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CALYSTEROL: A C_{29} CYCLOPROPENE-CONTAINING MARINE STEROL FROM THE SPONGE *CALYX NICAENSIS*[a]

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Abstract—From an extract of *Calyx nicaensis* calysterol (1), a new C_{29} cyclopropene-containing sterol, has been isolated and its structure elucidated.

Recently the occurrence in some marine organism of C_{29} and C_{30} sterols with biogenetically unusual side chains[1] has been reported. As a part of our studies on metabolites of Porifera,[2] we investigated the sterol fraction of some sponges in order to explain the origin of these unusual side chain modifications.

The present paper deals with the isolation and structure elucidation of calysterol (1) (23,24-ethylidencholesta-5,23-dien-3β-ol) present in the sponge *Calyx nicaensis*.

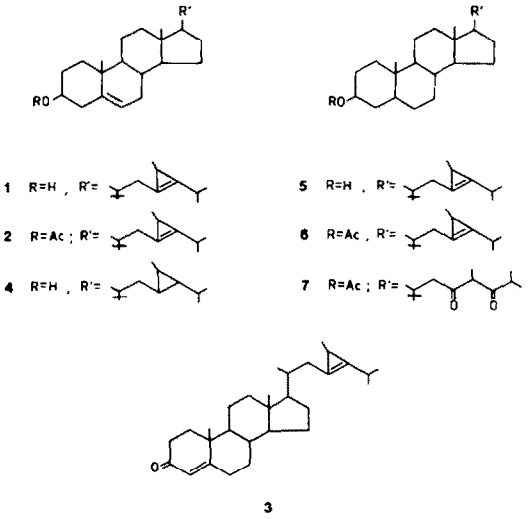
The unsaponifiable matter of the crude extract yielded a sterol fraction which was further resolved by acetylation and subsequent chromatography to a crystalline product (2) $C_{31}H_{48}O_2$, m.p. 104–106°, $[\alpha]_D$ −34·3. MS data [ions at *m/e* 452(M^+), 392 ($M^+ - CH_3COOH$), 313 (M^+ − s.c. − 2H), 253 (M^+ − s.c. − CH_3COOH − 2H) and 213 (M^+ − CH_3COOH − s.c. − C_3H_6)] indicated that 2 is a C_{29} acetyl sterol with two unsaturation linkages in the side chain and a double bond in the nucleus.[3] The intense ion at *m/e* 392 suggested a 3β-acetoxy-Δ^5-sterol in accordance with the $[\alpha]_D$ value and the NMR spectrum which included a broad doublet at δ 5·36 (H–C_6), a broad hump at δ 4·60 (H–C_3), a singlet at δ 2·01 (CH_3–C(=O)–) and two singlets at δ 0·71 (H_3–C_{18}) and at δ 1·02 (H_3–C_{19}).

Additional support for structure 2 was provided by hydrolysis to 1, $[\alpha]_D$ −29·3, m.p. 114–116°, M^+ 410. Oppenauer oxidation of 1 gave the α,β-unsaturated ketone 3, $[\alpha]_D$ +65·5, m.p. 55–56°, M^+ 408, whose CD curve is similar to that of cholest-4-en-3-one.

These results accounted for the structure of the nucleus, while the spectral data of 2 furnished the following informations concerning the nature of the C_{10} side chain. The NMR spectrum [δ 1·12 (6H, d, J 7 Hz, H_3–C_{26} and H_3–C_{27}), 0·98 (3H, d, J 6 Hz, H_3–C_{21} or H_3–C_{29}), 1·02 (3H, d, J 6 Hz, H_3–C_{29} or H_3–C_{21})] indicated the presence of four secondary Me groups, two belonging to an isopropyl function, as indicated by IR (ν_{max} 1380, 1370 cm^{-1}) and mass [intense ions at *m/e* 409 ($M^+ - C_3H_7$) and 349 ($M^+ - CH_3COOH - C_3H_7$)] spectra.

Further investigation of the NMR spectrum indicated that the isopropyl function was linked to a fully substituted olefinic carbon, since the methyne proton resonated as an heptet at δ 2·65. This assignement was confirmed by spin decoupling experiments: by irradiation at δ 2·65 the doublet at δ 1·12 was simplified into a singlet, while by irradiation at δ 1·12 the heptet at δ 2·65 collapsed into a singlet.

Moreover the absence of olefinic signals in the NMR spectrum of 2 (except that of H–C_6) indicated that the side chain double bond was tetrasubstituted.



In order to obtain additional information 1 was hydrogenated to give 4 whose TMS-derivative proved to be unitary by GLC, M^+ 412, $[\alpha]_D$ −17·5, m.p. 125–127°. The NMR spectrum of 4 exhibited a high field complex signal (3H) spread between δ 0·3–0·6, thus requiring the presence of a cyclopropane ring. Catalytic hydrogenation of 1 under more drastic conditions gave β-sitostanol, which was identified by co-GLC with an authentic sample and by GLC–MS.

The proposed structure was further substantiated by the following experiments and the presence of the methylcyclopropene group in the side chain confirmed.

Ketone 3 was reduced to 5, M^+ 412, as a mixture of diastereoisomers, which, without further purification, was acetylated and successively oxidized. The product (7) was shown to contain a partially enolized 2-Me-1,3-diketonic system by UV[λ_{max} 293 nm (ϵ = 2300)], IR (ν_{max} 1720, 1680 and 1580 cm^{-1}) and NMR [δ 2·1 (0·9 H, s, H_3–C_{29} of enolic form)] spectra.

The occurrence of calysterol in *Calyx nicaensis* confirms that in marine organisms sterols with unusual side chains may be quite widespread, the presence in 1 of a cyclopropene ring, which has been found only in some naturally occurring fatty acids, is remarkable.

In the hope of determining the origin of this side chain

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substitution pattern, the analysis of the minor sterols present in the sponge *Calyx nicaensis*, is currently in progress.

EXPERIMENTAL

The IR spectra were recorded on a Perkin–Elmer 157 Spectrophotometer. The NMR specrta were determined on a Perkin–Elmer R 32 using TMS as internal reference. The mass spectra were taken on an AEI MS 902 instrument. The optical rotations were measured with a Perkin–Elmer 141 polarimeter. TLC and PLC separations were effected using glass packed precoated silica gel plates. GLC analyses were performed with a C. Erba Fractovap GV chromatograph under the following conditions: the column was glass tubing 2 m long, 2·2 mm i.d., packed with 1 per cent SE–30 on chromosorb W(80–100 mesh), oven temperature 245°, flow rate 70 ml N_2/min. TMS derivatives were prepared by treatment of free sterol (4 mg) with Tri-Sil(Pierce) (0·5 ml) for 5 min at room temp.

Sponges (*Calyx nicaensis*) collected in the Bay of Taranto were supplied by Stazione di Biologia Marina del Salento (dir. Prof. P. Parenzan) and identified by Prof. M. Sarà (Università di Genova).

Isolation of **2**. Wet sponges (500 g, dry weight) were cut into small pieces and extracted with acetone (5 l. in two portions) and successively with $CHCl_3$ (5 l. in two portions). The combined extracts were concentrated leaving an aqueous suspension which was extracted with Et_2O. This yielded a residual yellow oil (2·5 g) which was saponified with a soln of 10 per cent KOH in 85 per cent EtOH under reflux for 2 hr; the unsaponifiable matter (1·4 g) was chromatographed on a SiO_2 column (140 g) using C_6H_6–Et_2O (9:1) as eluent. The combined sterol fractions were acetylated with Ac_2O in pyridine and the acetates (0·8 g) separated by PLC (8 plates) using 40–70° light petroleum–C_6H_6 (3:2) as eluent. After two migrations, the band R_f 0·5, visualized by heating a thin strip of each plate sprayed with a 5 per cent ceric sulphate in a 10 per cent H_2SO_4, scraped and eluted gave 0·7 g of **2**, m.p. 104–106°; $[\alpha]_D$ −34·3 (*c*, 1 in $CHCl_3$); $\nu_{max}^{CCl_4}$ 1736, 1240 cm^{-1}; (Found, C, 82·28; H, 10·72. Calc. for $C_{31}H_{48}O_2$ C, 82·24; H, 10·69%).

Alkaline treatment of **2**. To a soln of 10 per cent KOH in 85 per cent EtOH (10 ml) **2** (0·7 g) was added and the mixture refluxed for 2 hr. After cooling, the soln was concentrated and extracted with Et_2O. The ethereal extract was dried on $CaSO_4$ and taken to dryness *in vacuo* affording 0·64 g of 1 m.p. 114–116°; $[\alpha]_D$ −29·3 (*c*, 1 in $CHCl_3$); M^+ 410 *m/e*; $\nu_{max}^{CCl_4}$ 3670–3400 cm^{-1}; δ^{CDCl_3} 5·32 (1H, bd, H–C_6), 3·52 (1H, m, H–C_3), 2·60 (1H, heptet, J 7 Hz, H–C_{25}), 1·10 (6H, d, J 7 Hz, H_3–C_{26} and H_3–C_{27}), 1·05 (3H, s, H_3–C_{19}), 0·72 (3H, s, H_3–C_{18}), 0·96 (3H, d, J 6 Hz, H_3–C_{29} or H_3–C_{21}), 0·98 (3H, d, J 6 Hz, H_3–C_{21} or H_3–C_{29}). (Found C, 84·79; H, 11·31. Calc. for $C_{29}H_{46}O$ C, 84·81; H, 11·29).

Oxidation of **1**. To a soln of **1** (0·5 g) in 10 ml of freshly distilled cyclohexanone and 30 ml dry toluene, 0·7 g of aluminium isopropoxide was added. The mixture was refluxed for 2 hr. After removal of volatile solvents by steam distillation, the aqueous residue was extracted with $CHCl_3$. The organic phase, taken to dryness, afforded a residue which was chromatographed on PLC using C_6H_6–Et_2O (9:1) as eluent. The band R_f 0·6 (UV light) gave 0·25 g of **3**, m.p. 55–56°, $[\alpha]_D$ +65·5 (*c*, 1 in $CHCl_3$), M^+ 408 *m/e*, λ_{max}^{MeOH} 243 nm (ϵ = 17000), $\nu_{max}^{CCl_4}$ 1670 and 1620 cm^{-1}, δ^{CDCl_3} 5·73 (1H, s, H–C_4); $[\theta]$ −4870 at 318 nm (MeOH).

Hydrogenation of **1**. **1** (50 mg), dissolved in EtOH (10 ml), was hydrogenated in presence of Pd/C (5 mg) at room temp and 2 atm overnight. After removal of the catalyst and the solvent, the residue, recrystallized from EtOH, gave **4** (35 mg), M^+ 412 *m/e*, $[\alpha]_D$ −17·5 (*c*, 1 in $CHCl_3$), m.p. 125–127°.

1 (50 mg) was hydrogenated at 75° and 3 atm in EtOH (10 ml) on Pd/C for 18 hr. After removal of the catalyst by filtration, the residue was shown to comprise β-sitostanol, as the most abundant constituent, by comparison of its chromatographic (GLC and TLC) properties with those of an authentic sample and by GLC–MS.

Reduction of **3**. A mixture of **3** (0·25 g) and $NaBH_4$ (0·15 g) in dry pyridine (10 ml) was allowed to stand at room temp for 90 hr. After acidification with 2N H_2SO_4 and extraction with Et_2O, the organic phase was dried on $CaSO_4$ and taken to dryness. The residue (0·2 g), without a further purification, was acetylated and successively chromatographed on a SiO_2 column (7·5 g) using C_6H_6 as eluent to give **6** (0·12 g), M^+ 454 *m/e*, as a mixture of diastereoisomers (four compounds with very close R_f on TLC).

$KMnO_4$/$NaIO_4$ *Oxidation of* **6**. To **6** (0·12 g) in t-BuOH (10 ml), K_2CO_3 0·04 M (2·2 ml) and an aqueous soln (11 ml) 0·023 M in $KMnO_4$ and 0·093 M in $NaIO_4$ were added. The mixture was kept at room temp. for 18 hr. After acidification with 5N H_2SO_4, the soln was decolorized with $NaHSO_3$ aq and extracted with Et_2O. After drying over $CaSO_4$ the combined ethereal extracts were taken to dryness and purified on PLC using C_6H_6–Et_2O (95:5) as eluent. The band R_f 0·4 (UV light), scraped and eluted with Et_2O, afforded 0·05 g of **7**, M^+ 486 *m/e*.

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