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Structures of Two C-27 Steroids Constituting Asterosaponins A and B†

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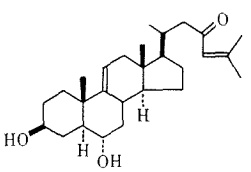
On acid hydrolysis, asterosaponins A and B afforded two C-27 steroids. One was found to be identical to marthasterone, 3β,6α-dihydroxy-5α-cholesta-9(11),24-diene-23-one. Another has been established as a hitherto unknown steroid, 3β,6α,23ξ-trihydroxy-5α-cholest-9(11)-en.

In 1964, Yasumoto *et al.*[1] isolated asterosaponins A and B as toxins from the Japanese starfish, *Asterias amurensis*. Further they confirmed that the both saponins 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 asterosaponins as inhibitors counteracting the action of the neural spawning-inducer[5] in the same organism and suggested that they may play a part to inhibit unfavorable spawning early in the breeding season.[6,7]

On acid hydrolysis, asterosaponins A and B afforded a mixture of aglycones being composed of 21~28 carbon and 2~3 oxygen atoms. Recently, we elucidated the structure of one of the aglycones in the hydrolyzate to be a novel steroid, 3β,6α-dihydroxy-5α-pregn-9(11)-en-20-one (**1**).[9,10] Quite independently, the occurrence of **1** has been reported by Sheikh *et al.*[11] and by Shimizu[12] as a constituent of the saponins of the starfish, *Acanthaster planci*. In further detailed investigations on the hydrolyzate, we succeeded in isolating two additional steroids from asterosaponins A and B. One was identified as marthasterone (**2**), which has been isolated by Turner *et al.*[13] from the *Marthasterias glacialis* saponins different from the asterosaponins.[14] Another was found to be a hitherto unknown steroid and was established as 3β,6α,23ξ-trihydroxy-5α-cholest-9(11)-en (**3**). A preliminary communication of the structure elucidation has been reported previously,[15] and here we wish to present the details of our experimental results.



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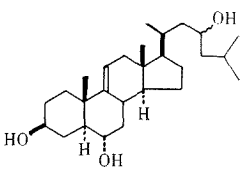

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On hydrolysis with 2 N hydrochloric acid, the asterosaponins gave water-insoluble aglycones which were purified by silica gel column chromatography using a solvent system of benzene-ethyl acetate. Each eluate was applied to thin-layer chromatography (TLC) with a solvent system of benzene-acetone (3: 2, v/v), which revealed the occurrence of two components (**2** and **3**) at *Rf*s 0.49 and 0.37.

The component (**2**) with *Rf* 0.49 possessed the molecular formula $C_{27}H_{42}O_3$. Its NMR, UV and mass spectra were completely identical to those reported for marthasterone, 3β,6α-dihydroxy-5α-cholesta-9(11), 24-diene-23-one.[13,16] Catalytic reduction of **2** with Pd-C gave the dihydro derivative (**4**). Identity of **4** with dihydromarthasterone, kindly supplied by Dr. Turner, was verified through gas-liquid chromatography (GLC) (as trimethylsilyl derivatives) and IR and mass spectral measure-

† Studies on Asterosaponins. Part IV. Part III, see reference 8).

ments. Thus, **2** was assigned to be marthasterone.

The component (**3**) with *Rf* 0.37 on TLC melted at 240~243°C. Its molecular formula was $C_{27}H_{46}O_3$. The mass spectrum of **3** revealed peaks at *m/e* 287 (M^+—side chain) and 211 (ring D cleavage and dehydration) which are characteristic to steroids. Acetylation of **3** furnished powdery triacetate (**5**). The NMR spectrum (Fig. 1) of **5** showed the

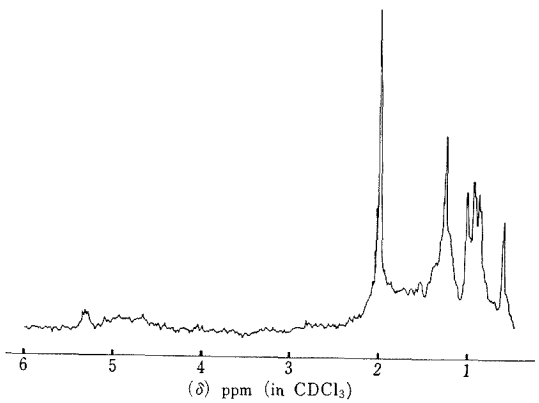

FIG. 1. NMR Spectrum of 3β,6α,23ξ-Trihydroxy-5α-cholest-9(11)-en Triacetate (**5**).

signals attributable to five methyls at δ 0.60, δ 0.85~0.95, δ 1.01, three acethyls at δ 0.20, three acetate methines at δ 4.20~5.10 and an olefinic proton at δ 5.32. 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 the molecule of **3**. The NMR spectral data of compound **5** were best explained by assuming **3** as a derivative of 3β,6α-dihydroxy-5α-cholest-9(11)-en based on Zurcher's method[17,18] (Table I). Then, **4** was treated with sodium borohydride to give tetrahydro-

TABLE I. CHEMICAL SHIFTS OF C-18 AND C-19 METHYL GROUPS IN COMPOUND **5** (ppm, Downfield from Internal TMS)

C-18 Methyl		C-19 Methyl	
Obsd.	Calcd.	Obsd.	Calcd.
0.60	0.60	1.01	1.01

marthasterone melting at 239~242°C. The synthetic compound showed the IR, NMR and mass spectra and retention time on GLC (as trimethylsilyl derivative) identical with those of **3**. Further, the melting point of **3** was not depressed on admixture with a synthetic sample. Thus, **3** has been demonstrated to be 3β,6α,23ξ-trihydroxy-5α-cholest-9(11)-en.

Compound **3** is unique in possessing a hydroxyl group at C-23, because 23ξ-hydroxylanosterol from the common fungus, *Scleroderma aurantium*,[19] has been the sole natural product with such a substitution hitherto encountered. The absolute configuration at C-23 in **3** is under investigation.

EXPERIMENTAL

Melting points were determined on a microscope hot stage and uncorrected. Optical rotations were measured with a JASCO DIP-S polarimeter. UV spectra were measured on a Cary-14 spectrophotometer. IR spectra were obtained with a JASCO IR-S spectrometer. NMR spectra were recorded on a JEOL-JNM-4H spectrometer at 100 MHz with tetramethylsilane as an internal standard. Mass spectra were measured with a Hitachi RMU-6L mass spectrometer operating with an ionization energy of 70 eV. High resolution mass spectra were obtained with a Hitachi RMH-2 mass spectrometer as direct probe sample using an ionization voltage of 70 eV. GLC analyses were performed on a Hitachi K-53 using a column (0.3×200 cm) packed with 3% OV-1 on Chromosorb at 260 or 280°C. The flow rate of the carrier gas, nitrogen, was maintained at 35 ml/min. Samples were dissolved in an appropriate amount of anhydrous pyridine and were trimethylsililated according to the method of Sweeley *et al.*[20] TLC was carried out with silica gel GF_{254} plates of 0.25 mm thickness, which were developed with a solvent system of benzene-acetone (3: 2 v/v) (A) or ethyl acetate (B). Spots were detected by spraying 10% (w/v) solution of phosphotungustic acid in ethanol or under a UV lamp (254 mμ).

Acid hydrolysis of crude asterosaponins. A crude mixture of asterosaponins (2.0 g), prepared as described previously,[10] was dissolved in 2 N hydrochloric acid (50 ml) and was heated at 100°C for 2 hr. The reaction mixture was then extracted with ethyl acetate (100 ml), and the extract was washed with saturated $NaHCO_3$ solution and water. After being dried over anhydrous Na_2SO_4, the solvent was evaporated *in vacuo* to give a brown gum (0.83 g). The gum was dissolved in ethyl acetate (2 ml) and applied onto a silica gel

column (1.2×22 cm), which was developed with ethyl acetate to collect 6.5-ml fractions. Each fraction was examined with TLC by use of Solvent A. Fractions showing *Rf* 0.49 were combined and evaporated *in vacuo* to afford powdery **2** (160.0 mg). On similar treatment, fractions with *Rf* 0.37 gave **3** (16.4 mg) as needles. The homogenity of **2** and **3** was checked by GLC (260°C). The trimethylsilyl derivatives of **2** and **3** showed peaks at retention times of 18.72 and 20.40 min, respectively.

Physicochemical properties of aglycone **2**. $[\alpha]_D^{25}$ +17.3° (*c*=0.75, chloroform); MS *m/e*: 414 (M^+), 399, 396, 381, 316, 298, 287, 269, 251, 211, 95: NMR $\delta_{Me_4Si}^{CDCl_3}$: 0.60 (3H, singlet), 0.90 (3H, doublet), 0.95 (3H, singlet), 1.90 (3H, singlet), 2.15 (3H, singlet), 3.30~3.80 (2H, multiplet), 5.32 (1H, doublet) and 6.02 (1H, singlet); IR ν_{max}^{Film} cm^{-1}: 3400, 1700, 1625; UV λ_{max}^{EtOH} mμ (ε): 237 (12000).

Catalytic reduction of **2**. Compound **2** (77 mg) was dissolved in ethyl acetate (6 ml) and hydrogenated over Pd-C catalyst (11 mg) under atmospheric pressure for 24 hr. The reaction mixture was filtered, and the catalyst was washed with ethyl acetate. The combined filtrates were evaporated *in vacuo* to afford powdery **4** (32 mg). Homogenity of **4** was checked by TLC with Solvent A (*Rf* 0.48) and B (*Rf* 0.42). Mp 165~167°C; $[\alpha]_D^{25}$ +200° (*c*=0.03, chloroform); MS *m/e*: 416 (M^+), 401, 398, 383, 365, 316, 285, 211, 95; IR ν_{max}^{Nujol} cm^{-1}: 3260, 1710.

Identity of **4** with dihydromarthasterone was verified through TLC with Solvents A and B and GLC analysis. Both trimethylsilyl derivatives of **4** and dihydromarthasterone showed identical retention times (17.50 min) on GLC (260°C).

Physicochemical properties of aglycone **3**. Mp 240~243°C; $[\alpha]_D^{25}$ +41.5° (*c*=0.26, ethanol); MS *m/e*: 416 (M^+), 400.3316 (M^+-H_2O), 385.3085 ($M^+-H_2O-CH_3$), 382.3227 ($M^+-2\times H_2O$), 367.2991 ($M^+-2\times H_2O-CH_3$), 287.2011 ($C_{19}H_{27}O_2$), 269.1911 ($287-H_2O$), 251.1805 ($269-H_2O$), 247.1690 ($C_{16}H_{23}O_2$), 229.1603 ($247-H_2O$), 211.1485 ($229-H_2O$), 95.0871 (C_7H_{11}); NMR $\delta_{Me_4Si}^{Pyridine\text{-}d_6}$: 0.60 (3H, singlet), 1.00~1.10 (12H, complex), 3.50~4.20 (3H, multiplet), 5.12 (1H, triplet); IR ν_{max}^{Nujol} cm^{-1}: 3350.

Acetylation of **3**. Compound **3** (5.1 mg) was dissolved in anhydrous pyridine (1 ml) to which was added acetic anhydride (0.5 ml). After standing overnight at room temperature, the reaction mixture was poured onto chilled water and extracted with benzene. The benzene layer was washed with dilute aqueous hydrochloric acid and water, dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to afford oily **5** (5.2 mg). MS *m/e*: 544 (M^+), 484, 469, 424, 409, 371, 364, 349, 311, 251, 211. NMR spectrum is shown in Fig. 1.

Borohydride reduction of **4**. Compound **4** (20.5 mg) was treated with sodium borohydride (3 mg) in ethanol (2 ml) overnight at room temperature. After addition of dilute acetic acid, the reaction mixture was extracted with chloroform. The chloroform layer was repeatedly washed with water, dried over anhydrous Na_2SO_4 and then evaporated *in vacuo* to dryness. The residue was recrystallized from ethyl acetate to afford needles (19 mg). TLC : *Rf* 0.48 (Solvent A) and 0.42 (Solvent B); mp 239~242°C; $[\alpha]_D^{25}$ +36.4° (*c*=0.25, ethanol). IR, NMR and mass spectra were identical with those of **3**.

TLC (Solvents A and B) and GLC (retention time, 6.70 min, as trimethylsilyl derivatives; 280°C) revealed identity of **3** with a synthetic sample. The melting point of **3** was not depressed on admixture with a synthetic specimen.

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