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STEROLS OF THE AMANSIEAE (RHODOMELACEAE: RHODOPHYTA)

GEORGES COMBAUT and PETER SAENGER*

Laboratoire de chimie des substances naturelles marines, Université de Perpignan, 66025 Cédex, France; *Research Associate, Queensland Institute of Technology, Department of Biology and Environmental Science, P. O. Box 2434, Brisbane, Australia

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Key Word Index—*Lenormandia prolifera, L. smithiae; Vidalia gregaria; V. fimbriata; Amansia glomerata; Osmundaria prolifera; Kuetzingia natalensis*; Amansieae; Rhodophyta; red algae.

Abstract—*Osmundaria prolifera, Kuetzingia natalensis, Halopythis pinastroïdes* and *Vidalia fimbriata* contain C_{27} sterols as the major sterols. Six other species of the Amansieae, belonging to the genera *Amansia, Vidalia, Lenormandia* and *Rythiphlaea*, contain C_{28}-sterols as the major sterols.

INTRODUCTION

Since the discovery that campesterol was the major sterol of the red alga *Rytiphlaea tinctoria* [1] and that 24-methylenecholesterol was the major sterol in *Vidalia volubilis* [2], we presumed it likely that the Amansieae contained other species capable of alkylation at C-24 of the sterol side chain [3]. We now report the sterol composition and content of seven additional species of the Amansieae belonging to the genera *Lenormandia* (*L. prolifera, L. smithiae*), *Vidalia* (*V. gregaria, V. fimbriata*), *Amansia* (*A. glomerata*), *Osmundaria* (*O. prolifera*) and *Kuetzingia* (*K. natalensis*). The results support our presumption and emphasize the significance of this group of algae for sterol studies.

RESULTS AND DISCUSSION

Because of the small amounts of dried material of each alga available (50–500 mg), separation of the sterol fraction was not feasible. Consequently, GC analysis of the crude lipid extracts was undertaken. The analytical conditions used were highly selective, readily separating 24-méthylenecholesterol from campesterol and brassicasterol from desmosterol. The GLC relative retention times (RR_t) of a mixture of authentic standards were cholesterol (RR_t 1.00), brassicasterol (1.11), campesterol (1.28), stigmasterol (1.40) and sitosterol (1.60). The sterols isolated from the brown alga *Cystoseira elegans* were shown by GC/MS of the TMSi derivatives to consist of 22-dehydrocholesterol (RR_t 0.91), cholesterol (1.00), brassicasterol (1.11), 24-methylenecholesterol (1.25) and fucosterol (1.60), while in the mixture of sterols isolated from *Halopythis pinastroïdes* cholesterol (1.00) and desmosterol (1.08) were identified by GC/MS analysis. The results obtained using the present analytical techniques (see Experimental) are given in Table 1. The eight algal species examined can be divided into three groups.

For the first group, C_{27}-sterols comprise the major sterols and in *O. prolifera, K. natalensis* and *V. fimbriata* cholesterol forms the major component with 22-dehydrocholesterol as the minor C_{27}-sterol. *H. pinastroïdes* also belongs to this group but it differs in containing a significant amount of desmosterol. Desmosterol is absent in the other species. For example, co-injection of sterols of 10% *H. pinastroïdes* extract with 90% *O. prolifera* extract showed a peak at RR_t 1.11 while a peak of RR_t 1.08 was evident when 30% *H. pinastroïdes* extract was co-injected with 70% *O. prolifera* extract. C_{28}-sterols are minor constituents in this algal group.

The second group is characterized by C_{28}-sterols forming the major constituents. In *L. prolifera, L. smithiae* and *A. glomerata*, 24-methylenecholesterol comprises approximately 50% of the total sterol content, with

Table 1. Percentage sterol composition of species of the Amansieae

Algae \ RR_t	C_{27}-sterols			C_{28}-sterols			C_{29}-sterols
	0.91	1.00	1.08	1.11	1.25	1.28	1.60
O. prolifera	5	70		7	12		5
K. natalensis	11	53		13	8		12
H. pinastroïdes	4	38	43		5		5
V. fimbriata	11	62		4		12	8
V. gregaria		42		4		38	9
L. smithiae		17		14	52		14
L. prolifera		38		8	52		2
A. glomerata		25		14	50		6

brassicasterol constituting the minor C_{28}-sterol. Cholesterol is relatively abundant in these three algae while C_{29}-sterols form a minor constituent in this group as they did in the first group. The third group contains only *V. gregaria* which has approximately equal amounts of C_{27}- and C_{28}-sterols, with cholesterol and campesterol comprising the major sterols in this species.

While we intend to continue the examination of further algae belonging to the Amansieae as these become available, the present results indicate the unusual sterol composition of some members of this group. *O. prolifera*, *K. natalensis*, *V. fimbriata* and *H. pinastroides* conform to the general sterol pattern of the red algae with C_{27}-sterols as the major components of this fraction [4, 5]. *A. glomerata*, *L. smithiae* and *L. prolifera*, and to a lesser extent *V. gregaria* conform with *R. tinctoria* and *V. volubilis* in possessing C_{28}-sterols as the major sterol constituents. Campesterol, brassicasterol and 24-methylenecholesterol have been reported as minor constituents in the red algae [5]; these C_{28}-sterols comprise a significant fraction of the total sterol content only in certain species of the Amansieae, and in the brown algae *Laminaria digitata* and *L. faeroensis* [6].

It seems doubtful that the sterols can be used at a taxonomic level below the subfamily. For example, the three species of *Vidalia* so far investigated show a heterogeneous pattern; *V. gregaria* has approximately equal amounts of C_{27}- and C_{28}-sterols while *V. fimbriata* and *V. volubilis* contain C_{27}- and C_{28}-sterols, respectively, as the major components. *Vidalia gregaria* is an adelpho-epiphyte, host specific to *O. prolifera*, which contains C_{27}-sterols as the major component and the possibility exists that the sterol composition of *V. gregaria* is influenced by its host.

EXPERIMENTAL

Extraction. *L. smithiae* (538 mg dry wt), *V. gregaria* (421 mg), *V. fimbriata* (48 mg), *O. prolifera* (490 mg), *L. prolifera* (525 mg) and *A. glomerata* (551 mg) were extracted with $CHCl_3$–MeOH (87:13). The Et_2O soluble portion of these extracts was submitted to GC analysis on 1% OV-1 in a glass column (2.80 × 3 mm i.d.) at 235°. The maximum efficiency was obtained with an N_2 flow of 30 ml/min (theoretical plates 2000/m). The sterol fraction of the brown alga *Cystoseira elegans* was submitted as its TMSi derivatives to GC/MS analysis (1% OV-1, 4 m × 3 mm glass column). The sterol at RR_t 0.91 was identified as 22-dehydrocholesterol (M^+ at m/z 456, base peak at m/z 111); 1.11 as brassicasterol (M^+ at m/z 470, base peak m/z 69); 1.25 as 24-methylenecholesterol (M^+ at m/z 470, ions due to loss of side chain at m/z 386 and 296). The sterol fraction of *Halopythis pinastroides* was submitted as its TMSi derivatives to GC/MS analysis (OV-1 10 m glass capillary column). The sterol at RR_t 1.08 was identified as desmosterol (M^+ at m/z 456, base peak m/z 129).

Algal material. Collection data for the algae are as follows: *Amansia glomerata* C. Ag., Swains Reefs, Queensland, Australia, 26th May, 1977; *Halopythis pinastroïdes* (Gmel.) Kuetz., Le Caro, France, April, 1976; *Kuetzingia natalensis* J. Ag., Inhaca Island, Mozambique, 31st January, 1971; *Lenormandia prolifera* (C. Ag.) J. Ag., Cape Paterson, Victoria, Australia, 10th January, 1969; *Lenormandia smithiae* (H. & H.) Falkbg., Port Elliot, South Australia, 7th December, 1967; *Osmundaria prolifera* Lam., Venus Bay, South Australia, 23rd November, 1967; *Vidalia fimbriata* (R. Br.) J. Ag., Inhaca Island, Mozambique, 31st January, 1971; *Vidalia gregaria* Falkbg., Eucla, Western Australia, 28th November, 1967.

REFERENCES

1. Alcaide, A., Devys, M. and Barbier, M. (1968) *Phytochemistry* **7**, 329.
2. Fattorusso, E., Magno, S., Santacroce, C., Sica, D., Impellizzeri, G., Mangiafico, S., Piattelli, M. and Sciuto, S. (1976) *Biochem. Syst. Ecol.* **4**, 135.
3. Combaut, G., Codomier, L., Teste, J. and Pedersen, M. (1981) *Phytochemistry* **20**, 1748.
4. Scheuer, P. J. (1973) *Chemistry of Marine Natural Products.* Academic Press, New York, pp. XI + 201.
5. Goodwin, T. W. (1974) in *Algal Physiology and Biochemistry* (Stewart, W. D. P. ed.) pp. 266–280. Blackwell, Oxford.
6. Patterson, G. W. (1968) *Comp. Biochem. Physiol.* **24**, 501.