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STEROLS OF THE UNICELLULAR ALGAE *NEMATOCHRYSOPSIS ROSCOFFENSIS* AND *CHRYSOTILA LAMELLOSA*: ISOLATION OF (24*E*)-24-*n*-PROPYLIDENECHOLESTEROL AND 24-*n*-PROPYLCHOLESTEROL

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Key Word Index—*Nematochrysopsis roscoffensis*; *Chrysotila lamellosa*; Chrysophyceae; Prymnesiophyceae; unicellular algae; sterols; (24*E*)-24-*n*-propylidenecholesterol; 24-*n*-propylcholesterol.

Abstract—Two rare C_{30}-sterols, (24*E*)-24-*n*-propylidenecholest-5-en-3β-ol and 24-*n*-propylcholest-5-en-3β-ol, and (24*S*)-24-ethylcholesta-5,22-dien-3β-ol (stigmasterol) are the major sterols of *Nematochrysopsis roscoffensis*, a Chrysophyte of the Sarcinochrysidales order. This unique sterol composition is different from the sterol contents of other Chrysophytes and justifies the peculiar position of the Sarcinochrysidales, which are by some characteristics morphologically and biologically related to the Phaeophyceae. The presence of (24*S*)-24-methylcholesta-5,22-dien-3β-ol (24-epibrassicasterol) as a major sterol in *Chrysotila lamellosa* is in accordance with the few previous results obtained from other Prymnesiophyceae, although the presence of the other major sterol, (24*R*)-24-ethylcholesta-5,22-dien-3β-ol (poriferasterol), has never been reported in these algae.

INTRODUCTION

Many marine invertebrates feed on unicellular algae, and it seems likely that the numerous exotic sterols found in these organisms might in fact be unchanged or modified dietary algal sterols [1, 2]. In an attempt to identify the origin of these unusual sterols, it appeared fruitful to investigate the composition of those algae which are the first and most important (with respect to their biomass) link in the marine food chains. We have previously identified two novel C_{30}-sterols, 24-*n*-propylcholesterol and (24*E*)-24-*n*-propylidenecholesterol, from a unicellular marine alga [3]. As this alga, most probably a Chrysophyte, is still unidentified, (Gayral, P., personal communication), it was of interest to examine an apparently closely related, but identified, species., i.e. *Nematochrysopsis roscoffensis* [4]. A sample of another unicellular alga, *Chrysotila lamellosa*, belonging to the Prymnesiophyceae, a group of uncertain taxonomic affinities, was also obtained. The investigation of the sterol composition of these two slow-growing algae might be helpful for testing the accuracy of the arguments based on morphology and biology which have been utilized to determine their systematic positions.

RESULTS

Free sterols and sterol esters were not analysed separately. The compositions of the total sterol fractions isolated from the unsaponifiable lipids of *N. roscoffensis* and *C. lamellosa* are listed in Table 1. The stereochemistry at C-24 of the 24-alkylsterols is often of interesting taxonomic value and has been determined for all the major sterols of each alga after isolation by argentation TLC. In the case of Δ^{22}-sterols, it is often difficult to determine unambiguously this configuration by ^{1}H NMR spectroscopy [5, 6]. The acetate of 24-epibrassicasterol (**2**) isolated from *C. lamellosa* was therefore hydrogenated, and the stereochemistry at C-24 was assigned by ^{1}H NMR spectroscopy on the resulting campestanyl acetate according to previously published data [5–8]; the doublets for the C-21 and C-27 methyl groups permitted the assignment of 24*S*-configuration [5, 7, 8]. These doublets were at δ0.891 and 0.798, respectively, identical to the chemical shifts of the C-21 and C-27 doublets of campestanyl acetate, but different from the chemical shifts of the C-21 and C-27 doublets of ergostanyl acetate (the 24*R*-epimer) which resonate at δ0.902 and 0.777. Stigmasteryl acetate (**8**) from *N. roscoffensis* and poriferasteryl acetate (7) from *C. lamellosa* could be differentiated by their melting points [9, 10].

The major sterol of *N. roscoffensis* was identical in all aspects (GC retention time, mass spectrum, ^{1}H NMR spectrum) to the (24*E*)-24-*n*-propylidenecholesterol (**10**) previously identified by us from an unidentified unicellular alga [3]. The mass spectrum of its acetate showed no molecular ion, in accordance with the structure of a 3β-acetoxy-Δ^5-sterol, due to the loss of acetic acid. The remaining ion at *m/z* 408, indicated the presence of a C_{30}-sterol with two double bonds. The base peak at *m/z* 296 suggested the presence of a $\Delta^{24(28)}$-double bond in the side chain [11]. The fragment at *m/z* 255 corresponding to the loss of the side chain and acetic acid indicated the presence of a C_{11}-side chain containing one double bond. The ^{1}H NMR spectrum was typical for a 3β-acetoxy-Δ^5-sterol, with two singlets at δ0.686 and 1.023 corresponding to the C-18 and C-19 methyl groups, respectively, and multiplets centred at δ4.62 and 5.38 corresponding to the protons at C-3α and C-6. The structure of the side chain

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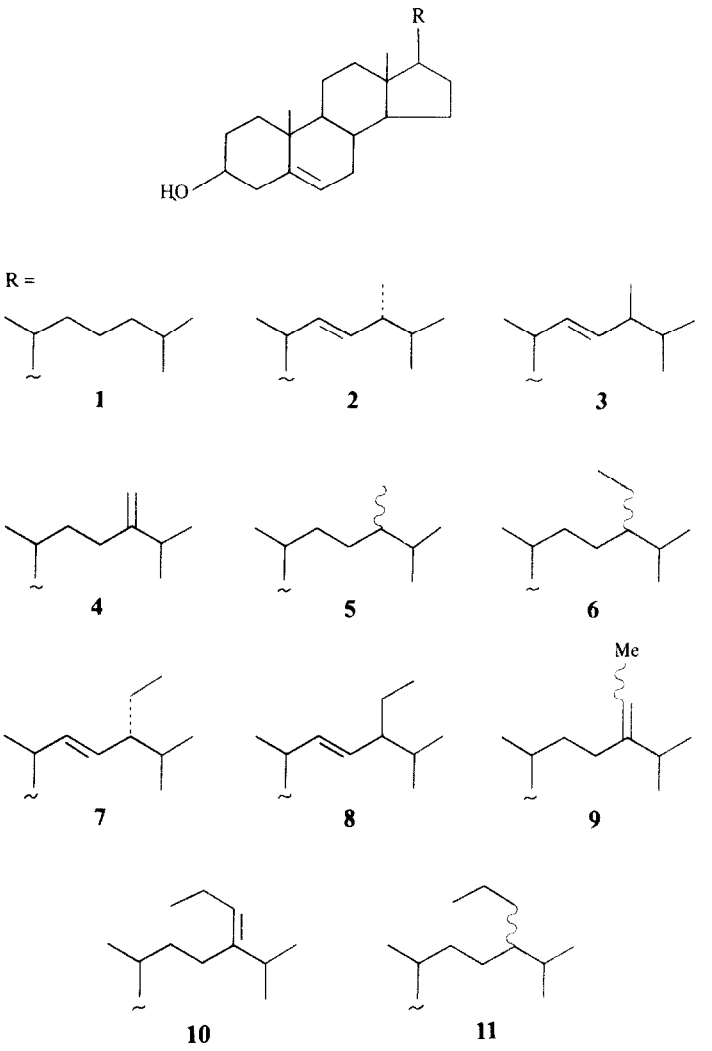
Table 1. Composition of the total sterol fractions from *Nematochrysopsis roscoffensis* and *Chrysotila lamellosa*

	*N. roscoffensis**† (%)	*C. lamellosa** (%)
Cholesterol (**1**)	12	—
Brassicasterol (**2**)	2	—
24-Epibrassicasterol (**3**)	—	49
24-Methylenecholesterol (**4**)	4	—
24-Methylcholesterol (**5**)		6
Sitosterol (**6**)	6	—
Stigmasterol (**8**)	25	—
Poriferasterol (**7**)	—	44
24-Ethylidenecholesterol (**9**)	2	—
(24*E*)-24-*n*-Propylidenecholesterol (**10**)	40	—
24-*n*-Propylcholesterol (**11**)	9	—

*Sterol content of *N. roscoffensis*: 0.7 mg/g, dry wt; *C. lamellosa*: 0.3 mg/g, dry wt.
†The composition of the sterol fraction was similar in two different cultures.



could be deduced from decoupling experiments. The presence of an isopropyl group was shown by irradiation of the allylic proton at δ2.20 which collapsed the two methyl doublets centred at 0.98. Irradiation of the olefinic proton at δ5.07 collapsed a signal corresponding to two allylic protons at 2.00. The irradiation of these allylic protons collapsed not only the olefinic triplet at δ5.07, but also the methyl triplet at 0.94, indicating the presence of an *n*-propylidene group at C-24. The chemical shift of the allylic proton at C-25 permitted the configuration of the $\Delta^{24(28)}$-double bond to be unambiguously assigned as *E*. This chemical shift (δ2.20) was identical to the chemical shift of the proton at C-25 of fucosterol (δ2.20), which has the same configuration, but different from that of the C-25 proton of 28-isofucosterol (δ2.80), which is the corresponding *Z*-isomer [11, 12].

The other C_{30}-sterol of *N. roscoffensis* was tentatively identified as 24-*n*-propylcholesterol (**11**) by the GC retention time and the mass spectrum of its acetate. The absence of the molecular ion was also in accordance with the structure of a 3β-acetoxy-Δ^5-sterol. The base peak at *m/z* 410 corresponding to the loss of acetic acid indicated the presence of a C_{30}-sterol with one double bond; the fragment at *m/z* 255 corresponding to the loss of acetic acid and the side chain showed the presence of a C_{11}-side chain without a double bond. Furthermore, its GC retention time was similar to that of the acetate of 24-propyl cholesterol, which has been previously described [3]. This sterol might reasonably arise from the hydrogenation of the $\Delta^{24(28)}$-double bond of the former *n*-propylidene sterol.

DISCUSSION

Chrysotila lamellosa belongs to the Prymnesiophyceae [13]. The presence of 24-epibrassicasterol in this alga is in full accordance with previous results on the sterol compositions of these algae. This still relatively less frequent sterol has been reported from a few other Prymnesiophyceae algae such as *Isochrysis galbana* [14], *Emiliana huxleyi* [15] and two *Hymemonas* species [14, 15], but in the latter case there was no indication about the stereochemistry at C-24. Furthermore, in contrast to the other Prymnesiophyceae algae from which only 24-epibrassicasterol has been reported, this sterol is accompanied by poriferasterol in *C. lamellosa*. This peculiar sterol composition of the Prymnesiophyceae all containing 24-epibrassicasterol is in full accordance with their taxonomic separation from the Chrysophyceae with which they were previously combined.

Nematochrysopsis roscoffensis belongs to the class Chrysophyceae. For numerous morphological and biological reasons, a novel order of the same class was created for this genus: the Sarcinochrysidales, which are related by many characteristics to the Phaeophyceae [16, 17]. The sterol composition of *N. roscoffensis* reinforces this classification. The major C_{29}-sterol of this alga is stigmasterol with the 24*S*-configuration, whereas in other Chrysophyceae such as *Ochromonas* spp., it is poriferasterol, the C-24 epimer [18]. Furthermore, the major sterols are the unique C_{30}-sterols (24*E*)-24-*n*-propylidenecholesterol and 24-*n*-propylcholesterol which have so far never been isolated from other Chrysophyceae. The stereochemistries of the $\Delta^{24(28)}$-double bond of 24-*n*-propylidenecholesterol from *N. roscoffensis* and of fucosterol, the major sterol of the brown algae, are similar. This fact is in good agreement with a possible relationship between the Sarcinochrysidales and the Phaeophyceae.

Qualitatively and quantitatively the sterol composition of *N. roscoffensis* is similar to that of an unidentified alga which was the first reported source of the two C_{30}-sterols [3]. These two algae, obtained from opposite sides of the Atlantic (the unidentified alga was from the Caribbean and *N. roscoffensis* from the Channel), are most probably closely related and belong to the same order.

The identification of unusual C_{30}-sterols in two unicellular algae from two different marine environments is of major importance for the elucidation of marine food chains. Since unicellular algae can be grown at least as unialgal cultures, it can be safely assumed that their sterols are synthesized *de novo*. The 24*E*-isomers of 24-*n*-propylidenesterols, or their 24*Z*-isomers, are frequently encountered as trace sterols in marine invertebrates. They were identified, for instance, in a scallop [19, 20] and in two sponges [21, 22]. The dietary origin of these sterols can be reasonably assumed; and a probable source could be unicellular Chrysophytes of the Sarcinochrysidales order.

EXPERIMENTAL

Cultures of algae. N. roscoffensis Chadefaud (No. 9) and *C. lamellosa* Anand. (No. 17) from the collection of Professor P. Gayral (Université de Caen, France) were grown in her laboratory using an enriched sea-water medium. Both strains were unialgal and free from protozoa and fungi.

Extraction and analysis of sterols. The cells (34 g dry wt for *N. roscoffensis*, 16 g for *C. lamellosa*) were extracted under reflux once with EtOH (40 ml) and 2 × with $CHCl_3$ (2:1, 200 ml). After filtration, the combined extracts were evapd to dryness *in vacuo* and hydrolysed for 1 hr under reflux in a 6% methanolic soln of KOH (100 ml). After addition of H_2O (200 ml), the unsaponifiable lipids were extracted with hexane (3 × 100 ml). The extract was dried over dry Na_2SO_4, filtered off and taken to dryness under red. pres. The sterols were isolated by TLC on silica gel using CH_2Cl_2 as eluant (2 migrations, R_f 0.36) and acetylated overnight at room temp. with Ac_2O in dry pyridine. The excess reagent was removed under a stream of N_2. The steryl acetates were purified by TLC (cyclohexane–EtOAc, 9:1, R_f 0.42). After GC/MS analysis, they were separated by TLC on $AgNO_3$–silica gel [23], using cyclohexane–toluene (7:3, 3 migrations) as eluant, into the following bands: band 1 (R_f 0.49) contained all steryl acetates possessing a saturated side chain and stigmasteryl or poriferasteryl acetate; band 2 (R_f 0.37) brassicasteryl or 24-epibrassicasteryl acetate; and band 3 (R_f 0.25) 24-*n*-propylidenecholesterol. The steryl acetates from band 1 were separated into steryl acetates possessing saturated side chains (R_f 0.27) and poriferasteryl or stigmasteryl acetate (R_f 0.12) on $AgNO_3$–silica gel plates using dry EtOH–free $CHCl_3$ as eluant.

GC was performed on a WCOT capillary glass column (OV-1, 25 m) using an on-column injector; the FID temp. was 310°; the oven temp. was monitored using the following programme: 60 to 220° (30°/min), 220 to 280° (3°/min) and 280 to 310° (5°/min). ^{1}H NMR spectra were recorded on a Bruker WP80 or W200 spectrometer in $CDCl_3$, using TMS as internal standard. GC/MS was done at 70 eV on a LKB 9000S spectrometer fitted with a 15 m capillary SE-30 WCOT column.

C. lamellosa sterols. 24-Epibrasicasteryl acetate: MS *m/z* (rel. int.): 380 (100), 365 (8), 337 (8), 255 (46), 228 (9), 213 (13). ^{1}H NMR (80 MHz): δ0.692 (3H, *s*, H-18), 0.824 (3H, *d*, *J* = 7 Hz, H-27), 0.833 (3H, *d*, *J* = 6 Hz, H-26), 0.913 (3H, *d*, *J* = 7 Hz, H-28), 0.988 (3H, *d*, *J* = 7 Hz, H-21), 1.024 (3H, *s*, H-19), 2.031 (3H,

s, acetate), 4.65 (1H, *m*, H-3α), 5.15 (2H, *m*, H-22, H-23), 5.38 (1H, *m*, H-6).

Campestanyl acetate: Obtained by catalytic hydrogenation, at room temp. and atmospheric pressure, of 24-epibrassicasteryl acetate in EtOAc using Adam's catalyst. ^{1}H NMR ($CDCl_3$, 200 MHz): δ0.645 (3H, *s*, H-18), 0.766 (3-H, *d*, *J* = 6 Hz, H-28), 0.798 (3H, *d*, *J* = 7 Hz, H-27), 0.815 (3H, *s*, H-19), 0.846 (3H, *d*, *J* = 7 Hz, H-26), 0.891 (3H, *d*, *J* = 6 Hz, H-21), 2.022 (3H, *s*, acetate), 4.68 (1H, *m*, H-3α).

Poriferasteryl acetate: Mp 145–146° from MeOH (lit. 146–147°, [10]). MS *m/z* (rel. int.): 394 (100), 379 (6), 351 (15), 255 (41), 228 (12), 213 (11). ^{1}H NMR ($CDCl_3$, 80 MHz): δ0.698 (3H, *s*, H-18), 0.795 (3H, *d*, *J* = 6 Hz, H-27), 0.807 (3H, *t*, *J* = 6 Hz, H-29), 0.847 (3H, *d*, *J* = 6 Hz, H-26), 1.018 (3H, *d*, *J* = 6 Hz, H-21), 1.024 (3H, *s*, H-19), 2.025 (3H, *s*, acetate), 4.67 (1H, *m*, H-3α), 5.10 (2H, *m*, H-22, H-23), 5.37 (1H, *m*, H-6).

N. roscoffensis *sterols*. *Stigmasteryl acetate*: Mp 141–142° from MeOH (lit. 141° [9]). MS *m/z* (rel. int.): 394 (100), 379 (7), 351 (17), 255 (52), 228 (11), 213 (13). ^{1}H NMR ($CDCl_3$, 80 MHz): δ0.697 (3H, *s*, H-18), 0.795 (3H, *d*, *J* = 6 Hz, H-27), 0.806 (3H, *t*, *J* = 6 Hz, H-29), 0.846 (3H, *d*, *J* = 6 Hz, H-26), 1.021 (3H, *d*, *J* = 6 Hz, H-21), 1.023 (3H, *s*, H-19), 2.030 (3H, *s*, acetate), 4.65 (1H, *m*, H-3α), 5.10 (2H, *m*, H-22, H-23), 5.37 (1H, *m*, H-6).

(24E)-24-n-*Propylidenecholesterol*: Mp 94–95° from MeOH. MS *m/z* (rel. int.): 408 (35), 393 (7), 296 (100), 281 (22), 255 (9), 253 (14), 228 (14), 213 (18). ^{1}H NMR ($CDCl_3$, 80 MHz): δ0.686 (3H, *s*, H-18), 0.940 (3H, *t*, *J* = 7 Hz, H-30), 0.980 (9H, *d*, *J* = 7 Hz, H-21, H-26 and H-27), 1.023 (3H, *s*, H-19), 1.99 (2H, *m*, H-29), 2.024 (3H, *s*, acetate), 2.20 (1H, *m*, H-25), 4.62 (1H, *m*, H-3α), 5.07 (1H, *t*, *J* = 7 Hz, H-28), 5.40 (1H, *m*, H-6).

24-n-*Propylcholesterol*: MS *m/z* (rel. int.): 410 (100), 395 (18), 255 (21), 213 (21).

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