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SHORT COMMUNICATION

TWO NEW STEROLS FROM *FUCUS EVANESCENS**

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Key Word Index—*Fucus evanescens*; Phaeophyta; sterols; 3,5,24-stigmastatriene-7-one; 5,24-stigmastadiene-3β,7α-diol.

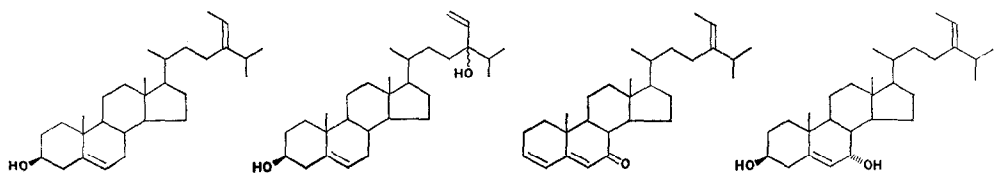
Abstract—Two new sterols, 3,5,(*E*)-24(28)-stigmastatrien-7-one and 5,(*E*)-24(28)-stigmastadien-3β,7α-diol, and friedelin were isolated from brown algae, *Fucus evanescens*.

INTRODUCTION

IT IS A fact that the major sterol of brown algae irrespective of species,[1] is a 24-ethylidensterol, fucosterol(I). However, previously we have found minor sterol components, saringosterol (II),[2] cholesterol and 24-methylenecholesterol in several brown algae.[3] By further related studies we have isolated another two new sterols from *Fucus evanescens* (Hibamata), whose structures are presented in this report.

RESULTS

From the nonsaponifiable fractions of benzene extracts of *F. evanescens*, most fucosterol was removed by crystallization from ethanol. The mother liquors were subjected to column chromatography on silicic acid and two new sterols (III and IV) were isolated, besides fucosterol(I), saringosterol(II), a triterpene, friedelin[4] and a diterpene, phytol.[5]



(I) (II) (III) (IV)

Sterol(III) was assumed to contain 3,5-dien-7-one steroid nucleus, as shown from IR bands at 1652, 1620 and 1590 cm^{-1} and UV absorption maximum at 280 nm (ϵ 28 000). These values, as well as NMR signals at 6·07 (2H, *s*) and 5·48 ppm (1H, *s*), were consistent with the published data on 3,5-stigmastadien-7-one.[6] Additional confirmation was obtained by direct spectral comparison with 3,5-cholestadien-7-one.[7] The presence of 24-ethylidene

* Part II in the series on "Studies on Steroids". For Part I see *J. Org. Chem.* **35**, 4145 (1970).

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moiety in the side chain was suggested from NMR signals at 5·12 (1H, *q*, $J = 7$ Hz) and 1·55 (3H, *d*, $J = 7$ Hz), and mass ion peak at *m/e* 310·2296 (theory for $C_{20}H_{30}O$, 310·2296) derived from MacLafferty rearrangement.[8,9] An isomeric structure (Z-type) can be safely excluded by the absence of NMR signal at 7·2 ppm due to C-25 proton.[10,11] Reduction of III with sodium borohydride gave the dienol, UV max. at 237 nm (ϵ 22 000), while catalytic hydrogenation with 10% Pd–charcoal afforded the hexahydro derivative (M^+ 414). From the above mentioned data, the structure of sterol(III) was concluded to be 3,5,(E)-24(28)-*stigmastatrien-7-one.* However, it is difficult to believe that a compound of this nature could exist, *per se*, in algae. Thus, we have the feeling that it was formed during the isolation procedure from 3β-hydroxy-5-stigmasten-7-one or its ester which may be the true compound present in algae.

Sterol(IV) contained an IR band at 3300 cm^{-1} and no characteristic UV absorption above 220 nm. Its NMR signals at 5·18 (1H, *q*, $J = 7$ Hz) and 1·57 ppm (3H, *d*, $J = 7$ Hz), and mass ion peaks at *m/e* 330 (M-98) and 312 ($M-H_2O$-98), were characteristic of a 24-ethylidensterol, while other signals at 5·60 (1H, *d*, $J = 5$ Hz), 3·85 (1H, *q*, $J = 5$ and 2 Hz), and 1·00 (3H, *s*) were superimposable to those of 7α-hydroxycholesterol,[12] assigned to C_6-H, C_7-H and C_{19}-methyl protons, respectively. Oxidation of sterol(IV) with manganese dioxide, followed by acid catalyzed dehydration, yielded sterol(III) thereby confirming the structure of sterol(IV) as 5,(E)-24,(28)-*stigmastadien-3*β,7α-*diol.*

EXPERIMENTAL

Isolation procedure. Fucus evanescens, identified by Dr. Y. Nakamura, Hokkaido University, was harvested at Muroran Bay, North Japan. The air-dried, chopped algae (20 kg) was refluxed with benzene and the extract (270 g) was saponified by refluxing in a mixture of NaOH (40 g), MeOH (300 ml), benzene (60 ml) and H_2O (100 ml), for 6 hr. The unsaponifiable fraction was recrystallized from EtOH and 22 g of fucosterol was obtained. The remaining solvent was evaporated to leave a pale brown oil (12 g), which was applied on a column of silicic acid (360 g). The column was eluted stepwise and each fraction was checked by TLC and GLC. Fractions 20–30 (*n*-hexane-benzene (1:1) contained Sterol(III), friedelin and phytol; 31–45 (benzene) had Fucosterol(I); 43–45 (benzene-Et_2O (7:3) Saringosterol(II); and 47–51 (benzene-Et_2O (1:1), Sterol(IV). Fraction 20–30 (3·6 g) was acetylated with Ac_2O and pyridine overnight at room temp. and separation of phytol acetate was achieved by column chromatography on silicic acid. From the residual fraction (260 mg), friedelin (10 mg) was obtained by crystallization from acetone. The mother liquid, after evaporation of solvents, was crystallized from light petroleum to afford pure compound III (200 mg). Compound III m.p. 85° $[\alpha]_D$ −180°, (Calcd. for $C_{29}H_{44}O$: C, 85·23, H, 10·85. Found: C, 84·75, H, 10·69%). M^+ 408·3390 (Theory, 408·3392). Fraction 47–51 (100 mg) was crystallized from EtOH to give pure compound IV (20 mg). Compound IV m.p. 180°, $[\alpha]_D$ −25·6°, M^+ 428 ($C_{29}H_{42}O_2$) Friedelin and phytol were identified by the direct comparison with authentic samples.

Reduction of III *with* $NaBH_4$. To the solution of III (10 mg) in 1 ml of MeOH, 50 mg of $NaBH_4$ was added and the mixture stirred at room temp. for 1 hr. The product showed a single peak on GLC, NMR signal at 3·80 (1H, *m*, C_7-H), UV absorption at 237 nm (ϵ 22 000) and IR band at 3350 cm^{-1}.

Catalytic hydrogenation of III. Compound III (10 mg) in EtOH (1 ml) was hydrogenated over 10% Pd-C (10 mg) at room temp. and atmospheric pressure. The products showed a single peak on GLC (1·5% OV-1 on Chromosorb W), M^+ 414 on mass spectrometry and no major maximum absorption in the UV.

Allylic oxidation and dehydration of IV. Sterol (IV) (5 mg) was refluxed with MnO_2 (100 mg) in 3 ml $CHCl_3$ for 48 hr. The product, having UV maximum at 237 nm, was refluxed in 3 ml EtOH containing conc. HCl (0·1 ml) for 2 hr. Filtration through silicic acid gave crude products (UV max at 281 nm), in which sterol (III) was definitely identified by GC-MS system using LKB-9000; column packing, 1·5% OV-1 on Gas Chrom P; column size, 150 cm × 4 mm i.d.; temp. 250°; 70 eV.

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