MARINE STEROLS. XII.* GLAUCASTEROL, A NOVEL C_{27} STEROL WITH A UNIQUE SIDE CHAIN, FROM THE SOFT CORAL SARCOPHYTON GLAUCUM**

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ABSTRACT

A minor C_{27} sterol, glaucasterol, was isolated from the soft coral Sarcophyton glaucum. Based on the spectroscopic evidence and the correlation to cholestanol and 26-nor-27-homocholestanol, its structure was proposed to be 24ξ,25ξ-24,26-cyclocholesta-5,22E-dien-3β-ol (1), the first example of a natural C_{27} sterol having a cyclopropane ring in the side chain.

INTRODUCTION

Recent studies on the complex sterol components from marine invertebrates, particularly from coelenterates and sponges, have identified an ever-growing number of biogenetically unusual sterols which have not been found in terrestrial organisms (1). The soft coral (Coelenterata) Sarcophyton glaucum, a common species in Indian and Pacific coastal waters, is a typical example shown to contain a variety of sterols. In previous studies, we found 19 sterols in S. glaucum collected from Ishigaki Island in southern Japan (2,3,4). Further examinations of minor components revealed that still more unidentified components were present in the sterol mixture of S. glaucum (4). In the present study, we isolated a unique new C_{27} sterol, glaucasterol, for which we propose the structure 1.

RESULTS AND DISCUSSION

Glaucasterol, mp 124-128°, was isolated in small amounts (0.8 mg) from 29.2 g of a sterol mixture from S. glaucum. The isolation was carried out by chromatography of the sterol acetate mixture on silver

nitrate-impregnated silica gel followed by a reversed phase partition chromatography of free sterols by Lipidex 5000 column. The relative retention time (RRT) to cholesterol on gas chromatography (GC) over 1.5% OV-17 column was 1.16, which is a rather large RRT for an unsaturated C_{27} sterol. Desmosterol (5,24-cholestadien-3β-ol), which also occurs in S. glaucum in small amounts (2), has a similar RRT (1.21) but is eluted faster on a $AgNO_3$-silica gel chromatography than 1.

The mass spectrum of 1 (Fig. 1) revealed the molecular formula $C_{27}H_{42}O$ (found, m/z 382.3240; calcd, m/z 382.3238) indicating that 1 is a triunsaturated C_{27} sterol. The presence of a conventional 3β-hydroxy-Δ^5-monounsaturated steroid ring was suggested by proton magnetic resonance (PMR) and mass spectra which satisfied the necessary conditions [chemical shifts of angular methyl groups (5): δ 0.694 (C-18), 1.006 (C-19); C-6 olefinic proton at δ 5.35; broad hydroxy methine at δ 3.5 (6); very weak molecular ion and strong ion at m/z 364 (M^+-AcOH) in the acetate of 1 (7)]. The ions at m/z 271.2065 (calcd, 271.2063, M^+-side chain, -2H), 255 (M^+-side chain, $-H_2O$), 253 (M^+-side chain, -2H, $-H_2O$) and 213 (ring D cleavage, $-H_2O$) are those generally found in Δ^5-sterols with an unsaturated side chain (7,8). The presence of a double bond at C-22 was indicated by the ions at m/z 300, 285, and 267, which are typical ions in Δ^{22}-sterols derived by cleavage at C-20 and C-22 with 1H transfer, and also with the loss of a methyl or a methyl plus H_2O (8). The C_8H_{13} side chain fragment was the base peak (calcd, m/z 109.1018; found, 109.1018). This indicates that the allylic cleavage in the diunsaturated side chain, other than at C-17 and C-20, is restricted. The presence of a conjugated

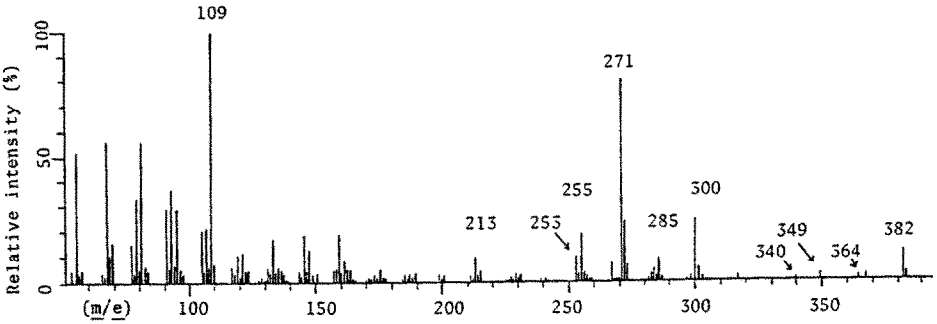


Figure 1. Mass spectrum of glaucasterol

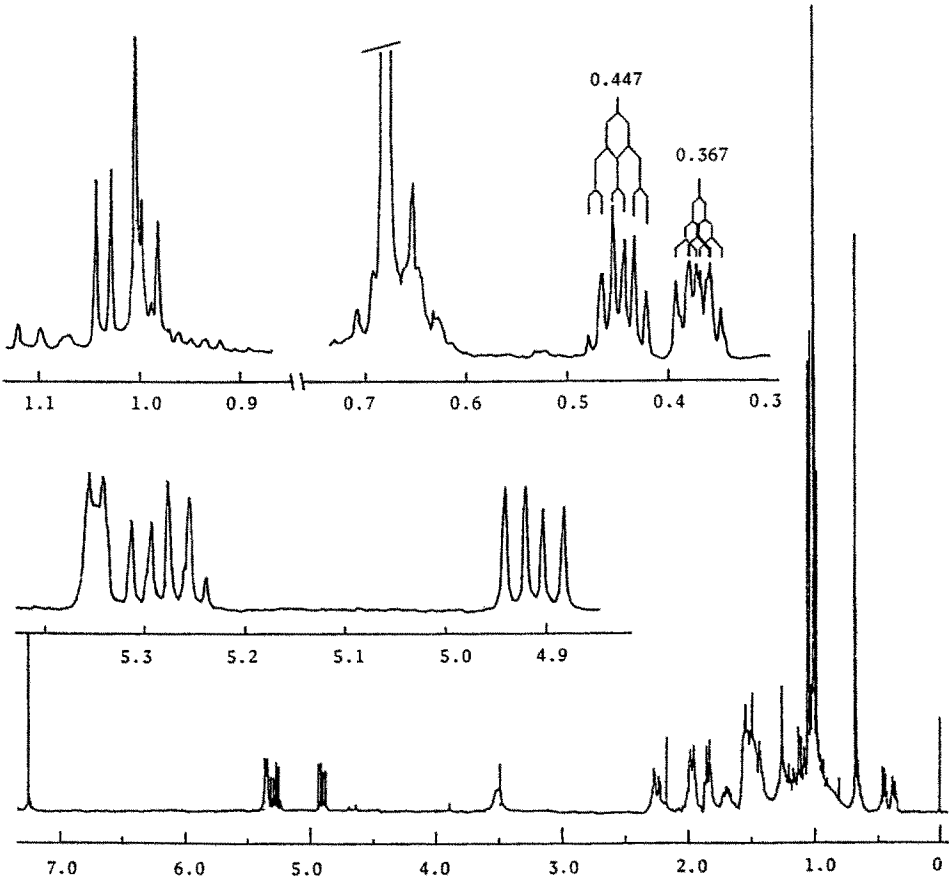


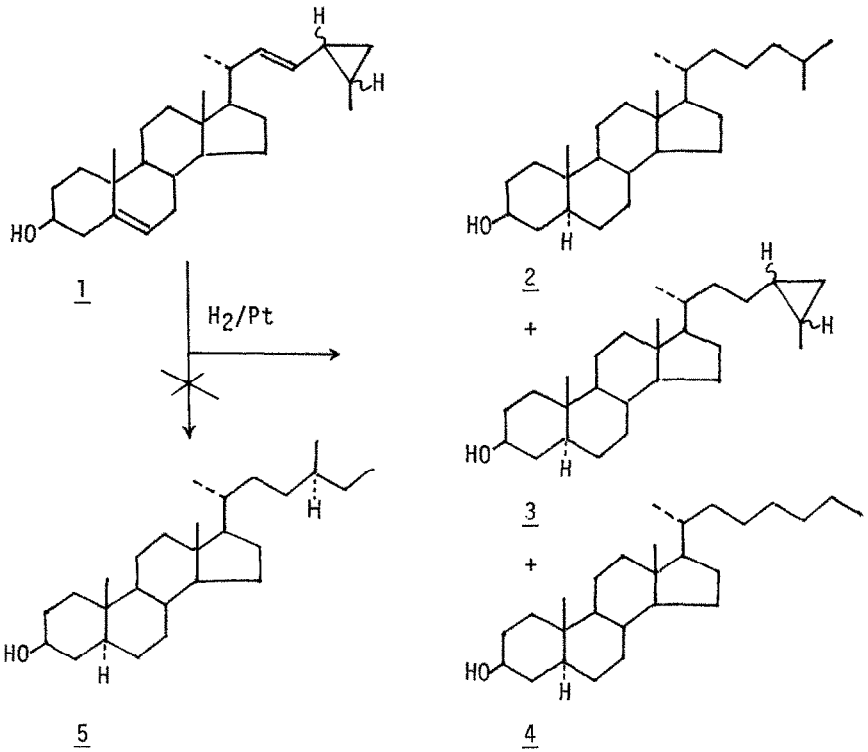
Figure 2. PMR spectrum of glaucasterol (400 MHz, in $CDCl_3$) (δ, ppm)

diene was excluded by the absence of the corresponding UV absorptions.

The 400 MHz PMR spectrum (in $CDCl_3$, Fig. 2) revealed the structure of the side chain in 1. It contained olefinic proton signals only for C-6 (δ 5.35, m) and C-22,23 [δ 5.285, 1H, dd, J=8.3, 15.1 Hz (22-H); 4.912, 1H, dd, J=8.3, 15.1 Hz (23-H)], indicating the presence of a cyclic ring in the side chain. The multiplet signals due to the protons in the cyclopropane ring were observed at δ 0.367 (1H, probably ddd, J=4.4, 5.0, 8.3 Hz) and at δ 0.447 (1H, probably ddd, J=8.3, 8.3, 4.4 Hz). Also, if we assume that glaucasterol (1) was derived from the conventional cholestane skeleton, then the presence of the two secondary methyl signals at δ 1.040 (J=5.86 Hz, C-21) and at 0.994 (J= 6.83 Hz) would indicate that 1 has an unusual C-24,26-cyclized side chain. The weak ion at m/z 340 in the mass spectrum of 1 is thus attributed to the cleavage at the cyclopropane ring.

The side chain structure in 1 was supported by several decoupling experiments. Irradiation at δ 5.285 changed the double doublet at δ 4.912 to a doublet (J=8 Hz). Irradiation at δ 4.912 changed the double doublet at δ 5.285 to a deformed doublet (J≃8Hz) while the shape of a multiplet signal at δ 0.95-1.0 was simplified. This coupling counterpart methine (24-H) of the olefinic proton at δ 4.912 was found at considerably high field (δ 0.99) since the irradiation at δ 0.99 simplified the signals at δ 0.367 and 0.447 , and a multiplet at δ 0.65 overlapped with the 18-methyl signal, and also changed the double doublet at δ 4.912 to a doublet (J=15 Hz). Also, from the transformation of the shapes of each signal by irradiation at δ 0.367 and 0.447, all the four protons on the cyclopropane ring (δ 0.367, 0.447, 0.99, and 0.65) were found to be coupled with each other.

The high field shift of the C-23 olefinic proton (δ 4.912), which in usual Δ^{22}-sterols occurs at δ 5.1-5.25 (9), was thus caused by the shielding effect of the neighboring cyclopropane ring. The large vicinal coupling constant (15.1 Hz) of 22,23-olefinic protons indicates the trans geometry of the C-22 double bond. It is also consistent with the fact that in 22-cis sterols, the 18-methyl signal occurs at lower field (δ 0.733)(10), while the 21-methyl signal occurs at higher field (δ 0.94-0.95)(11) than those in 1.



Another evidence for the structure 1 was obtained by its conversion into the known sterols by mild catalytic hydrogenation. Hydrogenation of 1 over PtO_2 in acetic acid-methanol gave three

products (2,3,4) in 10:5:1 ratio as examined by GC. Compounds 2 and 4 were identified as 5α-cholestan-3β-ol and 26-nor-27-homo-5α-cholestan-3β-ol (12), respectively, by direct comparison with the authentic samples by glass capillary GC and by GC-mass spectra. Occelastanol [5, (24S)-methyl-27-nor-5α-cholestan-3β-ol] (9) or its C-24 isomer was not found (13). This indicates that each one of the two strained allylic bonds in the cyclopropane ring was reductively cleaved by hydrogenation leading to 2 and 4 but not to 5 or its C-24 isomer (13). The structure of the second major product (3) was assigned as 24ξ,25ξ-24,26-cyclo-5α-cholestan-3β-ol since its mass spectrum showed ions due to the cleavage of the cyclopropane ring at m/z 344 (M^+-C_3H_6) and 329 (M^+-C_3H_6, -Me), and at m/z 316 and 301 which arose due to the cleavage at C-22 and C-23 with 1H transfer by McLafferty-type rearrangement (14, 15). The structure of glaucasterol was thus proposed to be 24ξ, 25ξ-24,26-cyclocholesta-5,22E-dien-3β-ol (1), the first member of a class of C_{27} sterols having a cyclopropane ring in the side chain (15).

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EXPERIMENTAL

Melting points were determined on a Kofler hot stage and are uncorrected. PMR spectra were recorded on a JEOL FX 500 spectrometer operating at 400 MHz, in $CDCl_3$ soln. with TMS as internal standard. Mass spectra were determined on a JEOL JMS D-300 spectrometer. Analytical GC was carried out on a Hitachi 163 gas chromatograph using a silicon OV-1 wall-coated open tubular glass capillary column (0.35 mm x 25 m), with N_2 carrier gas at a flow rate of 2.7 ml/min. at 250°. GC-mass spectrometry was carried out on a Shimadzu-LKB 9000 spectrometer using a glass column (3 mm x 2 m) packed with 2% OV-1 on 60-80 mesh Chromosorb W at 285°, with He carrier gas at a flow rate of 30 ml/min.

Isolation of glaucasterol (1) The fractions 123-125 (40 mg), obtained by $AgNO_3$-silica gel chromatography of the S. glaucum sterol acetate in a previous study (4), was a mixture of 24-methylene-5α-cholestan-3β-ol acetate (90%), 4α-methyl-24-methylene-5α-cholestan-3β-ol acetate (3%) and glaucasterol acetate (5%). It was hydrolyzed with 2.5% KOH in methanol in the usual manner and the free sterol mixture was chromatographed over a column of silica gel (50 g) with 5% ether in $CHCl_3$. The 4-demethylsterol fraction (31.8 mg), which was eluted after 4α-methyl-24-methylene-5α-cholestan-3β-ol, was subjected to Lipidex 5000 (Packard, 2.2 x 45 cm) column chromatography with a mixture of hexane-methanol (5:95) and the fractions were examined by GC. Glaucasterol was eluted slightly faster than 24-methylene-5α-cholestan-3β-ol. The fractions which contained only 1 were combined and recrystallized from methanol to give pure 1 (0.8 mg). For mp and PMR and mass spectra, see TEXT.

Hydrogenation of glaucasterol (1) A solution of 1 (0.1 mg) in 5% acetic acid in methanol (2 ml) was hydrogenated with ca. 1 mg of PtO_2 catalyst overnight. Filtration and evaporation of the solvent gave a solid residue which showed three peaks on glass capillary GC[peak 1 (RRT 1.02), 62%; peak 2 (RRT 1.08), 31%; peak 3 (RRT 1.12), 7%]. The peaks 1 and 3 were identified as 5α-cholestan-3β-ol and 26-nor-27-homo-5α-cholestan-3β-ol, respectively, by co-injection on GC and also by GC-mass spectra. Mass spectrum of peak 2 (24ξ,25ξ-24,26-cyclo-5α-cholestan-3β-ol), m/z 386 (M^+), 371 (M^+-Me), 353 (M^+-Me,-H_2O), 344 (M^+-C-25 to C-27), 329 (M^+-C-25 to C-27,-Me), 316 (M^+-C-23 to C-27,-H), 301 (M^+-C-23 to C-27,-H,-Me), 273 (base peak, M^+-side chain,-2H), 257 (M^+-side chain,-H_2O) (cf. references 14, 15)

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