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STEROLS OF *AGARUM CRIBOSUM*: DESMOSTEROL IN A BROWN ALGA

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Abstract—The sterol composition of the cold water brown alga *Agarum cribosum* was determined by GC–MS. Six of the seven sterols found were identified as stigmasta-5,(*E*)-24(28)-dien-3β-ol (fucosterol), 24-methylenecholest-5-en-3β-ol (24-methylenecholesterol), cholest-5-en-3β-ol (cholesterol), 3β-hydroxycholest-5-en-24-one (24-ketocholesterol), 24ξ-stigmasta-5,28-diene-3β,24-diol (saringosterol) and cholesta-5,24-dien-3β-ol (desmosterol).

INTRODUCTION

In our examination of the brown alga *Agarum cribosum* for possible chemical defensive agents, we have made a study of the major chemical constituents of this species. In this paper we report on its sterol composition. Of particular note is the detection in small amounts of the sterol desmosterol (cholesta-5,24-dien-3β-ol). This sterol is common in red algae but its presence has not been confirmed in brown algae although Patterson [1] tentatively identified it in *Laminaria digitata* and *L. faeroensis* by GLC retention times. Our mass spectral analysis positively confirms this sterol's presence in a brown alga.

RESULTS

The non-saponifiable lipids of *Agarum cribosum* (2.7 g) were separated by preparative HPLC and two sterol fractions with differing NMR spectra were obtained. The NMR spectrum of fraction 1 was similar to that of fucosterol but it also contained a peak at 4.8 ppm (δ) corresponding to the terminal methylene protons at C-24 of 24-methylenecholesterol. Both fractions were acetylated and each was analysed by high resolution glass capillary GC–MS. Fraction 1 (1.08 g) contained fucosterol and 24-methylenecholesterol; fraction 2 (19 mg) was a complex mixture containing at least 7 sterols which were identified by mass spectrometry.

Table 1 presents the molecular ions of the sterol acetates and their percentages of the total sterol composition. Identification of each sterol was made by the comparison of its MS with that of an authentic sample or, for 24-methylenecholesterol, by identity to the published spectrum [2].

Table 1. Sterol composition of *Agarum cribosum*

Sterol	Identity	$M^+ - 60$	% of total sterols (combined fractions 1 and 2)
I	Cholest-5-en-3β-ol (cholesterol)	368	<0.05
II	Cholesta-5,24-dien-3β-ol (desmosterol)	366	<0.05
III	24-Methylenecholest-5-en-3β-ol (24-methylenecholesterol)	380	10.80
IV	Stigmasta-5,(*E*)-24(28)-dien-3β-ol (fucosterol)	394	88.70
V	3β-Hydroxy-cholest-5-en-24-one (24-ketocholesterol)	382	<0.05
VI	24ξ-Stigmasta-5,28-diene-3β,24-diol (saringosterol)	410	0.40
VII	Unknown	410	<0.05

The acetate of sterol II (Table 1) appeared to be a dehydrocholesteryl acetate from its molecular ion. The major fragmentation ions were at *m/e* 351 (M − 60 − 15); 324 (M − 60 − 42); 281, 282, 283 (M − 60 − 69, $+H^{\cdot}$ or $-H^{\cdot}$); 253 (M − 60 − 112 − H_2) in agreement with the published spectrum of desmosteryl acetate [3]. There were marked differences between the spectrum of sterol II acetate and that of 22-dehydrocholesteryl acetate and 7-dehydrocholesteryl acetate. Sterol VII acetate had

a $M^+ - 60$ at 410 with major fragmentation ions at *m/e* 395 (M − 60 − 15) and 255 (M − 60 − 155). The spectrum was not similar to other published spectra of sterol acetates of MW of 470 and was not identified.

DISCUSSION

The identification of fucosterol and 24-methylenecholesterol and their percentage composition of the total sterol fraction are comparable to that found in many species of brown algae [4]. The percentage of cholesterol reported here is lower than the 0.4–8% reported in these same species but it is in reasonable agreement with the 0.05% reported by Safe *et al.* [5]. It is probable that more cholesterol was present in HPLC fraction 1 but was not detectable because of the large amounts of fucosterol and 24-methylenecholesterol. The amount of desmosterol found may also be less than actually present for the same reason. The chromatographic method used may preclude the accurate determination of some sterol percentages. However, preliminary chromatographic separation was essential in order to isolate the minor sterols from the two major ones. The more polar sterols, 24-ketocholesterol and saringosterol, are more likely to be present in HPLC fraction 2 and their values probably reflect the actual amounts. Saringosterol has been reported in brown algae in varying amounts [1, 4, 5]. Knights [6] postulated that. in *Ascophyllum nodosum*, it is actually an artifact formed from fucosterol during storage and 24-ketocholesterol, which has not been reported frequently, may also be an oxidation product of fucosterol.

It appears likely that desmosterol is present in other brown algae previously examined [1], but was not detected by the methods used [4, 5]. Its presence might be explained by it being an intermediate in the biosynthesis of the C-24 alkylated sterols. However, Goodwin [7] has postulated that the C-4 and C-14 demethylations of cycloartenol, the plant sterol precursor, occur *after* the alkylation at C-24. His proposed biosynthetic pathway is based upon the actual isolation from plants of C-24 alkylated cycloartenol derivatives. Desmosterol is proposed as the precursor of cholesterol [8].

Sterol VII remains unidentified. The MW of the sterol acetate suggested that it is an isomer of saringosterol; however, the MS fragmentation pattern was not at all similar to this compound and there was no evidence for a second hydroxyl group. The concentration of this material was quite small and the MS contained a high background so that further structural interpretation was not possible.

EXPERIMENTAL

General. ^{1}H NMR spectra were recorded on a JEOL JNM-MH 100 spectrometer. Preparative HPLC was carried out on a Waters Prep LC 500 with a Si gel column (two 1 ft cartridges; mesh size 120–140) and a RI detector.

Extraction and preparation of sterol fractions. Agarum cribosum was collected in the drift after a major storm at Rye Beach, New Hampshire, in October 1976. The seaweed was free of macroscopic epiphytes except on the holdfasts which were discarded. The seaweed was air-dried, ground in a Wiley Mill and refluxed in Et_2O (1.3 l./kg) for several days (×3). The Et_2O extracts were filtered, concd, dried ($MgSO_4$) to finally yield a green-brown oil (0.73%) which was saponified (15% KOH in MeOH) to give *ca* 0.15% of non-saponifiable oil. The non-saponifiable lipids were separated by prep.-HPLC using a 50% Et_2O–hexane solvent mixture over Si gel and the fractions monitored by NMR.

Identification of sterol components. The sterol fractions were converted to acetates (Py–Ac_2O) and analysed by high resolution glass capillary GLC at 150–250° (4°/min) over SE 54 with He [9, 10].

The concns of the sterols were determined by programmable integrator using 5α-cholestane (10 ng/μl) as an internal standard. The GC–MS-c system used to identify individual sterol acetates was a Varian Aerograph 1400 equipped with a glass capillary column (17 m × 0·32 mm i.d.) coated with SE-52 interfaced with a Finnigan 1015C quadrupole mass spectrometer (70 eV). The data were collected and edited by a DEC PDP8-E (16 K core) computer equipped with a System Industries System 150 data system, Diablo Series 30 disc drive, and Tektronic 4010 CRT display unit and 4610 copier.

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