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STEROL PROFILES OF RED ALGAE*

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Key Word Index—Gelidiales; Cryptonemiales; Gigartinales; Rhodophyceae; sterols.

Abstract—Twelve species of red algae belonging to the Orders Gelidiales, Cryptonemiales and Gigartinales were examined for sterols. Four species contained cholestan-3β-ol as the major sterol, accompanied by C_{26}, C_{28} and C_{29} stanols. Sterols not previously reported in algae were 24-dimethyl-5α-chol-22-en-3β-ol, cholest-22-en-3β-ol, cholest-7-en-3β-ol, 24ξ-methylcholest-22-en-3β-ol, 24-methylenecholestan-3β-ol, 24ξ-ethylcholestan-3β-ol and isofucostanol.

INTRODUCTION

Since the pioneering works by Tsuda and his coworkers [1–3] on the sterols of red algae (Rhodophyceae), it has been well documented [4] that the principal sterols in red algae are, with only one exception [5], C_{27} sterols, e.g. cholesterol (**3**), desmosterol and 22-dehydrocholesterol (**2**). This is in marked contrast with the sterols in other plants, including green and brown algae, which contain usually C_{28} and/or C_{29} sterols. This unique sterol composition of red algae may be therefore, of phylogenic importance and it has also prompted interest in the biogenetic sequence of the sterols.

As a continuation of our studies on algal sterols [6], we have now examined, using the GC-MS, the distribution of the sterols in twelve different species of red algae belonging to the Orders Gelidiales, Cryptonemiales and Gigartinales. The sterol compositions of some other red algae belonging to the Orders Bangliales, Rhodymeniales and Ceramiales have been recently reported [7–12].

RESULTS AND DISCUSSION

The red algae examined can be classified into three groups based on their sterol profiles (Table 1). One group (7 species) contained cholesterol (**3**) as the major sterol, accompanied by a variety of C_{26}–C_{29} sterols. The sterol mixtures of the second group (4 species) showed analogous GLC patterns to those of the first group (Fig. 1), but their MS revealed that most of them were in fact a series of 5,6-dihydrosterols (stanols) in which cholestanol (**10**) was the major one. The possible third group (only one species examined) contained 22-dehydrocholesterol (**2**) as the principal sterol.

The identification of each sterol was based upon its GC relative retention time and its MS fragmentation pattern [13–15]. The sterol profiles of the algae are summarized in Table 1.

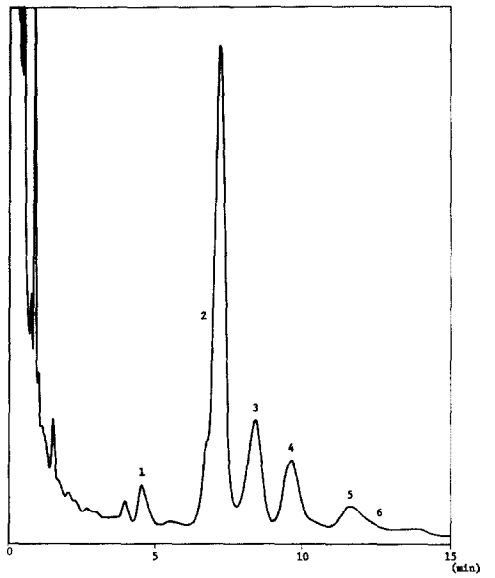

Fig. 1. GLC analysis of the trimethylsilyl ethers of the sterols isolated from *Gelidium amansii*.

In accordance with the previous results [4], cholesterol (**3**) is the most common sterol in red algae: all species presently examined contained cholesterol as the major (7 species) or minor (5 species) sterol. 22-Dehydrocholesterol (**2**), the principal sterol of *Hypnea japonica* had already been isolated from the same source by Tsuda *et al.* [3]. It is also the major sterol of *Porphyridium cruentum* (Order Bangiales) [11] and is found in various other species of red algae [8, 10, 12]. We have now found this sterol in two more species. Another frequently found C_{27} sterol, desmosterol [8–10, 12] was not detected in the present investigation.

The most significant feature of the present investigations is the demonstration of a series of stanols: 24-dimethyl-5α-chol-22-en-3β-ol (**8**), cholest-22-en-3β-ol (**9**), cholestan-3β-ol (**10**), 24ξ-methylcholest-22-en-3β-ol (**12**), 24-methylenecholestan-3β-ol (**13**), 24ξ-ethylcholestan-3β-ol (**14**), and isofucostanol (**15**). These stanols had been previously found in other marine sources, e.g.

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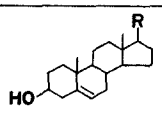
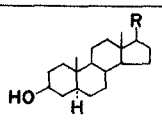
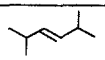
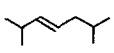
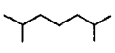
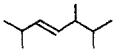
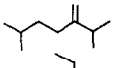
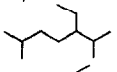
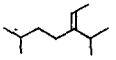
Table 1. Sterol profiles of red algae

Order Family	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Gelidiales																
Gelidiaceae	*Gelidium amansii* Lamouroux (Makusa)	+		+	+		+		+	+	⊕*	+	+	+	+	+
Gelidiaceae	*Gelidium subcostata* Okamura (Hirakusa)	+		+	+		+		+		⊕	+	+	+	+	
Gelidiaceae	*Pterocladia tenuis* Okamura (Obakusa)			+						+	⊕	+			+	
Cryptonemiales																
Callymeniaceae	*Callophyllis adhaerens* Yamada (Kurotosakamodoki)			+	+		+		+	+	⊕	+	+	+	+	+
Grateloupiaceae	*Carpopeltis cornea* Okamura (Tsunomukade)			⊕		+	+	+								
Grateloupiaceae	*Carpopeltis divaricata* Okamura (Hitotsumatsu)			⊕		+	+									
Gigartinales																
Solieriaceae	*Meristhotheca papulosa* J. Agardh (Tosakanori)			⊕			+									
Plocamiaceae	*Plocamium telfairiae* Harvey (Yukari)		+	⊕	+	+	+	+								
Phyllophoraceae	*Gymnogongrus flabelliformis* Harvey (Okitsunori)			⊕			+									
Phyllophoraceae	*Gymnogongrus divaricatus* (Oomataokitsunori)			⊕			+									
Phyllophoraceae	*Ahnfeltia parakoxa* Okamura (Harigane)			⊕		+	+									
Hypneaceae	*Hypnea japonica* Tanaka (Kagiibaranori)		⊕	+												

* ⊕ indicates the major sterol.

sponge [16] and/or jellyfish [17], but all of them except for cholestan-3β-ol appear to be new to algae. Although cholestan-3β-ol (**10**) has been identified as a minor sterol from *Rhodymenia palmata* (Order Rhodymeniales) [12], this stanol constituted the major sterol of four of the species now examined. Three of them belong to the Order Gelidiales, suggesting a chemotaxonomic significance. It should be noted that all algae classified in this group contain considerable amounts of cholest-7-en-3β-ol (**11**). Biogenetic hydrogenation of this sterol would give directly cholestan-3β-ol (**10**), which would otherwise be formed by reduction of cholesterol (**3**). Another noteworthy observation is that these algae grow in relatively deep sea and show remarkable parallelism of sterol profiles with that of a deep sea jellyfish [17]. From some species of Gelidiales, Tsuda *et al.* [1–3] had previously isolated cholesterol (**3**), representing the first finding of this sterol in the plant kingdom. Cholesterol (**3**) was indeed found in all species of Gelidiales now examined, but it constituted only a minor sterol component. In addition, Tsuda *et al.* [1–3] deduced the co-occurrence of 24-methylenecholesterol (**5**) (chalinasterol) from the characteristic infrared band due to the exomethylene group. However, the material definitely identified in the present work is 24-methylenecholestan-3β-ol (**13**).

R		
	**1**	**8**
	**2**	**9**
	**3**	**10, 11** (Δ^7)
	**4**	**12**
	**5**	**13**
	**6**	**14**
	**7**	**15**

Barbier *et al.* [12] have recently found in *Rhodymenia palmata* a C_{26} sterol (**1**), which has now been recognized as a widely distributed sterol in marine invertebrates. We have now detected this sterol, as well as its dihydro derivative (**8**) in at least three species of red algae. The occurrence of C_{26} sterol in several red algae seems to suggest that at least part of this may contribute to the exogenous source of C_{26} sterols in marine invertebrates.

EXPERIMENTAL

Red algae were collected at Sagami bay, Shizuoka prefecture, Japan, in May of 1971 and identified by Professor K. Iwamoto, Tokyo University of Fisheries. The air-dried algae (*ca* 25 g) was extracted with refluxing C_6H_6 (300 ml × 2) for 2 hr. Extracts (30–90 mg) were saponified in a mixture of 40% KOH (1 ml), C_6H_6 (0.5 ml) and MeOH (2.5 ml) at reflux for 3 hr. An aliquot (*ca* 1 mg) of the unsaponifiable material was treated with trimethylsilylimidazole (50 μl) in a sealed tube at 70° for 30 min. Resulting trimethylsilyl ethers of the sterols were analyzed by GC on 1.5% OV-17, (1.5 m × 3 mm, 250°). RR_t's of the TMSi derivatives of standard samples were: Cholestane, 1.0; 22-dehydrocholesterol, 1.84; cholesterol, 1.97;

brassicasterol, 2.26; cholest-7-en-3β-ol, 2.31; desmosterol, 2.36; campesterol, 2.57; 24-methylenecholesterol, 2.66; stigmasterol, 2.79; sitosterol, 3.18; fucosterol, 3.38; isofucosterol, 3.51. The TMSi derivatives were also analyzed by a GC–MS system on 3% OV-17 (1.5 m × 3 mm, 260°).

Sterols in Plocamium telfairiae. GC analysis showed the presence of five peaks which were assigned on the basis of their MS as cholesterol (**3**, major sterol, *ca* 50%), 24ξ-methylcholest-5,22-dien-3β-ol (**4**), 24-methylenecholesterol (**5**), 24ξ-ethylcholesterol (**6**) and isofucosterol (**9**). Peak 1 had a shoulder at shorter retention time and its MS showed ions at *m/e* 456 (M^+), 372 (C-20, 22 vinylic cleavage and transfer of hydrogen), 366 (M-TMSOH), and 327 (M-129) expected for 22-dehydrocholesterol (**2**). The MS of peak 5 was indistinguishable from that of fucosterol, but the slightly longer *RR*t indicated it to be isofucosterol.

Sterols in Gelidium amansii. GC showed the presence of six peaks (Fig. 1). Peak 1 showed parent ions at *m/e* 444 and 442 suggesting the presence of C_{26} sterol (**1**) and its dihydro analogue (**8**). The presence of saturated sterol nuclei with an unsaturated side chain was supported by a major peak at *m/e* (rel. int.) 257 (75) (M-side chain-TMSOH) and additional significant peaks at 374 (30) (C-20, 22 vinylic cleavage and transfer of hydrogen), 345 (40) (M-side chain-2H) and 255 (25) (M-side chain-2H-TMSOH). Contamination with the corresponding Δ^5-compound (**1**) was indicated by ions at *m/e* 442 (M^+), 352 (M-TMSOH), 313 (M-129) and 129. Peak 2 was cholestan-3β-ol (**10**) accompanied by cholesterol (**3**). The MS showed ions at *m/e* (rel. int.): 460 (60, M^+), 445 (82) (M-Me), 370 (30), M-TMSOH), 335 (45, M-Me-TMSOH), 305 (33), 230 (28), 217 (45), 216 (55) and 215 (100, M-side chain-TMSOH-42 mass units of part of ring D). The following peaks may be due to cholesterol-TMSi: *m/e* (rel. int.): 458 (25, M^+), 443 (10) ($M-CH_3$), 368 (55, M-TMSOH), 353 (30, $M-CH_3$-TMSOH), 329 (80, M-129) and 129 (85). In addition to these sterols (**3** and **10**), peak 2 appeared to contain cholest-22-en-3β-ol (**9**) which was indicated by the faster running shoulder in the GC and the prominent peaks at *m/e* 374 (C-20, 22 vinylic cleavage and transfer of hydrogen) and 257 (M-side chain-TMSOH). Peak 3 consisted of at least three components: cholest-7-en-3β-ol (**11**), 24ξ-methylcholest-22-en-3β-ol (**12**) and 24ξ-methylcholest-5,22-dien-3β-ol (**4**). A strong molecular ion peak at *m/e* (rel. int.) 458 (99) with base peak at 255 (M-side chain-TMSOH), as well as 229 (35) and 213 (42) were ascribable to **11**. The coexistence of **4** and **12** was evidenced from molecular ions at *m/e* 472 and 470 and other significant peaks at *m/e* 380, 257 and 129. Peak 4 was a mixture of 24-methylenecholestan-3β-ol (**13**) and unidentified materials. Significant ions at *m/e* (rel. int.) 472 (10, M^+), 457 (28, M-Me), 388 (82, $M-C_6H_{12}$), 345 (60, M-side chain-2H), 255 (M-side chain-TMSOH-2H) 217, 216 and 215 (60) were consistent with structure **13**. Concomitant peaks at *m/e* 470, 455, 380, 343 and 129 suggested the presence of 24-methylenecholesterol (**5**), but the weak intensity at *m/e* 386 and 341 (M-129) were incompatible with **5**. Peak 5 showed pairs of peaks at *m/e* 488, 486; 398, 396; and 383, 381; assignable respectively to M^+, M-TMSOH and M-Me-TMSOH of 24ξ-ethylcholestan-3β-ol (**14**) and 24ξ-ethylcholesterol (**6**). Characteristic ions for a fully saturated stanyl TMSi ether at *m/e* (rel. int.) 215 (100), 216 (55), 217 (48), 230 (31) and 305 (34) gave further support for **14**, while ions at *m/e* 357 (29, M-129), 255 (30, M-side chain-TMSOH) and 129 (92) were consistent with sterol (**6**). Peak 6 had ions at *m/e* 486 (M^+), 471 (M-Me), 388 (55) ($M-C_7H_{14}$), 345 (27, M-side chain-2H), 229 (19) 217, 216 and 215 (47), which were consistent with the structure of isofucostanol (**15**). However, this peak appeared to contain another component, as indicated by significant ions at *m/e* 394, 357, 255 and 129.

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REFERENCES

1. Tsuda, K., Akagi, S. and Kishida, Y. (1957) *Science*, **126**, 927; (1958) *Chem. Pharm. Bull.* **6**, 101.
2. Tsuda, K., Akagi, S., Kishida, Y., Hayatsu, R., and Sakai, K. (1958) *Chem. Pharm. Bull.* **6**, 724.
3. Tsuda, K., Sakai, K., Tanabe, K. and Kishida, Y. (1959) *Chem. Pharm. Bull.* **7**, 747; (1960) *J. Am. Chem. Soc.* **82**, 1442.
4. Patterson, G. W. (1971) *Lipids* **6**, 120.
5. Alcaide, A., Barbier, M., Potier, P., Magueur, A. M. and Teste, J. (1969) *Phytochemistry* **3**, 2301.
6. Ikekawa, N., Morisaki, N., Tsuda, K. and Yoshida, T. (1968) *Steroids* **12**, 41.
7. Saito, A. and Idler, D. R. (1966) *Can. J. Biochem.* **44**, 1195.
8. Gibbons, G. F., Goad, L. J. and Goodwin, T. W. (1967) *Phytochemistry* **6**, 677.
9. Alcaide, A., Devys, M. and Barbier, M. (1968) *Phytochemistry* **7**, 329.
10. Idler, D. R., Saito, A. and Wiseman, P. (1968) *Steroids* **11**, 465.
11. Beastall, G. H., Rees, H. H. and Goodwin, T. W. (1971) *Tetrahedron Letters* 4935.
12. Ferezou, J. P., Devys, M., Allais, J. P. and Barbier, M. (1974) *Phytochemistry* **12**, 593.
13. B. A. Knights (1967) *J. Gas Chromatog.* **5**, 273.
14. Brooks, C. J. W., Henderson, W. and Steel, G. (1973) *Biochim. Biophy. Acta* **296**, 431.
15. Morisaki, N. and Ikekawa, N. (1973) *J. Synth. Org. Chem. (Japan)* **31**, 573.
16. Erdman, T. R. and Thomson, R. H. (1972) *Tetrahedron* **28**, 5163.
17. Ballantine, J. A., Roberts, J. C. and Morris, R. J. (1975) *Tetrahedron Letters* 105.