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TRIPARANOL INHIBITION OF STEROL BIOSYNTHESIS IN *CHLORELLA EMERSONII**

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Abstract—Nine sterols, in addition to the five previously found, have been identified by means of GLC and mass spectral analysis in triparanol-treated cells of *Chlorella emersonii*. They are: 24-methylene-pollinastanol, 24-methylenecycloartanol, 24-dihydroobtusifoliol, cycloeucalenol, obtusifoliol, 14α-methyl-(24*S*)-5α-stigmast-8-en-3β-ol, 4α,14α-dimethyl-(24*S*)-5α-stigmast-8-en-3β-ol, 5α-ergosta-7,22-dien-3β-ol and (24*S*)-5α-stigmasta-7,25-dien-3β-ol. The presence of most of these sterols in both untreated and triparanol-treated cultures has led to a proposed scheme of sterol biosynthesis in *Chlorella emersonii*.

INTRODUCTION

IN A PRELIMINARY report[1] on triparanol-treated cultures of *Chlorella emersonii*, we identified two new sterols, 14α-methyl-5α-ergost-8-en-3β-ol and 14α-methyl-5α-ergosta-8,24(28)-dien-3β-ol in addition to three normally-occurring sterols—Δ^7-ergostenol, Δ^7-chondrillastenol, and chondrillasterol.[2] It was pointed out that in plants, sterol biosynthesis is thought to proceed similarly to that in animals—except that in plants, alkylation rather than reduction of the 24(25) double bond usually occurs. Due to this basic difference in sterol biosynthesis of animals and plants, it was of interest to determine the effect(s) of a Δ^{24}-sterol reductase inhibitor, triparanol, on sterol metabolism in a unicellular green alga, *C. emersonii*. In this paper we identify nine additional sterols from triparanol-treated cultures, many of which are also present in untreated cultures. A quantitative analysis of sterols in both types of cultures should aid in determining the effect of triparanol on sterol biosynthesis of *C. emersonii*.

Inasmuch as most of these new sterols can be detected in untreated cultures of *C. emersonii*, they appear to be biosynthetic precursors of the Δ^7-sterols. As a result, a proposed scheme of sterol biosynthesis in *C. emersonii* has been presented.

RESULTS AND DISCUSSION

The digitonin-precipitated sterols from triparanol-treated cultures of *C. emersonii* var. *emersonii* Shihira and Krauss were separated by means of Al_2O_3 column chromatography[1] and $AgNO_3$-silica gel column chromatography[3] (Fig. 1) and identified by means of GLC

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[1] P. J. DOYLE, G. W. PATTERSON, S. R. DUTKY and C. F. COHEN, *Phytochem.* **10**, 2093 (1971).
[2] G. W. PATTERSON, *Plant Physiol.* **42**, 1457 (1967).
[3] H. E. VROMAN and C. F. COHEN, *J. Lipid Res.* **8**, 150 (1967).

in four systems (Table 1), as well as by GLC–MS. The identities of five sterols, 24-methylenecycloartanol (VI), cycloeucalenol (VII), obtusifoliol (VIII), (24*S*)-5α-stigmasta-7,25-dien-3β-ol (XV) and 5α-ergosta-7,22-dien-3β-ol, were confirmed by direct comparisons of their MS and GLC behavior with authentic samples. For those sterols for which no known standards were available, relative retention times (RRT's) were used in establishing tentative structures.[4] MS data (Figs. 2–5) further support the assigned structures.

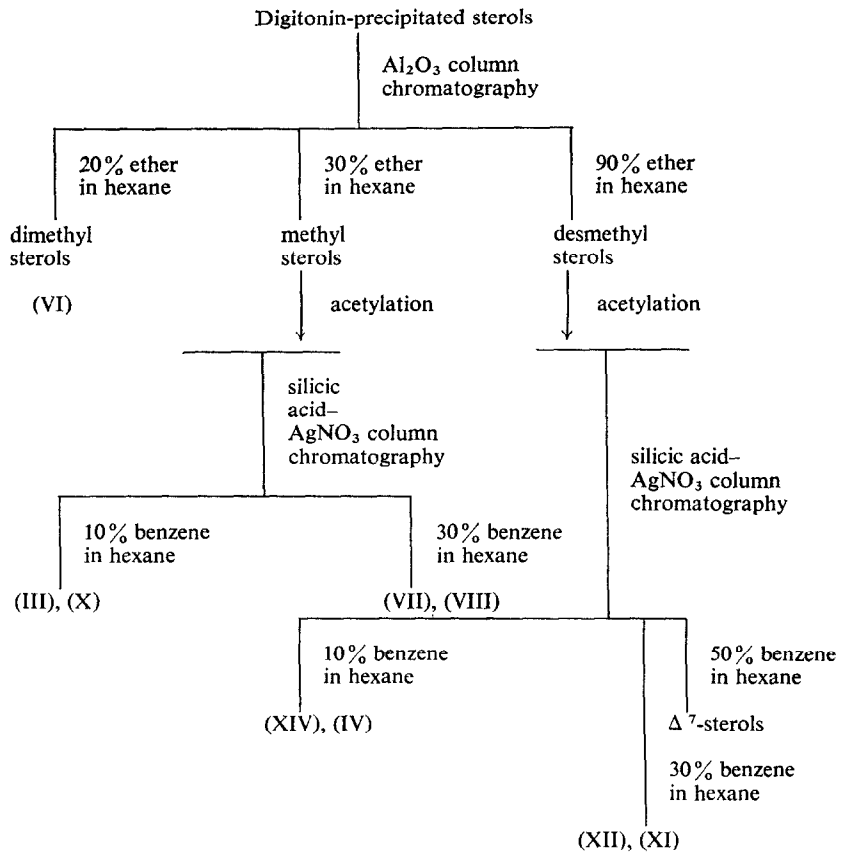

FIG. 1. SEPARATION OF *Chlorella emersonii* STEROLS USING ALUMINA AND $AgNO_3$–SILICA GEL COLUMN CHROMATOGRAPHY.

Of the newly-identified sterols examined in this study, two were present in sufficient amounts so that they could be isolated and certain physical properties obtained. The most easily isolated was 24-methylenecycloartanol, (VI) which eluted from the alumina column in the 20% ether in *n*-hexane fraction. This sterol gave a yellow color with Liebermann–Burchard reagent indicating the presence of a 14-methyl group.[5] Its RRT's in four GLC systems corroborate this structure. Melting point[6] and MS data agreed with the published

[4] R. B. CLAYTON, *Biochemistry* **1**, 357 (1962).
[5] B. L. WILLIAMS, L. J. GOAD and T. W. GOODWIN, *Phytochem.* **6**, 1137 (1967).
[6] G. OURISSON, P. CRABBE and O. R. RIDIG, *Tetracyclic Triterpenes*, p. 152, Holden-Day, New York (1964).

TABLE 1. RELATIVE RETENTION TIMES OF STEROLS FROM TRIPARANOL-TREATED *Chlorella emersonii*

Sterol acetates	Relative retention times Gas chromatographic columns*			
	QF-1†	Hi-Eff-8BP‡	PMPE§	SE-30‖
24-Methylenepollinastanol	1·58	1·68	1·63	1·46 (1·46)
24-Methylpollinastanol (from reduction of 24-methylenepollinastanol)		1·53		1·51 (1·51)
24-Methylenecycloartanol	2·22	2·08	1·95	1·98 (2·12)
Cycloeucalenol	1·82	1·84	1·69	1·70 (1·75)
Obtusifoliol	1·53	1·41	1·29	1·47 (1·52)
24-Dihydroobtusifoliol	1·54	1·28	1·21	1·50 (1·54)
4α,14α-Dimethyl-(24*S*)-5α-stigmast-8-en-3β-ol	1·84	1·53	1·43	1·88 (1·92)
14α-Methyl-(24*S*)-5α-stigmast-8-en-3β-ol	1·66	1·49	1·43	1·67 (1·67)
5α-Ergosta-7,22-dien-3β-ol	1·20	1·32	1·39	1·25 (1·25)
(24*S*)-5α-Stigmasta-7,25-dien-3β-ol	—	—	—	1·73 (1·73)

* Relative to cholesterol acetate.
† Column 1·8 m × 3·4 mm i.d., 1% QF-1 on 100–120 mesh Gas Chrom Q, 25 p.s.i., 231°.
‡ Column 1·8 m × 3·4 mm i.d., 3% Hi-Eff-8BP on 100–120 mesh Gas Chrom Q, 25 p.s.i., 238°.
§ Column 1·8 m × 3·4 mm i.d., 2% PMPE on 100–120 mesh Gas Chrom Q, 20 p.s.i, 250°.
‖ Column 1·8 m × 3·4 mm i.d., 3% SE-30 on 100–120 mesh Gas Chrom Q, 20 p.s.i., 244°.
Values in parenthesis are for the free sterols relative to free cholesterol.

data.[7] The second sterol isolated, obtusifoliol (VIII), had a m.p. of 135° that closely agreed with the reported m.p. 138–140°.[8] Its GLC and MS data were identical to those of authentic samples. Although physical properties were not obtained, a third sterol, cycloeucalenol,

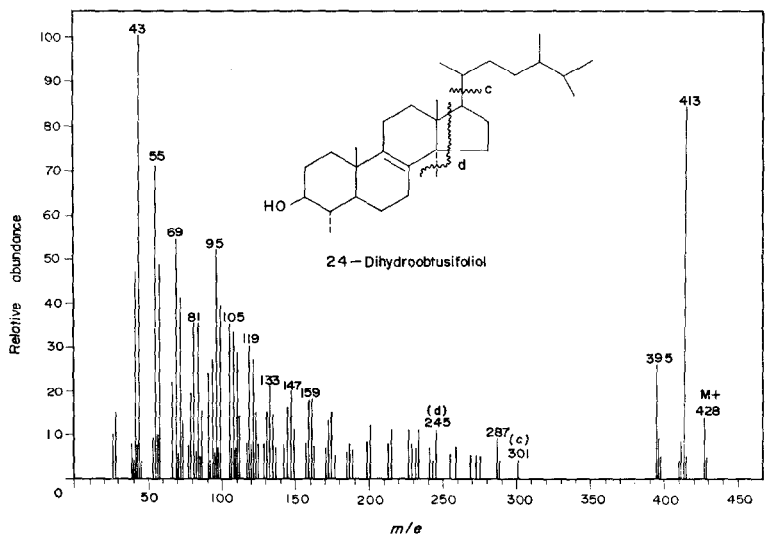

FIG. 2. MS OF 24-DIHYDROOBTUSIFOLIOL.

[7] H. E. AUDIER, R. BEUGELMANS and B. C. DAS, *Tetrahedron Letters* 4341 (1966).
[8] A. G. GONZALEZ and J. L. BRETON, *Anal. Fis. Quim.* **55B**, 93 (1959).

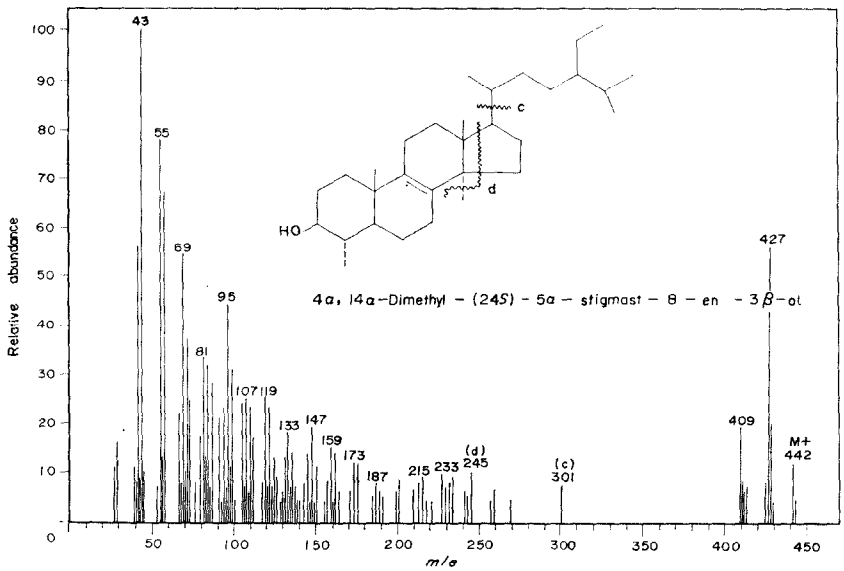

FIG. 3. MS OF 4α,14α-DIMETHYL-(24*S*)-5α-STIGMAST-8-EN-3β-OL.

(VII) was similar to the authentic compound in GLC and MS analyses. Compounds VII and VIII also gave yellow colors with Liebermann–Burchard reagent and so contained 14-methyl substituents.

Two sterols eluted with 30% ether in *n*-hexane on the alumina column had RRT's in four GLC systems that suggested the compounds were 24-dihydroobtusifoliol (III) and 4α,14α-dimethyl-(24*S*)-5α-stigmast-8-en-3β-ol (X). The MS of III (Fig. 2) gave a molecular

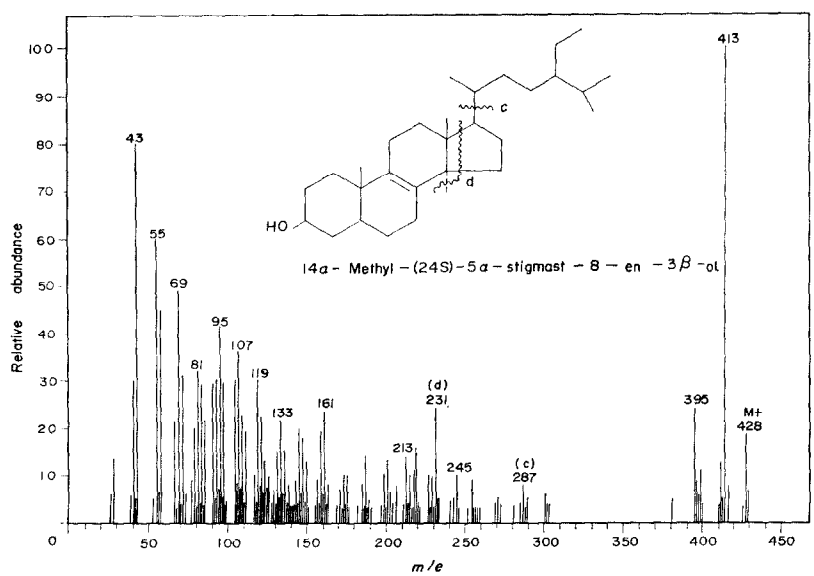

FIG. 4. MS OF 14α-METHYL-(24*S*)-5α-STIGMAST-8-EN-3β-OL.

ion peak at *m/e* 428 and prominent peaks at *m/e* 413 and *m/e* 395—indicating loss of CH_3 and $CH_3 + H_2O$, respectively. The peak at *m/e* 301 indicates loss of side chain, and the one at *m/e* 245 (M-183-15) reveals cleavage of the *D*-ring carbon bonds 13, 17 and 14, 15. The relatively few characteristic peaks in the mass spectrum is typical of Δ^8-sterols. GLC data (Table 1) show that the free sterol has a higher RRT than the corresponding sterol acetates[9]—strongly suggesting a methyl group at C-4. The assigned structure is in agreement with MS and GLC data.

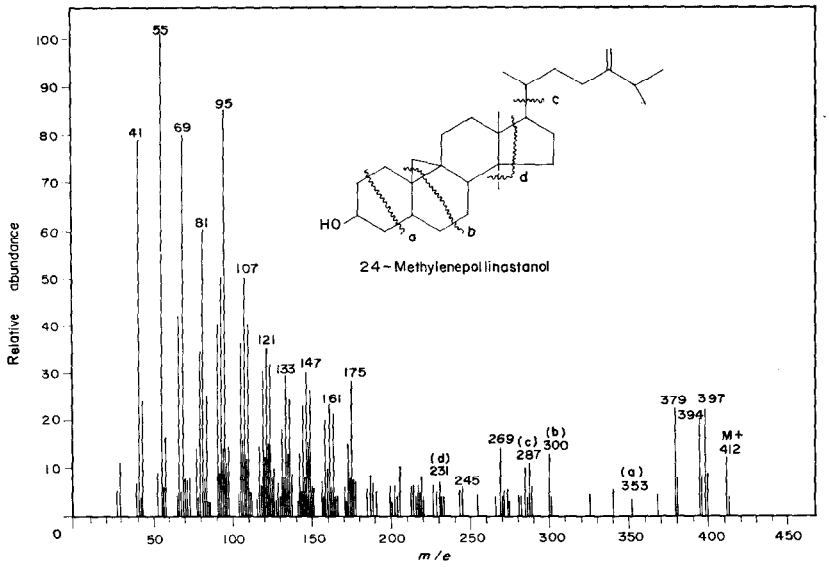

FIG. 5. MS OF 24-METHYLENEPOLLINASTANOL.

The MS of the 4α,14α-dimethyl-(24*S*)-5α-stigmast-8-en-3β-ol (Fig. 3) is similar to that of 24-dihydroobtusifoliol with the exception that it shows a molecular ion peak at *m/e* 442 with attendant peaks at *m/e* 427 and 409—i.e. loss of CH_3 and $CH_3 + H_2O$, respectively. The other major fragments and GLC data (Table 1; it has a higher RRT than its acetate) are similar to those of 24-dihydroobtusifoliol. MS data are consistent with the assigned structure, and the compound differs from 24-dihydroobtusifoliol only by having an ethyl rather than a methyl group at C-24.

Two sterols obtained from the 4-desmethyl fraction on alumina chromatography (Fig. 1) were isolated from the 10% ether in hexane fraction from $AgNO_3$–silica gel chromatography. We previously identified 14α-methyl-5α-ergost-8-en-3β-ol[1] (IV). The other compound, on the basis of GLC and MS analyses, is 14α-methyl-(24*S*)-5α-stigmast-8-en-3β-ol (XIV). The MS of XIV (Fig. 4) is also characteristic of a Δ^8-sterol. Its fragmentation pattern, after the initial loss of CH_3 and $CH_3 + H_2O$, exhibited peaks of identical mass like those of 14α-methyl-5α-ergost-8-en-3β-ol(IV). Cleavage of the 17–20 bond, to give a peak at *m/e* 287, indicates a ten-carbon side chain for XIV. Thus the assigned structure XIV differs from IV only in having an ethyl rather than a methyl substituent at C-24.

The sterol that was inseparable on a silver nitrate-impregnated silica gel column from the previously-identified 14α-methyl-5α-ergosta-8,24(28)-dien-3β-ol,[1] though readily separ-

[9] G. W. PATTERSON, *Analyt. Chem.* **43**, 1165 (1971).

ated by GLC, exhibited RRT's in four GLC systems which suggested it was 24-methylenepollinastanol. The change in the RRT of this sterol after catalytic reduction was identical to the differences of RRT's of 24-methylenecholesterol and campesterol on the SE-30 and the Hi-Eff-8BP columns—thus demonstrating that the compound contained a 24(28) double bond. Its GLC–MS (Fig. 5) gave a molecular ion peak at *m/e* 412 with other prominent peaks at 397, 394, 379 and 287, indicating losses of CH_3, H_2O, $CH_3 + H_2O$, and $C_9H_7^-$ (side chain), respectively. The expected major scission between carbon atoms 22 and 23, seen in the spectrum of 24-methylenecholesterol, does not occur to an appreciable extent. This lack of strong scission at 22–23 is typical of 24-methylene sterols with a 9,19-cyclopropane ring system. Fragments a and b (Fig. 5), which are common to the spectra of cycloeucalenol (VII) and 24-methylenecycloartanol (VI),[7] also support the presence of a 9,19-cyclopropane ring system. Fragment *c* indicates that the double bond is in the side chain and that the additional methyl substituent is in the sterol nucleus. This methyl group must be at C-14, since the fragments a, b, and c together eliminate the possibility of a 4-methyl group. Furthermore, the RRT's on an SE-30 column of the free sterol, relative to cholesterol and its acetate relative to cholesterol acetate, indicate the absence of a 4-methyl group, since 3β-hydroxy-sterols with methyl or dimethyl substituents at C-4 have RRT's slightly higher than the corresponding acetates on an SE-30 column[9] (see Table 1). Thus the data indicate that this sterol is 24-methylenepollinastanol.

5α-Ergosta-7,22-dien-3β-ol continuously eluted with chondrillasterol during fractionation. Its identity was confirmed by a direct comparison with an authentic sample of 5α-ergosta-7,22-dien-3β-ol by GLC and GC–MS. It is not surprising that small amounts of this sterol occur in *C. emersonii*, since it has also been isolated from a similar mixture of Δ^7-sterols in another green alga, *Oocystis polymorpha*.[10]

Another sterol was identified as (24*S*)-5α-stigmasta-7,25-dien-3β-ol (XV) on the basis of direct comparisons of RRT's and MS analyses with those of an authentic sample of (24*R*)-5α-stigmasta-7,25-dien-3β-ol isolated by Sucrow[11] from a mixture of Δ^7-sterols from pumpkin. This sterol was observed on GLC after repeated $AgNO_3$ silica gel column chromatography of the Δ^7-sterol fraction. The mass spectrum obtained by GC–MS did not show the presence of any other sterol in the scan of the RRT 1·73 peak. Mass spectra and RRT's of this compound and Sucrow's sterol were identical. This sterol, and other sterols isolated in this work, were assigned the 24*S* configuration solely because the sterols previously isolated from *C. emersonii* were established to have the 24*S* configuration.[2,12]

In addition to the identifications of sterols from triparanol-treated and untreated cultures of *C. emersonii*, a quantitative analysis of the sterols was performed using a gas chromatograph equipped with a Disc integrator (Table 2). There was a reduction in total sterol concentration of approximately 45% in the triparanol-treated culture. The concentration of each of the three major normally-occurring sterols, Δ^7-ergostenol, Δ^7-chondrillastenol and chondrillasterol, was considerably less than that of untreated cultures. Ten of the fourteen sterols identified in triparanol-treated *C. emersonii* cells were also present in untreated cultures.

From the preceding observations, it appears that triparanol blocks certain metabolic pathways of sterols in *C. emersonii*. One locus considered as a possible site of action for

[10] D. M. ORCUTT and B. RICHARDSON, *Steroids* **16**, 429 (1970).
[11] W. SUCROW and A. REIMERDES, *Z. Naturforsch.* **23b**, 42 (1968).
[12] M. J. THOMPSON, S. R. DUTKEY, G. W. PATTERSON, E. L. GOODEN, *Phytochem.* **11**, 1781 (1972).

triparanol was the sterol side chain at C-24. In vertebrates,[13] an insect,[14] and a nematode,[15] triparanol inhibited reduction of the 24(25) double bond—the terminal step in the production of cholesterol. In plants, sterol biosynthesis usually proceeds by alkylation at C-24, producing a sterol with a 9-carbon side chain, while in vertebrates a reduction of the 24(25) double bond occurs to give a sterol with an 8-carbon saturated side chain. If triparanol interfered with the alkylation process, isolation of sterols with 8-carbon side chains would be expected. Inasmuch as we detected none, it can be assumed that triparanol is not preventing the side chain alkylation in sterol biosynthesis in *C. emersonii*. However, the large reductions in concentrations of chondrillasterol (65%) and Δ^7-chondrillastenol (20%), on dry weight basis, indicate that triparanol is inhibiting alkylation that leads to sterols with 10-carbon side chains, and results in an increase in the percentage of sterols with a 9-carbon side chain. Along with this reduction in concentration of normal C-29 sterols is a 20-fold increase in the percentage of 24-methylene sterols which are thought to be substrates for the second transmethylation.

TABLE 2. QUANTITATION OF STEROLS FROM UNTREATED AND TRIPARANOL-TREATED CULTURES OF *Chlorella emersonii*

	Untreated		Triparanol-treated	
Sterols	% of sample	μg/g dry wt	% of sample	μg/g dry wt
24-Methylenecycloartanol	0·1	3	3·6	59
24-Dihydroobtusifoliol	t	t	0·1	2
4α,14α-Dimethyl-(24*S*)-5α-stigmast-8-en-3β-9l	t	t	t	t
14α-Methyl-5α-ergost-8-en-3β-ol	1·0	29	7·1	117
14α-Methyl-(24*S*)-5α-stigmast-8-en-3β-ol	0·0	0	0·4	7
14α-Methyl-5α-ergosta-8,24(28)-dien-3β-ol	0·0	0	2·9	48
Obtusifoliol	0·8	23	3·4	56
Cycloeucalenol	0·1	3	0·5	8
24-Methylenepollinastanol	0·0	0	9·3	153
Δ^7-Ergostenol	15·3	440	13·8	227
Δ^7-Chondrillastenol	6·9	199	9·6	158
Chondrillasterol	75·5	2180	47·0	770
5α-Ergosta-7,22-dien-3β-ol	0·1	6	1·0	16
(24*S*)-5α-Stigmasta-7,25-dien-3β-ol	0·0	0	t	t
Total	99·8	2883	98·7	1621

One of the more interesting and surprising effects attributable to triparanol was the 14-fold increase in the percentage of sterols with a methyl group at C-14. This would suggest that triparanol affects the biosynthesis of sterols at a site other than in the side chain. Triparanol has been reported to inhibit cholesterol biosynthesis between lanosterol and zymosterol in vertebrates.[16] In treated cultures the presence of nearly 4% of the total sterols as 24-methylenecycloartanol suggests that the effects of triparanol on sterol biosynthesis in *C. emersonii* extend even farther back than the 14-methyl sterol stages. Thus, although triparanol appears to exert an effect at several sites in phytosterol biosynthesis, the

[13] J. AVIGAN, D. STEINBERG, H. E. VROMAN, M. J. THOMPSON and E. MOSETTIG, *J. Biol. Chem.* **235**, 3123 (1960).
[14] J. A. SVOBODA and W. E. ROBBINS, *Science* **156**, 1937 (1967).
[15] R. J. COLE and L. R. KRUSBERG, *Life Sci.* **7**, 713 (1968).
[16] W. L. HOLMES and N. W. DITULLIO, *Am. J. Clin. Nutr.* **10**, 310 (1962).

mechanism of action is unclear. Further definitive work will be necessary before it will be possible to show the exact sites of triparanol action in *C. emersonii.*

The study with triparanol has been very useful in pointing to what may well be a pathway for phytosterol biosynthesis in *C. emersonii.* Isolation and identification of seven sterols new to science (III, IV, X, XI, XII, XIV, XV), from triparanol-treated cultures of *C. emersonii,* suggest a sterol biosynthetic pathway that deviates in the last steps from that postulated for higher plants.[17,18]

In the biosynthesis of phytosterols in most higher plants, it is quite evident, from isolations of 4α-methyl sterols, that demethylation of the 14α-methyl precedes demethylation of 4α-methyl group. In *C. emersonii,* demethylation at the C-4 position procedes demethylation of the 14α-methyl as is indicated by our finding of a large number of 14α-methyl sterols and 4,14-dimethyl sterols in both untreated and triparanol-treated cultures of *C. emersonii,* while no 4-methyl sterols without an accompanying 14-methyl were detected. The occurrence of a sterol lacking the 4-methyl but containing the 14-methyl has also been noted in tobacco where pollinastanol is an intermediate in cholesterol biosynthesis.[19]

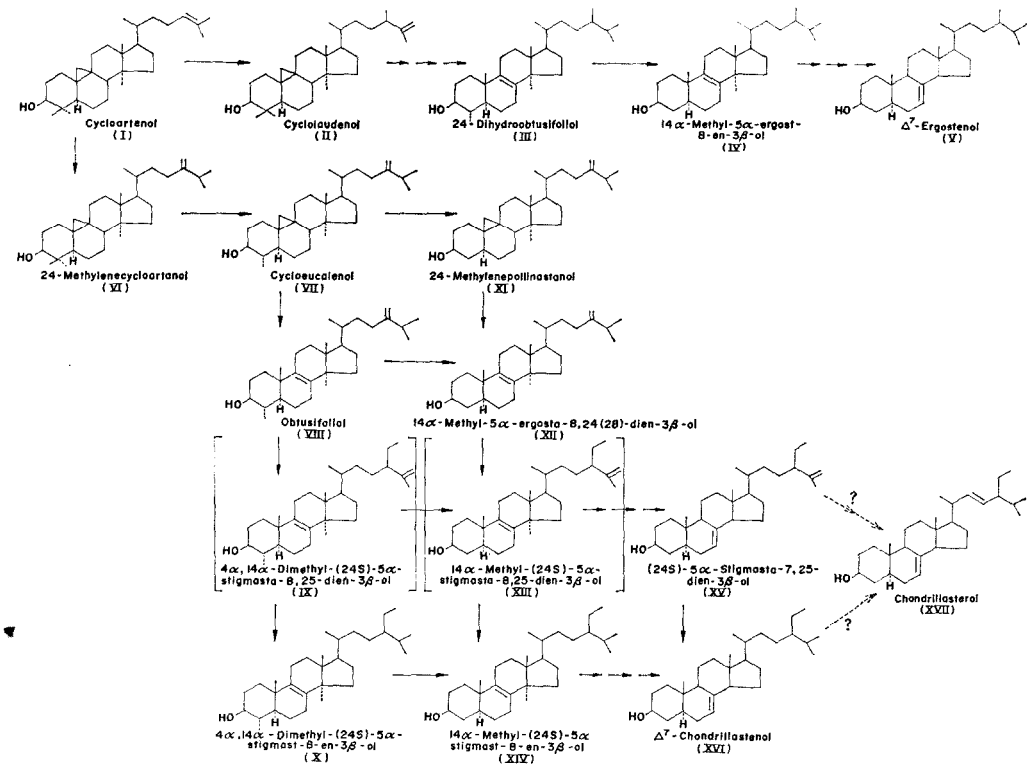

FIG. 6. A PROPOSED PATHWAY OF STEROL BIOSYNTHESIS IN *Chlorella emersonii.*

We have attempted to incorporate our results into a proposed scheme of sterol biosynthesis in *C. emersonii,* although other transformations within the scheme are possible. All sterols in Fig. 6 except I, II, IX and XIII, were identified in *C. emersonii.* Identification of 24-methylenecycloartanol (VI), cycloeucalenol (VII), and obtusifoliol (VIII) in triparanol-treated and untreated cultures of *C. emersonii* indicates that sterol biosynthesis, immediately

after the cyclization of squalene, is similar to that presently postulated in higher plants.[17] Cycloartenol (I) and 24-methylenecycloartanol (VI) have been found in other algae—*Ochromonas danica*, *Ochromonas malhamensis*, *Enteromorpha linza*, *Ulva lactuca*, and *Fucus spiralis*.[20] Failure to detect cycloartenol (I) in *C. emersonii* is not surprising, since it is not isolated from plants nearly as often nor in as large quantities as 24-methylenecycloartanol.

Even though we did not detect cycloartenol (I) in *C. emersonii*, it is shown in our scheme as a logical precursor to the 24-methylene sterols and to the naturally occurring Δ^7-C_{28} and Δ^7-C_{29}-sterols of *C. emersonii*—viz. Δ^7-ergostenol (V) and Δ^7-chondrillastenol (XVI). Tomita *et al.*,[21] from their study with *Chlorella vulgaris* (now called *Chlorella emersonii*[22]), grown in the presence of (CD_3)-methionine, concluded that a 24-methylene sterol is not involved in biosynthesis of the 24-methyl sterol, Δ^7-ergostenol, nor are 24-ethylidene sterols precursors for 24-ethyl sterols in *C. emersonii*. This is a valid conclusion provided that methionine is the only methyl donor in the alkylation process and that this is the only method of alkylating at C-24 in this alga. It is possible that there are other pathways to C-24 methyl- or ethyl-substituted sterols.

Cyclolaudenol (II), a C_{31} sterol isolated from the fern *Polypodium vulgare* L.[23] but not identified in this work, could occupy a link between cycloartenol (I) and 24-dihydroobtusifoliol (III) in the biosynthesis of Δ^7-ergostenol (V) in *C. emersonii*. Removal of the 4α-methyl of III would yield 14α-methyl-5α-ergost-8-en-3β-ol which, upon demethylation at C-14 accompanied by a shift in the Δ^8-bond, would yield Δ^7-ergostenol. We have not included any 24-methylene sterols as precursors to 24-methyl sterols, although it is possible that 14α-methyl-5α-ergosta-8,24(28)-dien-3β-ol (XIII) could be readily converted to Δ^7-ergostenol by *C. emersonii*.

Biosynthesis of the two most common naturally-occurring sterols with 10-carbon side chains in *C. emersonii*, Δ^7-chondrillastenol and chondrillasterol, differs from the scheme just proposed for sterols with 9-carbon side chains. It appears that 24-methylene sterols are intermediates in the biosynthesis of Δ^7-chondrillastenol (XVI) and chondrillasterol (XVIII). The two sterols in brackets (XIII and IX) have not been identified in any organism, but are proposed as possible intermediates in the conversion of 24-methylene sterols to 24-ethyl sterols. This obviates the involvement of 24-ethylidene intermediates which are not considered by Tomita *et al.*[21] to be involved in the biosynthesis of Δ^7-chondrillastenol and chondrillasterol. In *Nicotiana*, a $\Delta^{24(25)}$ double bond is postulated to be formed as a result of the second alkylation reaction[24] of the side chain, but the occurrence of Δ^{25} compounds in several other plants,[25,26] including *Chlorella emersonii*, leads us to believe that a Δ^{25} compound may be formed during the second alkylation reaction in some plants.

In addition to the identification of 24-methylenecycloartanol (VI), cycloeucalenol

[17] L. J. Goad and T. W. Goodwin, *Europ. J. Biochem.* **1**, 357 (1967).
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(VII), and obtusifoliol (VIII) in increased concentrations from triparanol-treated *C. emersonii*, 4α,14α-dimethyl (24*S*)-5α-stigmasta-8-en-3β-ol (X) was found in small amounts in both triparanol-treated and untreated cultures. Loss of the 4-methyl group of VII, VIII, and X produces 24-methylenepollinastanol (XI), 14α-methyl-5α-ergosta-8,24(28)-dienol (XII), and 14α-methyl (24*S*)-5α-stigmasta-8-en-3β-ol (XIV), respectively. Each of these three sterols was identified in the triparanol-treated cultures of *C. emersonii*, but not in the untreated cultures. A possible explanation for this observation could be that, in *C. emersonii*, the second alkylation reaction normally occurs at the obtusifoliol stage. Inasmuch as this alkylation appears to be inhibited by triparanol, obtusifoliol and cycloeucalenol would accumulate. These two sterols may then lose the 4α-methyl to yield 14α-methyl-5α-ergosta-8,24(28)-dien-3β-ol and 24-methylenepollinastanol, respectively. Although 24-methylenepollinastanol can be converted to 14α-methyl-5α-ergosta-8,24(28)-dien-3β-ol, the latter is prevented from further reaction by the postulated site of triparanol inhibition discussed earlier—namely, inhibition of removal of the 14-methyl group.

Identification of (24*S*)-5α-stigmasta-7,25-dien-3β-ol in triparanol-treated cultures of *Chlorella emersonii* is interesting, and its occurrence is supporting evidence of the possible intermediacy of the postulated compounds (IX and XIII) in sterol biosynthesis.

Introduction of a 22(23) double bond into the side chain of Δ^7-chondrillastenol (XVI) of (24*S*)-5α-stigmasta-7,25-dien-3β-ol (XV), plus reduction of the 25(26) double bond of the latter sterol, would yield chondrillasterol (XVII). The consistent failure to isolate any Δ^{22}-sterols, except chondrillasterol and 5α-ergosta-7,22-dien-3β-ol, indicates that the 22,23 double bond is introduced at a late stage of sterol biosynthesis.

EXPERIMENTAL

M.ps were obtained with a Fisher–Johns apparatus and are corrected. MS were recorded on an LKB Model 9000 gas chromatograph–mass spectrometer. The compounds were introduced into the ion chamber through a 0·75% SE-30 column. Ionization energy was 70 eV. MS were corrected for background. GLC analyses were made on Glowall Chromalab, Model A-110 and Model A-310 gas chromatographs equipped with argon ionization detectors and a Honeywell 12-in. recorder. Column packings were prepared as described previously.[1]

Cells of *Chlorella emersonii* var. *emersonii* Shihira and Krauss, Maryland Culture Collection No. 2, were grown on basal inorganic medium supplemented with 0·5% glucose as described previously.[1] Triparanol-treated cells were grown under identical conditions except for the addition of 5 ppm of triparanol to the culture medium. Average yield in the control cultures was 2·6 g/l. (dry wt) and in the treated cultures, 1·2 g/l. 390 g of untreated cells and 900 g of treated cells were extracted with $CHCl_3$–MeOH (2:1, v/v) after being lyophilized. After saponification, sterols were precipitated from the non-saponifiable fraction with digitonin, and the sterols were recovered by the method of Issidorides *et al.*[27] Total free sterols were separated into dimethyl-, methyl-, and desmethyl sterols on an alumina column.[1] After acetylation, sterol acetates were further separated by $AgNO_3$–silica gel column chromatography by the method of Vroman and Cohen.[3]

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Key Word Index—Chlorella emersonii; Chlorophyta; biosynthesis; sterols; triparanol.