4^=$ 40 0 % (calculated, 40 2 %) Mp 160-161°C. $[\alpha]_D^{25}$ +20° (c=0 5, methanol) The pmr spectrum (100 MHz, CD_3OD) of the salt revealed the presence of two methyls (δ 0 54, s, 3H, δ 0.97, s, 3H), an acetyl (δ 2 12, s, 3H), a secondary carbinol methine (δ 3.50, m, 1H), a

sulfate methine (δ 4 20, m, 1H) and an olefinic proton (δ 5 40, t, 1H)

When the salt of III was treated with ethyl acetate saturated with 2 N sulfuric acid, it afforded a steroid, which was identified as II through the measurement of retention times on gas liquid chromatography (ov-1 or SE-30 at 240°C, as trimethylsilyl derivative) as well as the Rf value on tlc

Oxidation of pyridinium salt of III with chromium trioxide in pyridine, followed by solvolysis with dioxane afforded diketone IV M^+ 330 2167 (calculated for $C_{21}H_{30}O_3$, 330 2193) $[\alpha]_{300}$ +570°, $[\alpha]_{264}$ -500° (c=0.085, ethanol) ν_{max} (KBr) 3450 and 1710 cm^{-1} The pmr spectrum of IV in deuterochloroform depicted



the presence of two methyls (δ 0 59, s, 3H, δ 0 91, s, 3H), an acetyl (δ 2.14, s, 3H), a secondary carbinol methine (δ 3.55, m, 1H) and an olefinic proton (δ 5.63, t, 1H) As in the case of 5α-pregn-9(11)-en-3,6,20,-trione (5), the chemical shift of the olefinic proton is located at a lower field (δ 5 63) as compared with that of II (δ 5 35) or the synthetic 6α-hydroxy-5α-pregn-9(11)-en-3,20-dione (V) (δ 5 40), which was prepared by partial oxidation of II with chromium trioxide in pyridine These observations suggest the presence in IV of the ketone group at C-6, which affects the chemical shift of the olefinic proton anisotropically Further, it has been known that the optical dispersion of 3-

keto-5α-steroids shows a single, positive Cotton-effect curve whereas that of 6-keto-5α-steroids shows the opposite sign (6) In this experiment, molecular rotation difference calculated from the optical rotatory dispersion of IV and that of II revealed a single, negative Cotton-effect curve On the basis of the data, the location of the keto group in IV has been unambiguously established at C-6 Thus, IV should be 3β-hydroxy-5α-pregn-9(11)-en-6,20-dione.

Chemical shifts of two quartenary methyls at C-18 and C-19 of IV showed good agreement with the values calculated according to the method of Zürcher (7,8), calculated values of the C-18 and C-19 methyls are δ 0 57 and 0 91, respectively On the other hand, the chemical shift of the C-19 methyl in V was observed at significantly low field (δ 1 16) Accordingly, the structure of III has been assigned to be 6α-hydroxy-3β-sulfo-oxy-5α-pregn-9(11)-en-20-one This compound is the first steroidal sulfate of invertebrete origin

It has been previously shown that in asterosaponin A (I) O-(6-deoxy-D-galactopyranosyl)-(1→4)-O-(6-deoxy-D-galactopyranosyl)-(1→4)-O-(6-deoxy-D-glucopyranosyl)-(1→4)-6-deoxy-D-glucose combines with the steroidal aglycone constituting an acetal O-glycoside linkage at the reducing terminal (9). Since the location in III of the sulfate group has been established in this experiment, the carbohydrate moiety must be attached to 6α-hydroxy group to form the acetal system in the original saponin I The configuration of the anomeric proton of the carbohydrate is under investigation.

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