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MINOR AND TRACE STEROLS OF *DUNALIELLA TERTIOLECTA**

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Abstract—The minor sterols have been isolated from the unicellular chlorophyte alga *Dunaliella tertiolecta* and identified by GC/MS and ^{13}C NMR as a mixture of 24-methyl and 24-ethyl Δ^7, $\Delta^{7,22}$ and $\Delta^{8,14}$-sterols.

INTRODUCTION

The marine unicellular chlorophyte *Dunaliella tertiolecta* Butcher is unusual in possessing no cell wall. Because sterols play an as yet undefined role in membrane structure a thorough study of the sterol profile of this organism may shed new light on the functional mechanism of membranes. in general. Recently we reported [1] that the major sterols of *D. tertiolecta* were ergosterol (**1**), and the more unusual C_{29} analogue (**2**). The stereochemical features of these sterols were established by ^{13}C NMR spectroscopy [2]. Using a specially designed culture tank for large-scale axenic cultivation of unicellular algae [3] we have prepared a large batch of *D. tertiolecta* cells for further examination of the minor and trace sterols.

RESULTS AND DISCUSSION

Si gel chromatography of the non-saponifiable lipids followed by acetylation yielded the usual mixture of steryl acetates [1] which was separated into fractions by argentation chromatography. In addition to the two major sterols already reported, ten other minor or trace components were identified and these are listed in Table 1.

The early monoene fraction (13 mg) contained two major components (RR_t 1.46, 42%; RR_t 1.78, 36%) together with traces of two others (RR_t 1.36, 13%; RR_t 1.68, 8%). Retention time values and GC/MS data (Table 1) identified the major sterols as 24-methyl- and 24-ethylcholest-7-en-3β-ol. By comparison with authentic 5α-cholest-7-en-3β-ol and from published data [4] the most intense resonances in the ^{13}C NMR spectrum of the mixture could be assigned to C-1–C-21 of a Δ^7 sterol (Table 2). Seven less intense signals (δ 39.16, 33.76, 31.55, 30.79, 20.52, 17.68, 15.50) were assigned to a saturated side-chain containing a 24*S*-ethyl substituent. The remaining six signals in the spectrum (δ 46.17, 29.07, 26.65, 23.10, 19.64, 12.33) were diagnostic for a saturated side-chain containing a 24-*S*-ethyl substituent. The resonances for C-22 (δ 33.84) and C-26 (18.98) overlap with those for C-4 and C-21. Thus the two major monoene sterols in *D. tertiolecta* are (24*S*)-24-methylcholest-7-en-3β-ol (**3**) and (24*S*)-24-ethylcholest-7-en-3β-ol (**4**). Signals for the two trace sterols were not observed in the ^{13}C NMR spectrum, but based on retention time and mass spectral data they are identified as the corresponding Δ^8 analogues.

The next fraction (21 mg) contained, in addition to **3** and **4**, an almost equimolar amount of a third component (RR_t 1.58, 25%) and traces of a fourth sterol (RR_t 1.26, 9%). The ^{13}C NMR spectrum of this fraction displayed all the resonances observed for the previous fraction as well as 10 new resonances (δ 138.14, 129.52, 51.24, 40.79, 31.85, 28.40, 25.41, 20.93, 12.44, 12.09) which could be accommodated by a Δ^{22} side-chain containing an 24*R*-ethyl substituent. The resonances for C-26 (δ 18.98) and C-27 (21.46) overlap with those for C-21 and C-11 of components **3** and **4**. Hence the third minor sterol is (22*E*, 24*R*)-24-ethyl-5α-cholesta-7,22-dien-3β-ol (**6**). Once again, resonances for the trace sterol could not be observed in the ^{13}C NMR spectrum but the GC/MS data (Table 1) indicated it was the 24-methyl analogue of **6**.

Fortuitously, this latter sterol occurred in the following fraction (7 mg) as the major component (37%) together with smaller amounts of **6** (29%). ^{13}C NMR analysis of this fraction (microcell, 5 μl $CDCl_3$) confirmed the presence of a Δ^7 sterol possessing a C-24 methyl Δ^{22} side-chain and key resonances at δ 17.64 (C-28) 33.11 (C-25) and 42.85 (C-24) established the sterol as (22*E*, 24*R*)-24-methyl-5α-cholesta-7,22-dien-3β-ol (**5**). The additional resonances for **6** could barely be discerned above the noise level. In addition to these two sterols this fraction also contained traces of four other components (RR_t 1.18, 2.5%; RR_t 1.33, 12%; RR_t 1.66, 10%; RR_t 1.92, 9.5%). The GC/MS data (Table 1) indicated the component with the shortest retention time was an ergostatrienol derivative containing at least two double bonds in the nucleus. The next two components each possessed a saturated side-chain (no loss of propyl) and were tentatively identified as 24-methyl- and 24-ethylcholesta-8,14-diene-3β-ol, respectively. The trace component with the longest retention time displayed a molecular ion (m/z 454) corresponding to a C_{29} diene. Fragment ions at m/z 356 (partial loss of side-chain), 313 (loss of complete side-chain with 2 H transfer), 273 (loss of ring D), 213 (loss of D ring plus acetate), and 255 and 253 (loss of side-chain plus acetate without and with 2 H transfer, respectively) were diagnostic [5] for 24-ethylidenecholesta-7,24(28)-dien-3β-ol. The next fraction (41 mg) contained almost pure **2** (>85%) and subsequent fractions (206 mg, combined) were a mixture of the major sterols **1** and **2**. The

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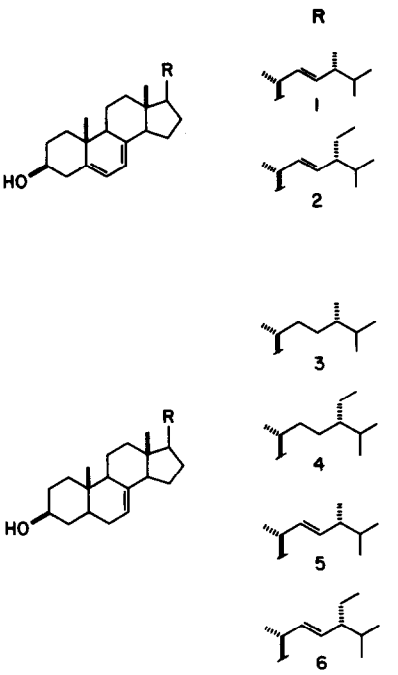
Table 1. GLC and GC/MS data for *Dunaliella tertiolecta* steryl acetates

	RR_t*	MS	Identification
Monoenes	1.36	442, 255, 213	(24ξ)-24-Methyl-5α-cholest-8-en-3β-ol
	1.46	442, 315, 273, 255, 213	(24*S*)-24-Methyl-5α-cholest-7-en-3β-ol (**3**)
	1.68	456, 255, 213	(24ξ)-24-Ethyl-5α-cholest-8-en-3β-ol
	1.78	456, 315, 273, 255, 213	(24*S*)-24-Ethyl-5α-cholest-7-en-3β-ol (**4**)
Dienes	1.26	440, 397, 337, 313, 273, 255, 213	(22*E*,24*R*)-24-Methyl-5α-cholesta-7,22-dien-3β-ol (**5**)
	1.33	440, 313, 253, 251	(24ξ)-24-Methyl-5α-cholesta-8,14-dien-3β-ol
	1.58	454, 411, 351, 313, 273, 255, 213	(22*E*,24*R*)-24-Ethyl-5α-cholesta-7,22-dien-3β-ol (**6**)
	1.66	454, 439, 313, 253	(24ξ)-24-Ethyl-5α-cholesta-8,14-dien-3β-ol
	1.92	454, 356, 313, 273, 255, 253, 213	(24ξ)-24-Ethylidene-5α-cholest-7-en-3β-ol
Trienes	1.18	438, 378, 363, 311, 253	$C_{28}\Delta^{7,9(11),22}$†
	1.22	438, 378, 363, 253	(22*E*,24*R*)-24-Methyl-5α-cholesta-5,7,22-trien-3β-ol (**1**)
	1.53	452, 392, 377, 253	(22*E*,24*R*)-24-Ethyl-5α-cholesta-5,7,22-trien-3β-ol (**2**)

* Relative to cholesteryl acetate = 1.00.
† Tentative identification.

Table 2. ^{13}C NMR spectral data (δ, TMS) for *D. tertiolecta* steryl acetates

	3	4	5	6
C-1	36.86	36.86	36.86	36.86
C-2	27.53	27.53	27.52	27.53
C-3	73.49	73.49	73.53	73.49
C-4	33.84	33.84	33.82	33.84
C-5	40.09	40.09	40.08	40.09
C-6	29.55	29.55	29.54	29.55
C-7	117.31	117.31	117.30	117.31
C-8	139.54	139.54	139.46	139.54
C-9	49.32	49.32	49.32	49.32
C-10	34.24	34.24	34.23	34.24
C-11	21.46	21.46	21.41	21.46
C-12	39.53	39.53	39.43	39.53
C-13	43.37	43.37	43.30	43.37
C-14	55.03	55.03	55.06	55.03
C-15	23.00	23.00	22.97	23.00
C-16	27.95	27.95	28.06	28.40
C-17	56.07	56.07	56.02	56.07
C-18	11.86	11.86	12.06	12.09
C-19	12.95	12.95	12.94	12.95
C-20	36.66	36.66	40.51	40.79
C-21	18.98	18.98	21.12	20.93
C-22	33.76	33.84	135.64	138.14
C-23	30.79	26.65	131.95	129.52
C-24	39.16	46.17	42.85	51.24
C-25	31.55	29.07	33.11	31.85
C-26	17.68	18.98	19.98	18.98
C-27	20.52	19.64	19.66	21.46
C-28	15.50	23.10	17.64	25.41
C-29		12.33		12.44



spectroscopic data for this mixture was the same as already described [1].

It is well established that sterols have a marked effect on the physical properties of membranes [6]. The nature of the sterol is critical, and recently Bloch *et al.* [7] have shown that certain structural and stereochemical features of the side-chain are important in the growth of *Saccharomyces cerevisiae*. The ^{13}C NMR data in this study confirm that the configuration at C-24 of *D. tertiolecta* sterols is the same as in sterols produced by other unicellular chlorophytes [8,9]. In addition, the sterol profile of *D. tertiolecta* is similar to other unicellular chlorophytes which do possess a cell-wall [8,9]. Apparently, no special requirement is necessary for the successful function of the cell. This also appears to hold for other membrane components. We (Wright, J. L. C., unpublished results) and others [10,11] have found that the hydrocarbon and fatty acid composition of *D. tertiolecta* is typical for a chlorophyte.

EXPERIMENTAL

Experimental procedures and culture conditions were the same as previously described [1]. Freeze-dried cells (80 g) of *Dunaliella tertiolecta* Butcher [12] yielded a sterol fraction which was acetylated in the usual manner. GLC analysis of this

acetylated mixture (513 mg) showed it to contain considerable amounts of phytyl acetate identified by ^{13}C NMR as the *trans*-isomer. The acetate mixture was sepd into fractions by chromatography on $AgNO_3$(20%)-Si gel ($C_6H_{14} \rightarrow C_6H_6 \rightarrow Et_2O$) (Table 1). Individual fractions were examined by GLC and GC/MS (data in Table 1) and also by ^{13}C NMR spectroscopy (data in Table 2).

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