THE ISOLATION AND CHARACTERIZATION OF (24*S*)-24-METHYLCHOLESTA-5,25-DIEN-3β-OL FROM THE SIPHONOUS MARINE ALGA *CODIUM BURSA*

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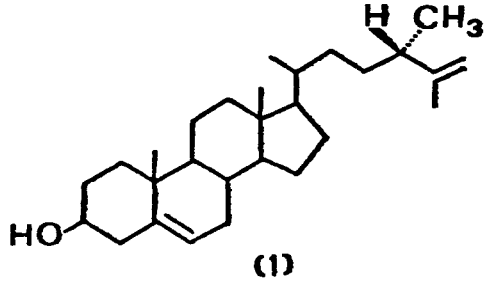
ABSTRACT.—(24*S*)-24-Methylcholesta-5,25-dien-3β-ol was identified in the sterol mixture isolated from the marine siphonous green alga *Codium bursa*. Its structure was elucidated by spectroscopic methods. The natural occurrence of this compound may be conclusive for the chemotaxonomic characterization of the genus *Codium* because of its rarity in nature.

The distribution of sterols in algae is receiving increasing attention (1) due to the variety of these compounds occurring in the different classes of algae (2). On the basis of the hypothesis that the biosynthetic pathways for sterols are rather similar in algae and higher plants (1), a more thorough knowledge of the sterol composition may lead to advances in taxonomy and plant evolution and provide clues to the functions of sterols in plants.

C_{27} sterols were identified in most members of the Rhodophyceae, with cholesterol as the main component, while the C_{28} and C_{29} are scarce (3). In the Phaeophyceae the presence of C_{29} fucosterol (4) as the predominating component may be considered to have phylogenetic significance since it is rarely found in other species of algae. By contrast, studies on the Chlorophyceae have been less intensive, and the complexity of their composition has led to incorrect identifications, which can be avoided only by the use of modern spectroscopic techniques. All the identified sterols have been found to contain alkyl groups at C_{24}, with a 24*S* configuration, which may be characteristic of the green algae (1). Thus 28-isofucosterol (5) was isolated from some members of the Ulotrichales (6), and a variety of 24-methyl and 24-ethyl-sterols (1,7) were found in some species of the Chlorococcales.



(1)

FIG. 1. (24*S*)-24-methylcholesta-5,25-dien-3β-ol isolated from Codium Bursa.

In this paper we report the isolation, from ether extracts of the marine alga *Codium bursa* (Chlorophyceae; Siphonales) (8), of a C_{28} sterol with a 24*S* methyl group and its structure and configuration elucidation as (24*S*)-24-methylcholesta-5,25-dien-3β-ol (**1**) on the basis of spectrometric parameters and suitable derivatives. The isolation of **1** appears significant for the characterization of the genus *Codium* because of the rareness of its occurrence; hitherto, the acetate of **1** has been reported as a minor component only in the sterol mixture from *Codium fragile* (9).

EXPERIMENTAL[1]

EXTRACTION.—*Codium bursa* was collected in the Bay of Milazzo (Messina). The algae (~10kg) were homogenized in acetone and filtered, and the residue was extracted for a second time with acetone. The extracts were concentrated under vacuum and extracted with petroleum ether (30–50°). The removal of the solvent yielded a dark green mass (25 g) which was saponified in 10% methanolic KOH (4 hs).

The unsaponifiable part, extracted with ethyl ether, was chromatographed on an alumina column (300 g), activity grade III, eluting with increasing percentages of diethyl ether in petroleum ether. The fractions eluted by 8:2 ether-petroleum ether yielded a sterol mixture (0.9 g; green color in the Liebermann-Burchard test). By subsequent Si gel tlc, (24*S*)-24-methylcholesta-5,25-dien-3β-ol was isolated (200 mg) from the most polar band and identified; mp 148°–50° (from EtOH); ir: νmax cm^{-1} 980, 1641, 3340; ^1H nmr: (δ, ppm) 0.67 (s, 3H, C-18), 1.02 (s, 3H, C-19), 0.96 and 1.01 (d, 3H, C-24), 0.87 and 0.98 (d, 3H, C-21), 1.62 (s, 3H, C-27), 4.65 (m, 1H, C-3β), 4.64 and 4.68 (2H, C-26), 5.35 (m, 1H, C-6).

HYDROGENATION.—Compound **1** (50 mg) in 15 ml of ethyl acetate was hydrogenated at room temperature and atmospheric pressure in the presence of 20 mg of 10% Pd/C. 24-Methylcholesta-5-ene-3β-ol (**2**) was obtained (50 mg) mp 150–3° (from EtOH). Nmr: (δ, ppm) 0.67 (s, 3H, C-18), 1.03 (s, 3H, C-19), 0.85 and 0.91 (d, 3H, C-26), 0.76 and 0.82 (d, 3H, C-27), 0.90 and 0.94 (d, 3H, C-21), 0.75 and 0.82 (d, 3H, C-28), 4.65 (m, 1H, C-3α), 5.34 (m, 1H, C-6).

ACETYLATION.—Under standard conditions (pyridine-acetic anhydride), 30 mg of **1** gave quantitatively the acetyl derivative (**3**) mp 118–121°; ir νmax cm^{-1} 980, 1645, 1750; ms: *m/e* (%) 440 (3), 380 (100), 313 (7), 255 (10), 228 (15), 213 (18); nmr: (δ, ppm) 0.66 (s, 3H, C-18), 1.02 (s, 3H, C-18), 1.02 (s, 3H, C-19), 0.94 and 1.01 (d, 3H, C-28), 0.89 and 0.96 (d, 3H, C-21), 1.61 (s, 3H, C-27), 2.06 (s, 3H, CH_3COO), 4.65 (m, 1H, C-3α), 4.68 (2H, C-26), 5.35 (m, 1H, C-6).

RESULTS AND DISCUSSION

The isolated sterol **1** mp 148°–50° (from EtOH), had the formula $C_{28}H_{46}O$ (M^+ 398), which indicates the presence of two unsaturations in the molecule. The ir spectrum shows a broad hydroxyl absorption at 3340 cm^{-1} and two bands, characteristic of a $>C=CH_2$ group at 1641 and 980 cm^{-1}. The presence of the isopropenyl group was confirmed by the nmr spectrum of **1**, which displayed two peaks at δ 4.65 and δ 4.68 and a three-proton singlet at δ 1.62. These resonances were lacking for the dihydro-derivative (**2**) obtained by catalytic hydrogenation of **1**. In addition, the nmr spectrum of **1** showed another olefinic proton resonance at δ 5.35, which is still present in the spectrum of **2**; therefore, the presence in **1** of a trisubstituted double bond inert to hydrogenation was deduced.

Compound **1** gave a monoacetyl derivative (**3**), whose nmr spectrum shows a signal at δ 2.06 for the acetyl group and a multiplet centered at δ 4.65 for the carbinolic proton. Conclusive information on the skeleton of **1** was deduced from the ms spectrum of **3**, which shows, beside the base peak at *m/e* 380 (M^+-acetate), ions at *m/e* 255 (M^+-lateral chain-acetate) and 213 (M^+-lateral chain-42-acetate), which suggest that **1** is a C_{28} steryl acetate containing a double bond in the lateral chain.

The trisubstituted double bond was located in position 5 by the presence of peaks at δ 0.67 and 1.02 for the C-18 and C-19 protons, respectively (10); the multiplet at δ 5.35 identifies the C-6 proton. The second double bond of the terminal methylene group therefore must be located at C-25 on the basis of the above-mentioned nmr parameters relative to the resonances of the two olefinic protons (in 26) and of the three methyl protons at C-27. Furthermore, the doublet at δ 0.96 and δ 1.01 is attributable to the protons of the methyl group at C-24.

The addition of the paramagnetic lanthanide reagent Eu(fod)$_3$ confirms the

[1]Mps are uncorrected. Nmr spectra were recorded in $CDCl_3$ at 60MHz. Chemical shifts are in ppm (δ) from TMS as internal standard. Ir spectra were determined as nujol mulls. Ms were recorded at 70 eV by direct inlet system. Cc was performed with Al_2O_3 and tlc with Si gel. Eu(fod)$_3$ induced shifts measurements (LIS) were carried out in solutions 0.3M, with increasing amounts of the lanthanide reagent up to a value of 0.4 L/S, at a probe temperature of 30–35°C. Eu(fod)$_3$ was gradually added from a standard solution (~300 mg/ml) by means of a 50 μl syringe. Each signal was followed, and the LIS were found to be directly proportional to the actual L/S ratio. The computer simulation of the experimental LIS was done on an IBM 370/115 computer; the LISCA program (14) was used for the interaction between the shift reagent and the hydroxyl group.

structural deductions (11). The computer simulation of the shifts induced by the complexation of lanthanide to 3β-hydroxyl group supported the assignments of the methyl resonances, the localization of the olefinic protons and, in particular, made it possible to define as 24*S* the configuration at C–24. Thus, **1** has the structure of (24*S*)-24-methylcholesta-5,25-dien-3β-ol. Moreover the acetyl derivative (**2**) had a mp of 122–3°, in good agreement with the value reported for the codisterol acetate (9), isolated as a minor component of the sterol mixture from the *Codium fragile.*

Since it is rarely found in nature, the isolation of this compound from two algae may prove to be important for the chemotaxonomic correlation of the genus *Codium.* Moreover, the occurrence of 25-methylene sterols in members of the order Siphonales is further confirmation of the hypothesis which suggests the derivation of the green siphonous algae from the Chlorococcales (9). Recent studies on the C–24 alkylation mechanism (12,13) have, in fact, revealed that the biosynthesis of 24-methyl-and 24-ethylsterols proceeds via 25-methylene sterols as intermediates. The study of the total composition of the steroid fraction of *Codium bursa* and other Codiaceae is in progress in order to improve such chemotaxonomic correlations.

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