Plant Physiol. (1967) 42, 1457–1459

Short Communication

Sterols of Chlorella. II. The Occurrence of an Unusual Sterol Mixture in Chlorella vulgaris[1, 2]

Glenn W. Patterson
Department of Botany, University of Maryland, College Park, Maryland 20740

Received December 27, 1966.

The first identification of a sterol in *Chlorella* was by Klosty and Bergmann (5) in 1952 when they isolated ergosterol from *Chlorella pyrenoidosa*. More recently, Otsuka (6) identified ergosterol and a Δ^5 sterol in *Chlorella ellipsoidea*. Ergosterol was the principal component of the mixture. Recent work in this laboratory has been centered about the identification of sterols from other species of *Chlorella*. A mixture of the Δ^5 sterols, poriferasterol, clionasterol, and 22-dihydrobrassicasterol was found (7) in *C. ellipsoidea* and *C. saccharophila*, but no ergosterol was detected. *Chlorella vulgaris* was shown to contain chondrillasterol as its principal sterol component, and although 2 other sterols were present in significant quantities, they were not isolated. Since plant sterols are known to have an effect on animal metabolism, and algae are being considered as future sources of food, it is important that we know the sterol composition of these algae. This paper describes the isolation and identification of each of the 2 unknown sterols occurring in *C. vulgaris*.

Cells of *Chlorella vulgaris* Beyer., Emerson's strain, were grown heterotrophically on basal inorganic medium containing 0.5 % glucose in 15-liter carboys equipped with bubbling tubes for air (3). The cells were harvested in a Sharples Super Centrifuge and freeze-dried before extraction. Average yield was 3 grams dry weight per liter. Lipid material was extracted from the cells with acetone in a soxhlet apparatus, saponified under nitrogen, and the non-saponifiable matter extracted with ether in a liquid-liquid extraction apparatus. The non-saponifiable lipid was fractionated as described by Heftmann et al. (3) on Woelm Grade III neutral alumina. The fraction containing sterols was acetylated and rechromatographed under the same conditions described above. The fraction containing the mixture of sterol acetates was subjected to column chromatography on Anasil B which was added to the 3 cm × 40 cm column in a slurry of *n*-hexane. The sterol acetates were added to the column in a minimum amount of *n*-hexane and eluted with 2 % ether in *n*-hexane. Gas chromatography was used to assay the 15-ml fractions which were collected. The 3 sterols isolated were named sterol 1, sterol 2, and sterol 3 in the order of their elution from the Anasil column. Pure sterol 1 was obtained from the Anasil column only with great difficulty. It was more readily obtained by preparative gas chromatography of the unresolved sterol 1-sterol 2 mixture. The trapped sterol was purified by alumina fractionation, digitonin precipitation and regeneration of the free sterol followed by recrystallization in methanol and acetone. Subsequent gas chromatographic analysis showed no impurities even when sterol 1 was injected in amounts sufficient to produce full-scale deflection of the recorder.

Nearly 100 mg of the sterol mixture was obtained from 100 g of *C. vulgaris*. The approximate composition of the sterol mixture was sterol 1, 10 %; sterol 2, 25 %; and sterol 3, 65 %. Sterol 3 has previously been identified as 24β-ethyl-$\Delta^{7,22}$ cholestadienol or chondrillasterol (7), a sterol which also occurs in another unicellular green alga, *Scenedesmus obliquus* (2). Sterols 1 and 2 also gave rapid Liebermann-Burchard reactions indicative of Δ^7 double bond and failed to show the typical ultraviolet absorption spectrum of a $\Delta^{5,7}$ sterol. Thus these sterols, like chondrillasterol, are Δ^7 sterols. The infrared spectra of sterols 1 and 2 were essentially identical. They were also similar to the spectrum of chondrillasterol except for the absence of the strong band at 10.3μ. The presence of this absorption band indicates the presence of a trans double bond at C-22 (4). The infrared spectrum of Δ^7 chondrillastenol, produced by Raney Nickel hydrogenation of chondrillasterol, was identical to that of sterol 1.

Gas chromatographic retention times (table I) indicate that sterol 1 is a Δ^7 C-29 sterol and sterol 2 is a Δ^7 C-28 sterol since the GLC retention times of sterol 1 coincide with those of 24β-ethyl-Δ^7 cholestenol (Δ^7-chondrillastenol) and the retention times of sterol 2 coincide with those of 24β-methyl Δ^7 cholestenol (Δ^7 ergostenol).

In table II, melting point and optical rotation data of *Chlorella* sterols are compared with values obtained from the literature for Δ^7 ergostenol (1) (also called fungisterol) and those obtained for Δ^7 chondrillastenol synthesized from chondrillasterol. The values

---

[1] This is Scientific Article A1276, Contribution No. 3804 of the University of Maryland Agricultural Experiment Station.

[2] This work has been supported in part by grant from the National Aeronautics and Space Administration.

Table I. *GLC Relative Retention Times of* Chlorella *Sterols and Certain Known Sterols*

Compound	Relative retention time*		
	SE-30**	QF-1***	NGS†
Chondrillasterol	3.06	4.00	11.8
Δ^7 Chondrillastenol	3.47	4.48	14.1
Sterol 1	3.47	4.46	14.2
Δ^7 Ergostenol	2.82	3.80	11.0
Sterol 2	2.82	3.80	11.1

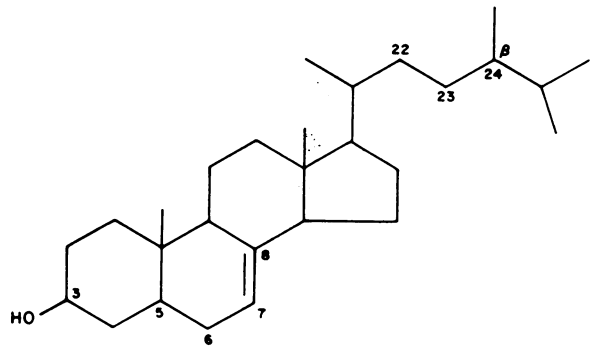
* Relative to cholestane.
** Column 6 ft × 3.4 mm ID, 2 % SE-30 on 100-140 mesh Gas-chrom Q, 20 psi, 236°, cholestane time 7 min.
*** Column 6 ft × 3.4 mm ID, 1 % QF-1 on 100-140 mesh Gas-chrom P, 20 psi, 236°, cholestane time 3 min.
† Column 6 ft × 3.4 mm ID, 1 % NGS on 100-140 mesh Gas-chrom P, 20 psi, 216°, cholestane time 4 min.

obtained for sterol 2 are essentially identical to those found in the literature for Δ^7 ergostenol. Agreement is also excellent in the comparison of melting points of sterol 1 and Δ^7 chondrillasterol.

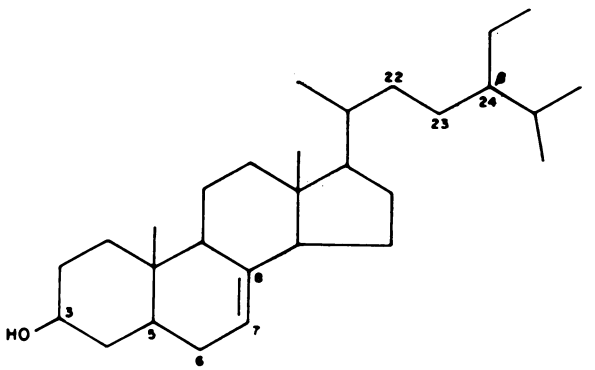
A mass spectrum of the acetate of sterol 1 was obtained. The experimental molecular weight determination of 456 corresponds to that calculated for Δ^7 chondrillastenyl acetate. Therefore, sterol 1 is 24β-ethyl Δ^7 cholestenol (Δ^7 chondrillastenol) and sterol 2 is identical to Δ^7 ergostenol (24β-methyl-Δ^7 cholestenol).

This is the first reported isolation of Δ^7 chondrillastenol from natural sources. This is also the first reported isolation of Δ^7 ergostenol from a green plant. It is of interest to note that gas chromatographic analysis of the sterol fraction from *Scenedesmus obliquus,* in which chondrillasterol was first isolated from a plant (2), in addition shows peaks with identical relative retention times to that of Δ^7 ergostenol and Δ^7 chondrillastenol. It seems quite probable that these 2 sterols are also companions of chondrillasterol in *Scenedesmus.*

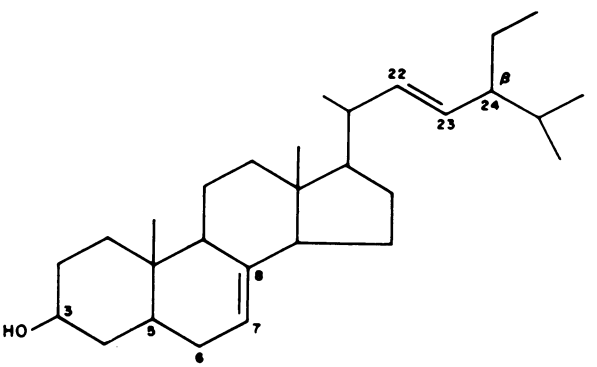
The sterols of *Chlorella vulgaris* (fig 1) are identical to those previously isolated from *Chlorella*



Δ^7 ERGOSTENOL



Δ^7 CHONDRILLASTENOL



CHONDRILLASTEROL

Table II. *Melting Point and Optical Rotation Data for* Chlorella *Sterols and Certain Other Sterols*

Sterol	% of Total Sterol	M.P. Sterol	M.P. Acetate	Optical rotation (at 25 C in $CHCl_3$) Sterol
Chondrillasterol (Sterol 3)	65	169–70	174–5	± 0
Δ^7 Chondrillastenol		140–1	160.5–162	+ 5
Sterol 1	10	140–2	161–163	...
Δ^7 Ergostenol		148	160	0
Sterol 2	25	148–9	159–161	— 2

ellipsoidea except that *C. vulgaris* contains Δ^7 sterols while those of *C. ellipsoidea* are Δ^5 sterols. In all living organisms studied which synthesize Δ^5 sterols, Δ^7 sterols are precursors in the biosynthesis of the Δ^5 sterols. *Chlorella vulgaris* differs from *C. ellipsoidea* in its apparent inability to convert the steroid Δ^7 double bond to the Δ^5 double bond as most plants do.

Acknowledgments

The author thanks M. J. Thompson for his aid with the optical rotations and the infrared spectra, and Dr. G. W. A. Milne for the molecular weight analysis.

Literature Cited

1. Bergmann, W. 1960. Sterols: their structure and distribution. In: Comparative Biochemistry. Vol. III. M. Florkin and H. Mason, eds. Academic Press, New York.
2. Bergmann, W. and R. J. Feeney. 1950. Sterols of algae. I. The occurrence of chondrillasterol in *Scenedesmus obliquus*. J. Org. Chem. 15: 812–14.
3. Heftmann, E., B. E. Wright, and G. U. Liddel. 1960. The isolation of Δ^{22}-stigmasten-3β-ol from *Dictyostelium discoideum*. Arch. Biochem. Biophys. 91: 266–70.
4. Jones, R. N. 1950. The stereochemical configuration of the Δ^{22} ergostenyl side chain. J. Am. Chem. Soc. 72: 5322.
5. Klosty, M. and W. Bergmann. 1952. Sterols of algae. III. The occurrence of ergosterol in *Chlorella pyrenoidosa*. J. Am. Chem. Soc. 74: 1601.
6. Otsuka, H. 1963. Contents of sterols in *Chlorella* cells at different developmental stages. Plant and Cell Physiol. 4: 293–97.
7. Patterson, G. W. and R. W. Krauss. 1965. Sterols of *Chlorella*. I. The naturally occurring sterols of *Chlorella vulgaris, C. ellipsoidea* and *C. saccharophila*. Plant Cell. Physiol. 6: 211–20.