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STEROLS OF SOME RED ALGAE*

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Abstract—The sterol composition of 17 red algae has been determined. Only C-27 sterols have been found in substantial amounts; details of the structural elucidation of liagosterol (cholesta-5,23-diene-3β,25-diol) are given. The possible taxonomic significance of the sterol distribution is briefly discussed.

INTRODUCTION

The major sterols of red algae are C-27 compounds. Cholesterol predominates, but in several species desmosterol has been also detected [1–10], while the rare 22-dehydrocholesterol is present in relatively large amounts only in *Hypnea japonica* [11] and *H. musciformis* [8].

In some cases, traces of C-26, C-28 and C-29 sterols have been also found [6, 7, 12]. However, these results are open to suspicion, since it is actually impracticable to free a macroscopic alga from contaminant microscopic fauna and flora. *Rytiphlea tinctoria*, in which C-28 compounds constitute more than half of the sterol fraction [13], appears to be the sole real exception to the general rule that Rhodophyta are incapable of alkylation at C-24.

As part of a chemotaxonomic investigation of red algae, we have now examined the sterol content of 17 macroscopic marine species belonging to the division Rhodophyta, class Florideophyceae. Amino acids and low MW carbohydrates of the same algae have been also analyzed and the results reported in the preceding paper.

RESULTS

The unsaponifiable fraction from the $CHCl_3$ extract of each alga was chromatographed on SiO_2 and the sterol fraction, after acetylation, resolved into individual components by SiO_2–$AgNO_3$ column chromatography. In addition to known steryl acetates, which were identified by comparison of their physical properties (mp, $[\alpha]_D$, MS, IR, NMR and co-GLC) with those of authentic specimens, two further polar compounds (*cis*- and *trans*-liagosteryl acetate) have been isolated and characterized.

Only compounds present in substantial quantities were considered in this survey (Table 1); sterols found in trace amounts in several algae, as revealed by the mass spectra of the crude mixtures of acetates, could derive from foreign organisms.

* Part 2 in the series "Constituents of Red Algae". For Part 1 see preceding paper. The trivial names of the sterols used in the text have the following systematic names: cholesterol = cholest-5-en-3β-ol; desmosterol = cholesta-5,24-dien-3β-ol; 22-dehydrocholesterol = cholesta-5,22-dien-3β-ol; liagosterol = cholesta-5,23-diene-3β,25-diol.

Table 1. The distribution of sterols in some red algae

Species	Sterol (mg/kg dry alga) Cholesterol	Desmosterol	22-Dehydrocholesterol	Liagosterol*
Nemalionales				
Helminthocladiaceae				
1. *Liagora distenta* (Mert.) C. Ag.	35	—	—	25
Chaetangiaceae				
2. *Scinaia furcellata* (Turn.) Bivona	103	—	—	59
Gelidiales				
Gelidiaceae				
3. *Gelidium latifolium* (Grev.) Thuret et Bornet	144	—	—	—
4. *Pterocladia pinnata* (Huds.) Papenfuss	220	—	—	—
Cryptonemiales				
Cryptonemiaceae				
5. *Grateloupia proteus* Kütz.	130	—	—	—
Corallinaceae				
6. *Amphiroa beauvoisii* Lamx.	68	—	—	—
Gigartinales				
Gigartinaceae				
7. *Gigartina acicularis* (Wulf.) Lamx.	46	49	—	—
8. *Gigartina teedi* (Roth) Lamx.	73	25	—	—
Nemastomaceae				
9. *Schizymenia dubyi* (Chauv.) J. Ag.	71	140	—	—
Hypneaceae				
10. *Hypnea musciformis* (Wulf.) Lamx.	19	—	83	—
Sphaerococcaceae				
11. *Caulacanthus ustulatus* (Mert.) Kütz.	68	—	—	—
Ceramiales				
Ceramiaceae				
12. *Centroceras clavulatum* Mont.	370	—	—	—
Rhodomelaceae				
13. *Lophocladia lallemandi* (Mont.) Schmitz	112	—	—	—
14. *Laurencia paniculata* (C. Ag.) J. Ag.	195	—	—	—
15. *Laurencia obtusa* (Huds.) Lamx.	307	—	—	—
16. *Alsidium corallinum* C. Ag.	39	—	—	—
17. *Acantophora najadiformis* (Delile) Papenfuss	170	—	—	—

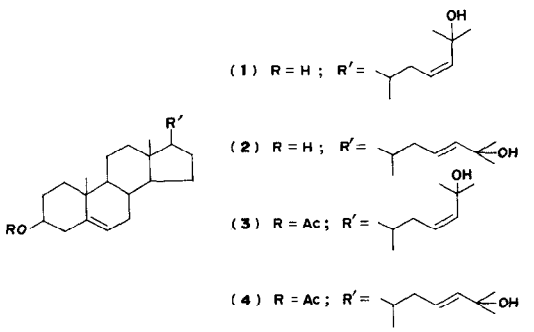
* *cis* Plus *trans* isomer.

cis-*Liagosterol* (**1**)

This hitherto unknown sterol (**1**) has been isolated, as the 3-acetyl derivative (**3**), from *Liagora distenta* and *Scinaia furcellata*. Assignment of structure (**3**) was based on the following evidence. From elemental analysis and mass spectrometry, the molecular formula $C_{29}H_{46}O_3$ was deduced. In the mass spectrum the molecular ion is absent; the fragmentation pattern suggests a C-27 steryl acetate possessing a labile hydroxyl group [*m/e* 424 (M^+-H_2O), 409 (M^+-H_2O–Me), 382 (M^+–HOAc), 364 (M^+-H_2O–HOAc); the transition 424 → 364 is attested by the metastable ion $m^* = 312{\cdot}5$], a double bond in the nucleus and another in the side chain [*m/e* 253 (M^+–HOAc–side chain–2H), 213 (M^+–HOAc–side chain–42)] [14].

The IR spectrum confirmed the presence of an OH group ($\nu_{max}^{CHCl_3}$ 3450 cm^{-1}) which must be hindered since it was not acetylated under mild conditions. Evidence for the position of the OH which, on the basis of the MS data, should be located in the side chain, was provided by the NMR spectrum: a 6H singlet at δ 1·28 is diagnostic for the presence of a $(Me)_2$–COH group. As a consequence, the hydroxyl must be linked at C-25 and taking into consideration the fact that the MS showed an ion at *m/e* 283 (M^+–HOAc–$C_6H_{11}O$), indicative for the presence of a C-23 double bond [14], (**3**) must incorporate an allylic alcoholic function. Also the other significant signals in the NMR spectrum are in agreement with the proposed structure: δ_{CDCl_3} 0·70 (3H, *s*, H_3C-18), 0·95 (3H, *d*, *J* 6 Hz, H_3 C-21), 1·03 (3H, *s*, H_3C-19), 2·02 (3H, *s*, MeCO), 4·65 (1*H*, *m*, C-3), 4·82–5·42 (3H, complex signals, HC-6, HC-23 and HC-24).

To confirm the structure of the side chain and to establish the position of the double bond in the nucleus, (**3**) was hydrogenated in the presence of Pd/C, thus yielding cholesteryl acetate in 30% yield. The configuration of the double bond must be *cis*, on account of the absorption at 735 and 755 cm^{-1} in the IR spectrum (CS_2).



trans-*Liagosterol* (**2**)

From both *L. distenta* and *S. furcellata* an additional steryl acetate (**4**) was isolated in small amount. Since it was not obtained in a pure state adequate mp and $[\alpha]_D$ data could not be obtained, but spectral data indicate a close similarity with **3**. In fact, its MS differs from that of the latter only in the relative intensities of peaks and in the presence of a metastable ion $m^* = 346{\cdot}8$ (382 → 364) instead of $m^* = 312{\cdot}5$. Moreover, the NMR spectrum [δ_{CDCl_3} 0·69 (3H, *s*), 0·91 (3H, *d*, *J* 6 Hz), 1·0 (3H, *s*), 1·31 (6H, *s*), 2·01 (3H, *s*), 4·66 (1H, *m*), 5·15–5·70 (3H, complex signals)] is quite similar to that of **3**, apart from the signals of the olefinic protons. Also the IR spectrum of (**4**) closely resembles that of **3**, from which it differs mainly in the lack of absorption at 735 and 755 cm^{-1} and in the presence of a band at 980 cm^{-1}. All these data suggest that the two compounds differ only in the stereochemistry of the double bond in the side chain and that (**4**) is the *trans* isomer. At this stage it is impossible to assess whether or not both diastereoisomers are natural products, as one of them could be an artifact formed from the other during the isolation.

DISCUSSION

From the literature data it appears that the sterol profiles of red algae are relatively simple (but see [15, 16]), since only three C-27 compounds (cholesterol, desmosterol and 22-dehydrocholesterol) are usually present in significant amounts. Though an additional sterol (liagosterol) has been found in some Rhodophyta, our results confirm the overall simplicity of the sterol patterns of red algae. Concerning the chemotaxonomy of these compounds, cholesterol and 22-dehydrocholesterol have no systematic value, since the former is too widespread and the latter confined to the single genus *Hypnea*. By contrast, desmosterol, previously found in relatively large amounts in some members of the Rhodymeniales and the Ceramiales and now identified in the families Gigartinaceae and Nemastomaceae, and particularly liagosterol, possibly characteristic of the order Nemalionales, seem to have some taxonomic significance.

EXPERIMENTAL

Extraction and fractionation of sterols. Each alga (usually 500 g fr. wt), collected as previously described [17], was freeze-dried and extracted with $CHCl_3$ (3 × 600 ml) at room temp. Combined extracts were evaporated to dryness and the residue was refluxed with 10% KOH in 50% EtOH (50 ml) for 3 hr. After extraction with Et_2O the organic phase, taken to dryness, was chromatographed on a SiO_2 column using C_6H_6–Et_2O (4:1) as eluent. The sterol fraction was collected and, after acetylation with Ac_2O–C_5H_5N for 12 hr at room temp., fractionated on a SiO_2–$AgNO_3$ (2:1) column (eluent: 40–70° light petrol–C_6H_6 7:3). The isolated steryl acetates were recrystallized from EtOH and identified by comparison with authentic samples.

Isolation of cis- *and* trans-*liagosteryl acetates.* The unsaponifiable fraction (2·5 g) from the $CHCl_3$ extract of *Liagora distenta* (5 kg of fr. material) was chromatographed on a SiO_2 column (250 g; C_6H_6–Et_2O 8:2) collecting fractions of 150 ml. Fractions 4–7 yielded cholesterol (50 mg); from the fractions 11–16 an oily product (81 mg) was obtained, which was successively acetylated with Ac_2O–pyridine (2 hr at laboratory temp.) and fractionated by SiO_2 PLC [eluent: C_6H_6–Et_2O (8:2)] The band R_f 0·6 was scraped and eluted with Et_2O, thus obtaining *cis*-liagosteryl acetate (**3**) (25 mg) which was recrystallized once from EtOH; mp 114–119°, $[\alpha]_D$ −39·4° (*ca* 0·2 *in* $CHCl_3$) (Found: C,78·33; H,10·71. $C_{29}H_{46}O_3$ requires: C,78·68; H,10·47%).

Elution of the band R_f 0·54 yielded crude *trans*-liagosteryl acetate (**4**) (11 mg) which was used for spectral measurements without any further purification.

Scinaia furcellata (500 g fr. alga) worked up as above afforded cholesterol (7 mg), **3** (2 mg) and **4** (2 mg).

Catalytic hydrogenation of **3**. Hydrogenation of a 13 mg sample of **3** in EtOAc (5 ml) with 10% Pd/C at room temp. and press. yielded a product which was purified by SiO_2 PLC (eluent: C_6H_6). From the band R_f 0·5 cholesteryl acetate (4 mg) was obtained and identified (GLC, $[\alpha]_D$, mp, MS) by comparison with an authentic sample.

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