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STEROLS OF THE BROWN TIDE ALGA *AUREOCOCCUS ANOPHAGEFFERENS*

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Key Word Index—Brown tide; *Aureococcus anophagefferens*; Pelagophyceae; chrysophytes; chemotaxonomy; algal sterols; 24-propylidenecholesterol.

Abstract—*Aureococcus anophagefferens*, an alga responsible for "brown tide", contains 24-methylenecholesterol as 48% of the free sterols and (*E*)- and (*Z*)-24-propylidenecholesterol as 32% and 12% respectively. This is the first isolation of appreciable amounts of (*Z*)-24-propylidenecholesterol from an alga and suggests that *Aureococcus anophagefferens* may be the source of the (*Z*)-24-propylidenecholesterol isolated from various marine invertebrates. 24-Propylidenecholesterol is proposed to be a chemotaxonomic marker for the Pelagophyceae, a new class of chrysophytes which includes the Sarcinochrysidales.

INTRODUCTION

Aureococcus anophagefferens is a picoplanktonic alga responsible for "brown tides" along the New York and southern New England coasts [1–3]. This organism has recently been classified together with several other aberrant chrysophytes as a member of a new alga taxon, the Pelagophyceae [4]. The sterols of three other members of this group have been examined and all have been found to contain the unusual marine sterol 24-propylidenecholesterol, almost exclusively as the *E*-isomer (**1**) [5–8]. 24-Propylidenecholesterol (**1**) has also been isolated in trace amounts from numerous marine invertebrates where it appears to be of dietary origin [9–24]. This sterol and its reduced forms have been used as geochemical biomarkers [25–27]. In particular, the 24-*Z* isomer of 24-propylidenecholesterol (**2**) has often been detected in sediments and marine invertebrates, but a plausible algal source has not been identified until now.

RESULTS

The sterols of cultured *Aureococcus anophagefferens* were separated by HPLC (Table 1), and the sterols were identified from their ^{1}H-NMR spectra. The major sterol was 24-methylenecholesterol (**3**) (48%), followed by (**E**)-24-propylidenecholesterol (**1**) (32%) and (*Z*)-24-propylidenecholesterol (**2**) (12%). These two isomers could be easily distinguished by the olefinic signals of their 24-propylidene groups {**1**: 5.06 (*t*); **2**: 5.01 (*t*)}, and their even more characteristic allylic H-25 signals {**1**: 2.19 (sept.); **2**: 2.80 (sept.)} [5].

DISCUSSION

Although (*Z*)-24-propylidenecholesterol (**2**) has previously been isolated from a marine chrysophyte [6] (identified as *Chrysoderma mucosa* [28], but reclassified as *Pulvinaria* sp. [29]), in that case it constituted only 0.22% of the total sterols. This, together with the benthic habitat of *Pulvinaria* sp., render this species unlikely to be the source of the (*Z*)-24-propylidenecholesterol (**2**) found in marine invertebrates and sediments. *Nematochrysopsis roscroffensis* and *Sarcinochrysis marina*, the only other two algae reported to contain 24-propylidenecholesterol, are similarly benthic organisms. The detection of (*Z*)-24-propylidenecholesterol (**2**) as a major sterol in *A. anophagefferens* is significant since this species occurs in the water column and is very prolific, capable of reaching densities of 3×10^6 cells ml^{-1} during a bloom [1]. Therefore *A. anophagefferens* may be the source of the (*Z*)-24-propylidenecholesterol (**2**) found in sediments and filter-feeding organisms, and may also represent the major source of the more abundant 24-*E* isomer (**1**). Interestingly, the scallop *Placopecten magellanicus*, from which 24-propylidenecholesterol was first isolated 25 years ago, contained the 24-*E* and 24-*Z* isomers in a ratio close to that found in *Aureococcus* [12].

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Table 1. Sterols of *Aureococcus anophagefferens*

Sterol	HPLC retention time (min)	Percentage of total sterols
24-Methylenecholesterol (**3**)	41.5	48.4
Unidentified sterol	48.5	2.8
(*E*)-24-Propylidenecholesterol (**1**)	50.5	32.0
(*Z*)-24-Propylidenecholesterol (**2**)	52.0	12.4
Dihydrobrassicasterol (**4**)	54.0	4.4
Sterol content: 0.35% dry wt		

Regardless of whether *Aureococcus* represents the major trophic source of 24-propylidenecholesterol in the food chain, this sterol appears to be a specific biomarker for the Pelagophyceae to which *A. anophagefferens* belongs. Until now, this sterol had only been found in members of the Sarcinochrysidales (*Pulvinaria* sp., *Nematochrysopsis roscoffensis*, and *Sarcinochrysis marina*) [5–8]. (It should be noted that although two publications [6, 8] mention the occurrence of 24-propylidenecholesterol in *Sarcinochrysis marina*, no published sterol analysis of this species exists.) Recently the class Pelagophyceae has been expanded to include the Sarcinochrysidales [29]; 24-propylidenecholesterol has never been detected in an algal species outside this new class. Conversely, all members of this class examined to date (*Aureococcus anophagefferens*, *Pulvinaria* sp., *Nematochrysopsis roscoffensis* and possibly *Sarcinochrysis marina*) contain 24-propylidenecholesterol. The sterol composition of algal species is often of chemotaxonomic value [30]. Because of its apparent specificity to the Pelagophyceae, 24-propylidenecholesterol resembles another unusual C30 sterol dinosterol, which is a specific biomarker for dinoflagellates [31, 32]. Sterol analysis of the members of the Pelagophyceae will be necessary in order to assess the generality of this taxonomic marker.

EXPERIMENTAL

Culture conditions

Cultures of *Aureococcus anophagefferens* CCMP-1706 were grown at 18° with aeration in 12 l glass carboys containing artificial seawater [33] supplemented with F/2 trace metals [34] using a 14:10 h light/dark cycle (160 μE m^{-2} s^{-1}). Cell growth was measured using *in vivo* fluorescence and by visual cell counts under phase contrast. Cells were harvested in stationary phase by centrifugation (14,000 × g) and lyophilized. Lyophilized cells (142 mg) were obtained from 8 l containing 3×10^6 cells ml^{-1}.

Sterol analysis

The lyophilized cells were extracted overnight at room temp. with 10 ml EtOAc twice. The extracts were concentrated under reduced pressure and fractionated by TLC on silica gel (hexanes–EtOAc 2:1). The sterol band was treated with acetic anhydride–pyridine to form the acetates which were purified by TLC (hexanes–EtOAc 4:1) and saponified to recover the free sterols. The sterols were separated using a Waters HPLC system (6000A pump, R401 differential refractometer) equipped with two Altex Ultrasphere ODS 5-μm columns (10 mm i.d. × 25 cm) in series using a flow rate of 3 ml MeOH min^{-1}. The relative proportions of sterols were determined from the HPLC integrals. The absolute amount of sterols (500 μg) was estimated by comparison of the HPLC integrals to that of a standard (cholesterol) of known weight. The sterol fractions were evaporated with a stream of N_2 and characterized by ^{1}H-NMR (300 MHz, $CDCl_3$) spectroscopy. The NMR spectra matched those of known samples: (*E*)-24-propylidenecholesterol (**1**) [5]; (*Z*)-24-propylidenecholesterol (**2**) [5]; 24-methylenecholesterol (**3**) [35]; dihydrobrassicasterol (**4**) [36].

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