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Identification of Sterols and Alcohols Produced by Green Algae of the Genera *Chlorella* and *Scenedesmus* by Means of Gas Chromatography—Mass Spectrometry

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ABSTRACT. Sterols and alcohols isolated from a collection of industrially important fresh-water green algae *Chlorella kessleri*, *Scenedesmus acuminatus*, *S. acutus* and *S. acutus* var. *Tomaselli* cultivated heterotrophically or autotrophically under pilot plant conditions, were identified and quantified by gas chromatography—mass spectrometry. Previously demonstrated sterols with double bonds in position 5 and 7 were detected. In addition, cholesta-5,7,22-trien-3β-ol that had not yet been described in green algae was found in *S. acutus*. Alcohols were found only in *C. kessleri* cultivated under heterotrophic conditions. Some saturated and unsaturated alcohols were detected for the first time in green algae.

The sterol content of algae was discussed in several review articles (Patterson 1971; Goodwin 1974). Major sterols produced by the genera *Chlorella* and *Scenedesmus* have 28 and 29 carbon atoms and contain methyl or ethyl groups on carbon $C_{(24)}$ (fungisterol, chondrillasterol, schotenol). Using gas chromatography—mass spectrometry (GC—MS) ergosterol, ergostadienol, corbisterol, 7-ergosterol, 5-ergosterol, poriferosterol and clionasterol were gradually detected as minor components (Bélanger *et al.* 1973; Paoletti *et al.* 1976; Milkova *et al.* 1977).

Paoletti *et al.* (1976) described three aliphatic alcohols in *Scenedesmus quadricauda* and *Spirulina platensis* that have not been characterized in more detail. Volkman *et al.* (1981) described alcohols with an even number carbon atoms (C_{14}—C_{30}) in wax of marine algae. Alcohols with an even number of carbon atoms (C_{14}—C_{22}) were detected in photosynthetic microorganisms of the genera *Euglena* and *Chroomonas* (Hilenski *et al.* 1976; Nichols *et al.* 1983).

In this work we studied the occurrence of sterols and aliphatic alcohols in two industrially important genera of green algae (*Chlorella* and *Scenedesmus*). In addition, in the genus *Chlorella* we compared the production of these compounds under heterotrophic and autotrophic cultivation conditions.

TABLE I. Conditions of gas chromatography—mass spectrometry used to determine sterols and alcohols

Apparatus and analyzed compounds	HP 5995 B	
	sterols	alcohols
Column type	WCOT	WCOT
Stationary phase	SE-30	SE-30
Length, m	12.5	25
ID, mm	0.25	0.25
Thickness of stationary phase, μm	0.25	0.25
Injector temperature, °C	300	250
Initial column temperature, °C	240	100
Temperature increase, K/min	1	5
Final column temperature, °C	280	280
Helium flow, mL/min	0.54	0.54
Split	1 : 47	1 : 47
Ionization energy, aJ	11	11
Mass range, m_u	40—600	40—600
Sweep rate, $m/z \times 1/s$	680	680

MATERIAL AND METHODS

Cultures. Chlorella kessleri (cultivated both autotrophically and heterotrophically), *Scenedesmus acuminatus* (cultivated autotrophically) and *Scenedesmus acutus* (cultivated autotrophically) were prepared in the *Department of Autotrophic Microorganisms* of the *Institute of Microbiology, Czechoslovak Academy of Sciences* (Třeboň, Czechoslovakia) in the form of dried algal biomass (about 5 % moisture). *Scenedesmus acutus* var. *Tomasseli* (cultivated autotrophically) was a gift of the *Institute of Algology, Bulgarian Academy of Sciences*, Sofia.

Preparation of the sterol and aliphatic alcohol fraction. Algal biomass (10 g) was saponified by 2-h boiling with 300 mL 2 M NaOH in 50 % ethanol. The reaction mixture was diluted with 1 L water and extracted with three 500-mL portions of diethyl ether. Combined organic extracts were washed with three 300-mL volumes of water, dried with sodium sulfate and evaporated to dryness. Preparative TLC (plates 200 × 200 mm, Silica gel H, *Merck*, system light petroleum—diethyl ether, 3 : 1) yielded the sterol fraction (R_F 0.35—0.50). In *C. kessleri* cultivated under heterotrophic conditions the alcohol fraction was also isolated (R_F 0.5—0.6).

Identification of sterols and alcohols. Analytical methods used for the determination of sterols and alcohols by GC—MS method are summarized in Table I. Sterols were analyzed as trimethylsilyl derivatives (Brooks *et al.* 1973). Aliphatic alcohols were determined as the corresponding trimethylsilyl ethers (Odham and Stenhagen 1972). UV spectra were measured in the Cary 118 (Varian) apparatus in ethanol within the range of 200—350 nm.

RESULTS AND DISCUSSION

Sterols were identified on the basis of fragmentation of their mass spectra according to Brooks *et al.* (1973) and Knights (1967). The distribution of sterols (Table II) is characterized here primarilly by the presence of 7-ergo-

TABLE II. Sterol content in genera *Chlorella* and *Scenedesmus* (mass %)

Systematic name	Trivial name	*C. kessleri*[a]		*S. acuminatus*	*S. acutus*	*S. acutus* var. *Tomaszekii*
		H	A			
24 ξ-Methylcholesta-5,22-dien-3β-ol	brassicasterol[b]	0	0	1.0	0	0
24 ξ-Methyl-5α-cholesta-7,22-dien-3β-ol	ergostadienol[b]	1.7	2.8	1.5	0	14.0
24 ξ-Methylcholest-5-en-3β-ol	campesterol[b]	0	0	0	0.9	0
24 ξ-Methyl-5α-cholest-7-en-3β-ol	fungisterol[b]	57.8	53.0	23.0	36.5	0
24 ξ-Ethyl-5α-cholesta-7,22-dien-3β-ol	chondrillasterol[b]	10.4	—	65.8	32.9	2.4
24 ξ-Ethyl-5α-cholest-7-en-3β-ol	schotenol[b]	30.1	23.8	6.7	27.7	55.7
24 ξ-Methyl-5α-cholest-8(14)-en-3β-ol	8(14)-ergostenol[c]	0	4.3	0	0	0
24 ξ-Ethyl-5α-cholest-8(14)-en-3β-ol	8(14)-stigmastenol[c]	0	9.3	0	0	0
4 ξ-Methyl-24ξ-ethyl-5α-cholesta-7,22-dien-3β-ol	4-methylchondrillasterol[c]	0	6.8	0	0	0
Cholesta-5,7,22-trien-3β-ol	cholestatrienol[d]	0	0	0	0	27.3
Not identified	—	0	0	2.0	2.0	0

[a] H — heterotrophic, A — autotrophic cultivation.
[b] Described in green fresh-water algae (Patterson 1971; Goodwin 1974).
[c] Described only after inhibition (Patterson *et al.* 1973).
[d] Not yet described in green algae.

stenol (23—57 %) and 7-chondrillastenol (6—55 %). Small quantities of other sterols are present in *C. kessleri*, among them three unusual sterols, 24-methyl- and 24-ethyl-5α-cholest-8(14)-en-3β-ol and 4-methylchondrillasterol. These sterols are usually found primarily in algae cultivated in the presence of inhibitors of sterol biosynthesis (Patterson *et al.* 1973; our unpublished results).

TABLE III. Alcohol content in *C. kessleri* cultivated under heterotrophic conditions (mass %)

Alcohol (trimethylsilyl ether)	RRT[a]	M^+	%
Previously described (in photosynthetic microorganisms):			
1-Tetradecanol[b]	0.834	286	3.02
1-Hexadecanol[b]	0.821	314	9.94
1-Oktadecanol[b]	0.958	342	1.60
Phytol[c]	1.000	368	73.07
Not yet described (in photosynthetic microorganisms):			
Decen-1-ol	0.265	228	1.90
1-Decanol	0.273	230	1.56
2-Undecanol	0.297	244	0.22
2-Tridecanol	0.388	272	0.24
1-Dodecanol	0.452	258	1.94
1-Tetradecanol[d]	0.577	286	0.43
1-Pentadecanol	0.717	300	0.80
Hexadecen-1-ol	0.774	312	0.62
Eikosen-1-ol[d]	0.866	368	0.38
Eikosadien-1-ol	1.007	366	2.46
Eikosen-1-ol[d]	1.043	368	0.17
Eikosen-1-ol	1.072	368	1.06
Not identified	1.179	456[e]	0.37
Not identified	1.201	143[e]	0.20

[a] Relative retention time related to phytol (28.90 min).
[b] Volkman *et al.* (1981); Nichols *et al.* (1983).
[c] Paoletti *et al.* (1976).
[d] Branching of the chain not determined.
[e] Highest ion detected.

The identification of cholesta-5,7,22-trien-3β-ol that has not yet been described in green algae is of primary importance. It has been found only in the red alga *Porphyridium cruentum* (Beastall *et al.* 1971) and also in marine animals, such as molluscs (*Cacospongia mollior* — Cafieri and De Napoli 1976; *Axinella cannabina* — Cafieri *et al.* 1975; *Terpos zeteki* — Delseth *et al.* 1979*a*; *Biemna fortis* — Delseth *et al.* 1979*b*).

Its identification was further confirmed by its UV spectrum which exhibits a pronounced absorption at 293, 282.5 and 272 nm, indicating the presence of a conjugated homoannular diene, as measured in the model UV spectrum of ergosterol. Cafieri *et al.* (1975) and Cafieri and De Napoli (1976) described a very low intensity of the molecular ion of cholesta-5,7,22-trien-3β-ol. This ion was also not detected in this work but its characteristic fragmentation

and presence of ions *m/z* 364 and 253 are in agreement with the proposed structure.

Interpretation of the mass spectra of trimethylsilyl ethers (Odham and Stenhagen 1972) and retention characteristics of the alcohol fraction isolated from *C. kessleri*, and mass spectra and retention characteristics of standards of alcohols (C_{12}—C_{20} even saturated and C_{16} and C_{18} monoene) a total of fifteen alcohols were identified, among them phytol as the major one. It is apparently formed by degradation of chlorophyll (Paoletti *et al.* 1976). Among other aliphatic alcohols, five saturated alcohols with an even number carbon atoms, three with an odd number carbon atoms, three monoene and one diene alcohol were detected. The hydroxyl group is always in position 1 of the chain, only two unsaturated alcohols have hydroxyl group in position 2. Two 1-tetradecanols and three eikosene-1-ols differing in branching were described but could not be accurately determined by the GC—MS method. Alcohols detected in *C. kessleri* are summarized in Table III. In green algae (*Chlorophyceae*) aliphatic alcohols were not described, only three incompletely characterized alcohols were briefly mentioned by Paoletti *et al.* (1976). In nature aliphatic alcohols are present primarilly in the form of waxes and are assumed to play a physiological role, serving as energy reserves of the cell (Withers and Nevenzel 1977).

Determinable amounts of aliphatic alcohols (200 ppm, as related to dry mass) were detected only in heterotrophically cultivated *C. kessleri*, whereas in autotrophic cultures of *C. kessleri*, *S. acutus* and *S. acuminatus* these compounds were not detected (if they are present, then only in quantities lower than 10 ppm as related to the dry mass). The results referred to here clearly demonstrate the utmost significance of the way of cultivation of algae (hetero- and autotrophy) for the production of alcohols and are in agreement with the data of Antia *et al.* (1974), who detected an increased production of waxes by photosynthetic microorganisms cultivated under heterotrophic conditions.

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