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STEROLS OF *POLYNEURA HILLIAE*

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Abstract—The major sterol of *Polyneura hilliae* is a C_{27}-sterol (cholesterol). The composition of total sterols is nearly identical in the three types of thalli investigated (male and female gametophytes and tetrasporophytes) but four additional sterols are present in female gametophytes.

INTRODUCTION

In continuation of our earlier investigations on Delesseriaceae sterols initiated with *Phycodrys rubens* (Hudson) Batters [1], the sterol composition of *Polyneura hilliae* (Greville) Kylin was studied. The Delesseriaceae are considered one of the most advanced families in the Rhodophyceae [2, 3]. Since it was possible to separate male and female gametophytes from tetrasporophytes, sterol characterization in the different phases was undertaken.

RESULTS AND DISCUSSION

Tetrasporophytes and male gametophytes contained identical sterols: the major sterol was cholest-5-en-3β-ol (C27: cholesterol), the other sterols present were 24-methylcholest-5-en-3β-ol (24-methylcholesterol), 24-ethylcholesta-5,22-dien-3β-ol (stigmasterol or poriferasterol) and 24-ethylcholest-5-en-3β-ol (sitosterol or clionasterol) plus two stanols, cholestan-3β-ol and 24-ethylcholestan-3β-ol. The percentage compositions of the sterol mixtures were different in the two types of sexual thalli. Cholesterol was 76.3% of the sterol mixture in the tetrasporophytes and 51.5% in male gametophytes; 24-methylcholesterol was more abundant in male gametophytes (20%) than in tetrasporophytes (7.9%).

Female gametophytes contained the same sterols except for cholestanol and four additional sterols in small quantities: cholesta-5,24-dien-3β-ol, cholest-7-en-3β-ol, 24-methylcholesta-5,24(24′)-dien-3β-ol (24-methylene cholesterol) and 24-methylcholestan-3β-ol (Tables 1 and 2).

We did not find C_{26}-sterols such as those reported in *Phycodrys rubens* [1] or in *Delesseria sanguinea* [4]. Brassicasterol, epibrassicasterol, fucosterol and isofucosterol known in *P. rubens*, and brassicasterol and isofucosterol present in *D. sanguinea*, were absent in *P. hilliae*. There was no C_{30}-sterol in *P. hilliae* in contrast to *P. rubens* [1] which contains 24-propylidene cholesterol.

As shown previously in *P. rubens* [1] and *D. sanguinea* [4], the major sterol of *P. hilliae* was cholesterol. Other sterols common to the three species of Delesseriaceae were 24-ethylcholesterol and cholestanol (except in female gametophytes of *P. hilliae*). 24-Ethylcholesta-5,22-dien-3β-ol was present only in *P. rubens* and *P. hilliae* and 24-methylcholesterol in *D. sanguinea* and *P. hilliae*. A number of sterols were restricted to *P. hilliae*. Two stanols, were identified: 24-ethylcholestanol in the three types of thalli studied and 24-methylcholestanol in female gametophytes. Furthermore 7-cholestenol and desmosterol (cholesta-5,24-dien-3β-ol) were found in the female gametophytes of *P. hilliae*, whereas 22-dehydrocholesterol was identified in *P. rubens* and *D. sanguinea*.

EXPERIMENTAL

The *P. hilliae* samples were collected in May 1992 at Grandcamp, Calvados, France. After rinsing with seawater, the thalli were sorted out into tetrasporophytes, female gametophytes and male gametophytes, and dried at 40°. Dried samples (6 g tetrasporophytes; 6 g female gametophytes; 4.8 g male gametophytes) were extracted in CH_2Cl_2–MeOH (2:1) using a Soxhlet extractor for 36 hr, then by reflux in CH_2Cl_2–MeOH (1:1) for 1 hr. After evapn of the solvent, the extract was treated by KOH 6% in MeOH for 1 hr. The nonsaponifiable frs were extracted by hexane, Et_2O then CH_2Cl_2, acetylated (Ac_2O in pyridine), purified by TLC (silica gel HF_{254}, CH_2Cl_2). Sterols were analysed by GC and GC-MS on a capillary column (J&W, DB5, 30 m × 0.32 mm) with He carrier gas (flow rate 0.5 b), injector temp. of 275°. The temp. was programmed after a 2-min hold at 260°, to 300° at either 2 or 5° min^{-1}. Detection was FID or EIMS (70 eV). Peak areas were used to assess relative amounts of different sterols. Sterols were identified from their spectral data and RR_t by comparison with authentic steryl acetates (cholesterol, 24-methylcholesterol and 24-ethylcholesterol) and with lit. data [4–7].

Table 1. Sterol content of *Polyneura hilliae*

Sterols	RR_t	Relative %		
		Tetrasporophytes	Male gametophytes	Female gametophytes
Cholesterol	1.00	76.3	51.5	89.7
Cholestan-3β-ol	1.02	1.8	4.1	—
24-Methylcholesterol	1.13	7.9	20	2.2
24-Ethylcholesta-5,22-dien-3β-ol	1.17	4.1	7.3	1.2
24-Ethylcholesterol	1.29	9	15.5	4.3
24-Ethylcholestan-3β-ol	1.31	0.7	1.6	0.2
Cholesta-5,24-dien-3β-ol	1.03			0.9
Cholest-7-en-3β-ol	1.05			0.5
24-Methylcholesta-5,24(28)-dien-3β-ol	1.12			0.7
24-Methylcholestan-3β-ol	1.15			0.1

Table 2. Amounts of major sterols in *P. hilliae* (mg 100 g^{-1} dry wt)

	Tetrasporophytes	Male gametophytes	Female gametophytes
Cholesterol	5.2	3.1	6.1
24-Ethylcholesterol	1.7	0.2	0.8

Quantitative analyses of cholesterol and 24-ethylcholesterol (major sterols) were performed using a cholestane int. standard and two calibration curves for cholesterol and 24-ethylcholesterol with increasing quantities of the selected sterol. Ratios were determined by integrating the areas of cholesterol or 24-ethylcholesterol and cholestane peaks. Ratios of sterols analysed from extracts gave measurements of cholesterol and 24-ethylcholesterol [8].

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