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# TWO NEW STEROLS FROM *CHLORELLA ELLIPSOIDEA**

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**Abstract**—Eight sterols were observed in *Chlorella ellipsoidea* and the four major components were identified as ergosterol, 5α-ergost-7-en-3β-ol, 22-*trans*-ergosta-5,8(9),22-trien-3β-ol and ergosta-5,8(9)-dien-3β-ol This is the first report of the latter two sterols from green plants

## INTRODUCTION

SEVERAL recent publications have indicated that sterol composition may be of value in distinguishing between closely related species of a genus in some green algae was well as providing valuable insight into the relationships between the classes of green algae [1-4] Sterol composition may also aid in the placement of algal species in which the divisional status is in doubt [5] Work with *Chlorella* has indicated that the genus can be divided into three groups based on the presence of either $\Delta^5$-, $\Delta^7$-, or $\Delta^{5,7}$-sterols in the organism [4] There are only two *Chlorella* species containing $\Delta^5$-sterols, *C ellipsoidea* and *C saccharophila* [1] Since the Indiana culture collection of algae had another isolate of *C ellipsoidea* (No 246) in addition to the one studied previously (No 247) it was of interest to us to determine the sterol composition of No 246 which is supposedly quite similar or even identical to No 247 In addition, Otsuka has reported[6] ergosterol and some $\Delta^5$-sterols from her *C ellipsoidea* (origin unknown) This is another area where biochemical data may aid in the taxonomy of a difficult genus

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[3] PATTERSON, G W (1969) *Comp Biochem Physiol* **31**, 391
[4] PATTERSON G W (1971) *Lipids* **6**, 120
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[6] OTSUKA H (1963) *Plant Cell Physiol* **4**, 293

## RESULTS AND DISCUSSION

The appearance of *Chlorella ellipsoidea*, Gerneck, Indiana Culture Collection No 246 in culture was markedly different from that of *C ellipsoidea* Gerneck Indiana Culture Collection No 247, which was previously examined for sterol composition [1] The No 246 culture was of a darker green color, foamed less during culturing and appeared to grow much more rapidly than the No 247 culture The obvious visual differences in these organisms and the pronounced biochemical differences described in this paper, indicate that further work may show that these organisms should be regarded as separate species

TABLE 1 COMPOSITION AND GAS CHROMATOGRAPHIC RELATIVE RETENTION TIMES* OF STEROLS FROM *Chlorella ellipsoidea*

| Sterol acetates | % of total sterol | GLC systems† SE30 | QF1 | HiEff-8BP | PMPE |
|---|---|---|---|---|---|
| Ergosta-5,8(9) 22-Trien-3β-ol | 32 6 | 1 14 | 1 07 | 1 13 | 1 15 |
| $RR_t$ 1 22 | 0 3 | 1 22 | 1 22 | 1 17 | 1 17 |
| Ergosterol | 34 8 | 1 22 | 1 22 | 1 36 | 1 33 |
| 5α-Ergosta-7 22-dien-3β-ol | 2 2 | 1 25 | 1 20 | 1 32 | 1 37 |
| Ergosta-5,8(9)-dien-3β-ol | 9 8 | 1 33 | 1 28 | 1 36 | 1 36 |
| $RR_t$ 1 38 | 2 4 | 1 38 | 1 36 | 1 70 | 1 64 |
| Ergosta-5 7-dien-3β-ol | 0 8 | 1 42 | 1 46 | 1 64 | 1 58 |
| Ergost-7-ene-3β-ol | 17 1 | 1 47 | 1 42 | 1 60 | 1 63 |

* Relative retention time of sterol acetate as compared to cholesterol acetate
† Conditions as described previously [7]

The digitonin-precipitated sterol from No 246 made up 0 24% of the dry weight of the organism which is about normal for unicellular green algae A GLC analysis on SE30 revealed four peaks with retention times relative to cholesterol of 1 14 1 22 1 33 and 1 46 Column chromatography on Woelm Grade II alumina failed to reveal any sterols with methyl groups at C-4 Acetylation and column chromatography on Anasil B (Analabs Hambden, Conn ) followed by repeated chromatography on $AgNO_3$- silica gel columns produced the four major sterol acetates in increased purity in the following order from the latter column $RR_t$s 1 46, 1 33, 1 14 and 1 22 Also from the $AgNO_3$-silica gel column during this separation, other sterols were observed with $RR_t$s of 1 42, 1 38, 1 25 1 22 (a different sterol from the original sterol with $RR_t$ 1 22 and with a different elution volume on $AgNO_3$-silica gel column) All members of this second group of sterols were present in much smaller quantities than those of the first group The GLC characteristics of the 8 sterols observed along with their relative quantity in the plant are presented in Table 1

The first sterol acetate eluted from the $AgNO_3$-silica gel column was identified as ergost-7-en-3β-acetate and had a $RR_t$ of 1 46 on an SE30 column and its $RR_t$s on other GLC systems (Table 1) agree with those previously published for these systems [7] Its physical characteristics $[\alpha]_D$ 0 , m p 159 160 and its MS were also in accord with published data [2 8]

The second sterol acetate eluted had a $RR_t$ of 1 22 and was contaminated with a sterol acetate with a $RR_t$ of 1 33 It is interesting that this sterol acetate ($RR_t$ 1 22) has a $RR_t$

[7] PATTERSON G W (1971) *Anal Chem* **43**, 1165
[8] ORCUTT D M and RICHARDSON B (1970) *Steroids* **16**, 429

identical to ergosterol acetate on an SE30 or QF1 column but exhibited significantly lower retention times on the more polar columns, indicating it to be a less polar sterol acetate than ergosterol acetate These GLC retention times do not match that of any known sterol A GC–MS of the sterol acetate indicated it to be a C-28 sterol with a saturated side chain and one nuclear double bond The location of this double bond has not been determined since insufficient material was available for further work but GC and MS data indicates that it is not located at the 5(6), 7(8), 8(9) or 8(14) positions

Only a small amount of the sterol acetate with a $RR_t$ of 1 33 was obtained in pure form, since it was difficult to separate from the previous compound and it was also readily oxidized to more polar metabolites GC–MS analyses indicated that it also was a C-28 sterol with a saturated side chain and a diunsaturated nucleus, although no conjugated double bond could be detected by UV spectroscopy The presence of a 5(6) double bond was suggested by the lack of a significant molecular ion when the acetate was analyzed by GC–MS [9] Its GLC characteristics matched that of no known sterol acetate, but they differed from that of the sterol acetate with a $RR_t$ of 1 14 (on SE30 column) only by a factor caused by the presence of a 22(23) double bond in the latter [7] Both compounds will be discussed later

The sterol acetate next eluted from the $AgNO_3$–silica gel column had a $RR_t$ of 1 25 (Table 1) and was identified as 5α-ergosta-7,22-dien-3β-acetate by comparative $RR_t$s and MS with authentic compound The compounds with $RR_t$s of 1 14 and 1 40 were eluted next and could not be completely separated by column chromatography The GLC data for the peak with $RR_t$ of 1 40 were very similar to those of 5α-ergosta-7,24(28)-dien-3β-acetate and ergosta-5,7-dien-3β-acetate,[7] and the GC–MS of this peak indicated that this peak probably consisted of an *ca* 1 1 mixture of these sterol acetates

The sterol acetate, $RR_t$ 1 14 and representing about 33% of the total sterol from the No 246 culture, recrystallized from methanol gave a sample of 97% purity by GLC analyses Its GLC characteristics were similar but not identical to those of brassicasterol acetate and its $RR_t$s were noticeably higher on the more polar columns GC–MS analysis of this acetate showed the lack of molecular ion but with major peaks at *m/e* 378 (M–MeCOOH), 363 [M–(MeCOOH + Me)], 253 [M–(MeCOOH + $C_9H_{17}$)] and other significant peaks above *m/e* 200 at *m/e* 337, 313, 237 and 211 The physical properties of this compound are acetate, m p 127–130°, $[\alpha]_D$ −23°, free sterol 127–129°, $[\alpha]_D$ −7° The compound showed no UV absorption above 220 nm Its IR spectrum showed absorption bands at 967 $cm^{-1}$ (disubstituted double bond) and 808 $cm^{-1}$ (trisubstituted double bond, though at lower frequency than the normal 837 $cm^{-1}$ typical for a $\Delta^5$-bond) The NMR spectrum further confirmed these olefinic protons in that the *trans* olefinic protons appeared at $\delta$ 5 25 and the proton of the trisubstituted double bond at $\delta$ 5 47 Since neither the NMR nor the MS indicates the presence of a cyclopropane ring, a tetrasubstituted double bond must be present in the sterol because the MS shows a molecular ion at *m/e* 396 for this $C_{28}$ sterol The fragmentation pattern further supports the presence of a $C_9H_{17}$ side chain containing a $\Delta^{22}$-bond Since the large M-60 peak in the MS of the acetate derivative and the NMR and IR spectra suggest the presence of a $\Delta^5$-bond (9) and UV analyses indicate no conjugated diene system, then the third double bond can only be at the 8(9) or 8(14) position A catalytic reduction of the acetate with $PtO_2$ in a 1 1 mixture of cyclohexane-acetic acid yielded ergost-8(14)-en-3β-acetate (confirmed by GLC, NMR, IR and MS analyses) The NMR spectrum of the acetate of the free sterol exhibited the methyl resonances

[9] KNIGHTS, B A (1967) *J Gas Chromatog* **5**, 273

almost in identical positions as those for 22-*trans*-ergosta-4,7,22-trien-3-one except for the shift further downfield of 2–4 Hz for the C-19 and C-18 methyls, respectively The calculated values[10] for the C-18 and C-19 methyl resonances for an ergosta-5,8(9),22-trien-3$\beta$-ol of $\delta$ 0 650 (39 Hz) and 1 166 (70 Hz) respectively were in agreement with the observed values of $\delta$ 0 666 (40 Hz) and 1 192 (71 5 Hz) while the calculated values for ergosta-5,8(14),22-trien-3$\beta$-ol were grossly different Furthermore, the $RR_t$s of 22-*trans*-ergosta-5,8(9),22-trien-3$\beta$-ol calculated by the method of Clayton[11] on our four GLC systems in Table 1 are in agreement with the values obtained with the isolated compound while the values calculated for 22-*trans*-ergosta-5,8(14),22-trien-3$\beta$-ol do not agree with those of the isolated compound The data definitely indicate that the sterol with a $RR_t$ of 1 14 is 22-*trans*-ergosta-5,8(9),22-trien-3$\beta$-ol

The compound with a $RR_t$ of 1·33 and which was shown by GLC analyses to differ from the sterol with $RR_t$ of 1 14 only in the absence of the $\Delta^{22}$-bond is then identified as ergosta-5,8(9)-dien-3$\beta$-ol The GC–MS of this sterol supports the assigned structure The last two sterol acetates eluted from the $AgNO_3$–silica gel column were 22-dihydroergosterol acetate ($RR_t$ 1 42) and ergosterol acetate ($RR_t$ 1 22) These identifications were made on the basis of GLC $RR_t$s (Table 1) and on the physical characteristics of ergosterol and ergosterol acetate which agreed with those of authentic ergosterol

The $\Delta^7$- and $\Delta^{5,7}$-sterols, with or without side chain double bonds isolated here are common in many *Chlorella* species and in many fungi However, previous work with *Chlorella ellipsoidea* Gerneck, Indiana Culture Collection No 247, described that organism as containing only $\Delta^5$-sterols [1] The sterol composition for No 246 just described is further evidence of the biochemical differences in these organisms

Ergosta-5,8(9),22-trien-3$\beta$-ol was recently identified for the first time in the fungal component of a lichen [12] To our knowledge it has not been previously reported to occur in green plants The occurrence in nature of ergosta-5,8(9)-dien-3$\beta$-ol is reported for the first time The presence of $\Delta^{5,8(9)}$-sterols in *Chlorella ellipsoidea* Indiana culture collection No 246 indicates a pathway of biosynthesis different from that now thought to occur in most plants or animals studied

## EXPERIMENTAL

*Chlorella ellipsoidea* Gerneck, Indiana Culture Collection No 246 was grown on basal inorganic medium supplemented with 0 5% glucose as described previously [13] Lipids were extracted from freeze-dried cells and $CHCl_3$–MeOH (2 1) and saponified The non-saponifiable fraction was precipitated by digitonin and the sterol recovered as described by Doyle *et al* [13] GLC analyses were made on the total fraction using 3% SE30 followed by chromatography on Woelm Alumina as described by Doyle *et al* [13] The free sterols were then acetylated overnight using $Ac_2O$–pyridine–$C_6H_6$ (5 3 2) The sterol acetates were then separated using $AgNO_3$-silica gel column chromatography GLC analyses were made on Glowall Model A-110 and Model 310 gas chromatographs Sterols isolated were identified by using GLC $RR_t$s on four columns relative to cholesterol acetate for acetate derivatives or cholesterol for free sterols and the $RR_t$s were the same in either case [7] M ps of the sterols and their acetate derivatives were obtained on a calibrated Fisher-John s m p apparatus NMR spectra were recorded at 60 mCi with a Varian A-60A NMR spectrometer using $CDCl_3$ as the solvent and TMS as an internal standard MS were recorded on an LKB Model 9000 GC–MS The compounds were introduced into the ion chamber or into the ion chamber through the GLC column which was 0 75% SE30 The ionization energy was 70 eV

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