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STEROLS OF *GONIOTRICHUM ELEGANS*

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Abstract—The sterol fraction was examined from cultures of the filamentous red alga *Goniotrichum elegans.* Brassicasterol accounted for nearly half of the total sterol and was accompanied by cholesterol (24%) as well as a number of minor components. This is the first record of brassicasterol as a major sterol in the Rhodophycophyta. The occurrence of this C_{28} major sterol may be of taxonomic importance in determining the relationship of the Goniotrichales to the other red algae all of which have C_{27} major sterols.

INTRODUCTION

The red algae (Rhodophycophyta) are usually divided on the basis of morphology and life histories into two subclasses, the Bangiophycidae and Florideophycidae. Cholesterol (cholest-5-en-3β-ol) has been considered, with a few exceptions to be characteristic of the Division [1]. However, nearly all of the previous studies concern representatives from the Florideophycidae, and only two of the four orders within the Bangiophycidae have been surveyed. Those genera within the order Bangiales usually exhibit desmosterol (cholesta-5,25-dien-3β-ol) and more rarely cholesterol as the major sterol component (R. Carlile, personal communication and refs. [2–5]), while those within the Porphyridiales have 22-dehydrocholesterol cholesta-5,22-dien-3β-ol) as the major sterol [6, 7].

Since desmosterol and 22-dehydrocholesterol are the most common major sterols in the Bangiophycidae, it was apparent that cholesterol was probably not characteristic of the subclass and that further examination of representatives of the other two orders within the Bangiophycidae was appropriate. Therefore, we chose to analyse the sterols of *Goniotrichum elegans* (Chauv.) Zanardini, a member of the order Goniotrichales, with the goal of providing information about the distribution of sterols within this order and broadening our understanding of sterol composition within the Bangiophycidae.

RESULTS AND DISCUSSION

Although the total sterol yield was low, two sterols were detected in appreciable amounts along with a number of minor components. The major sterol, comprising 44% of the total, showed a retention time relative to cholesterol of 1.12 on SE-30 and 1.09 on QF-1. These retention times are diagnostic for brassicasterol (24-methylcholesta-5,22-dien-3β-ol). The identification of this sterol was confirmed by its MS. A molecular ion at *m/e* 398 infers a C_{28} diunsaturated sterol, while peaks at *m/e* 383 (M − 15)and 300 [M − 15 + (C_{23-27} + 2H)] in combination with a strong ion at 255 (M − side chain + HOH) are indicative of both a C_{22} double bond and an alkyl group at C_{24}. Although there was insufficient sterol in the sample to obtain it in pure form for determination of the C_{24} absolute configuration by ^{1}H NMR spectroscopy, the sterol was assumed to be brassicasterol.

A second peak with a retention time relative to cholesterol of 1.01 on SE-30 and 1.02 on QF-1 was identified as cholesterol. Although this peak accounted for 24% of the total sterol there was insufficient amounts to obtain a definitive MS. However, since the retention times on both column packings were very close to cholesterol, we are confident in identifying it as this compound rather than cholestanol. Two minor peaks comprising 12 and 5% of the total sterol were tentatively identified as 24-dihydro-lanosterol and 24-methylene-cholesterol on the basis of their retention times on SE-30.

The identification of brassicasterol in this alga is both of general interest and taxonomic significance. Although this sterol has been reported as a minor component in other red algae, this is the first record of it occurring as the major sterol of any alga of the Rhodophycophyta. In addition, the presence of brassicasterol in *Goniotrichum*, if characteristic of the Goniotrichales, would set the order apart from other algae within the Bangiophycidae and from the red algae in general.

Since *Goniotrichum* is easily cultured, it should be possible to determine the relationship of cholesterol to brassicasterol synthesis by the application of specific inhibitors and labeled precursors. The biosynthetic scheme thus generated could then be used to determine whether sterols in *Goniotrichum* are synthesized as in animals or via a series of C_{24} alkyl sterols as in higher plants [8] and green algae [9].

EXPERIMENTAL

Algal culture. Stock cultures of *Goniotrichum elegans* were purchased from the Culture Collection of Algae at the University of Texas (UTEX 1957). These were grown in modified

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Erdschriebers media [10] substituting disodium glycerophosphate for Na_2HPO_4 and buffered with Tris to pH 7.8. Culture vessels were filled to a depth of 3 cm with media and lighting was indirect via a shaded window. The light period was not controlled and approximates that of College Place, Washington during the months of December and January. The algae were harvested by filtration and dried before extraction of the lipids.

Extraction and purification of sterols. The lipid fraction was extracted with $CHCl_3$–MeOH (2:1) for 24 hr in a Soxhlet followed by saponification for 1 hr in 15% KOH dissolved in 95% EtOH. The nonsaponifiable lipids were removed by separation against Et_2O for 24 hr in a liquid–liquid separator. Purification of the crude sterol fraction was accomplished by CC on a short (3 cm) alumina column with hexane as the solvent. After several mls of hexane had run through the column, the alumina was removed, dried and the sterols eluted from it with Et_2O followed by $CHCl_3$. The sterols were redissolved in hexane and refrigerated until use.

GLC and GC–MS. GLC was performed with 1% SE-30 and 1% QF-1, both on Gas Chrom Q (Applied Science), packed in 183 cm × 4 mm coiled glass columns. Column oven temp. was 250° for SE-30 and 220° for QF-1 with a N_2 at 30 ml/min. Injector temp. was 260° and the detector temp. was 300°. Detection was by hydrogen flame ionization. The relative amount of each sterol was determined as a percentage of the total sterol with the aid of a Varian model CDS 111C chromatographic data system. Sterols were identified according to their retention times relative to cholesterol [11].

MS were determined with a Hewlett–Packard model 5985A GC–MS. A 183 cm × 4 mm column packed with 1% OV-17 was used at a column temp. of 265°. Injector temp. 250° and N_2 at 30 ml/min. The source temp. of the mass spectrometer was 200° with a source voltage of 70 eV.

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