Phytochemistry, 1980, Vol. 19, pp. 783–785. Pergamon Press Ltd. Printed in England.

EFFECT OF CULTURAL CONDITIONS ON THE STEROLS AND FATTY ACIDS OF GREEN ALGAE*

DAN C. WRIGHT, LINDA R. BERG and GLENN W. PATTERSON

Department of Botany, University of Maryland, College Park, MD 20742, U.S.A.

(*Received* 29 *June* 1979)

Key Word Index—Green algae; sterols; fatty acids; autotrophic; photoheterotrophic; heterotrophic growth.

Abstract—Six species of green algae were grown autotrophically, photohetrotrophically, and heterotrophically and their fatty acid and sterol compositions determined. Sterol composition was higher in autotrophic than in heterotrophic plants by a factor of from 2 to over 20 in five of the six species studied. Relative amounts of various sterols did not change significantly with cultural conditions. In five of the species studied, autotrophic growth produced a significant increase in the relative proportion of linolenic acid compared to that in heterotrophic or photoheterotrophic growth. This increase was usually accompanied by a corresponding decrease in oleic or linoleic acids or both.

INTRODUCTION

Many green algae are capable of growing both autotrophically and heterotrophically. There have been several reports of the effects on fatty acid composition of growing green algae heterotrophically, photoheterotrophically, and autotrophically [1–4]. Generally, there is a significant decrease in the amount of polyunsaturated fatty acids under conditions of heterotrophism [1–3]. The decrease in linolenic acid is especially notable. These results are in agreement with data on the leaves of higher plants [2]. In a study of chloroplasts fatty acids of light- and dark-grown wheat seedlings, it was determined that both the total fatty acid and polyunsaturated fatty acids were lower in the dark-grown wheat [5]. Although the results in similar studies are well documented [6–8], the specific role of polyunsaturated fatty acids in photosynthesis remains in question.

In a number of studies on the differences between green and etiolated higher plants, a direct relationship emerges between the amount of photosynthetic activity and the rate of sterol synthesis [9]. Both green and etiolated seedlings produce sterols, and reports thus far indicate there is no difference in the kind of sterols they produce. The etiolated plants simply do not produce as much sterol. These generalizations have been substantiated in bean seedlings [10] and radish seedlings [11]. In a different study of sterols in bean leaves, there was little difference between etiolated and light-grown leaves, except the percentage of cholesterol was somewhat higher in the etiolated leaves [12].

Numerous studies have been conducted with *Euglena gracilis*. When cultured autotrophically it is green, while under conditions of complete heterotrophism it is white. One research group reported that the sterol composition of white *E. gracilis* was different from that of green *E. gracilis* [13]. In a subsequent study they determined that the precusors of sterol synthesis were also different quantitatively and qualitatively due to the presence or absence of light [14]. However, an investigation of *Euglena* mutant strains found no difference in sterol content regardless of growth conditions. These mutant strains produced ergosterol in the light or dark [15]. Studies concerning the effect of environmental conditions on sterol composition of green algae have not been conducted.

The work described here was designed to determine if any patterns could be discerned in both fatty acid and sterol composition due to differences in cultural conditions. Six different species of green algae were used because of the physiological variability within the group [16].

RESULTS AND DISCUSSION

When grown heterotrophically, all six algae had less total sterol than when grown autotrophically or photoheterotrophically (Table 1). All six algae except *B. cinnabarinus* had less total lipid when grown heterotrophically than when grown autotrophically or photoheterotrophically. Except for *C. ellipsoidea* and *A. braunii* these algae had more total fatty acid when grown photoheterotrophically than autotrophically or heterotrophically. These data suggest that the amount of total lipid and sterol of these algae is determined to a greater extent by the availability of light and/or photosynthesis than the availability of organic nutrients such as glucose. However, the percentage of total fatty acid of these algae is determined by the availability of organic nutrients such as glucose in addition to light and/or photosynthesis.

The percentages of individual sterols in the algae shown in Table 2 show little change from one growth condition to the next. The percentage of any particular sterol changes less than 5% between the different growth conditions for the different algae in all cases except *B. cinnabarinus* in which the percentage of chondrillasterol increased from 70.1% in autotrophic growth conditions to 81.5% in heterotrophic growth

*Scientific Article No. A2587, Contribution No. 5626 of the Maryland Agricultural Experiment Station.

conditions and the percentage of chondrillast-7-enol decreased from 15.8 to 5.1% correspondingly. Since algae grown either autotrophically or heterotrophically synthesize sterols at almost identical percentages relative to one another (Table 2), the inhibition of sterol biosynthesis due to heterotrophic growth conditions must occur prior to the formation of cycloartenol. Since no sterol intermediates accumulate (Table 2), desaturations and demethylations of the sterol molecule are not inhibited by heterotrophic growth conditions.

The changes in the percentage of different fatty acids shown in Table 3 are complex. In all six algae except *S. quadricauda*, the percentage of linolenic acid was less when grown heterotrophically than when grown autotrophically. However, this decrease in the percentage of linolenic acid was not correlated with a decrease in total unsaturation as evidenced by changes in the ratio of saturated to unsaturated fatty acids (*s/u*). The *s/u* ratio increased only in *O. marsonii* and *S. quadricauda* out of the six algae studied. Instead, the decrease in linolenic acid was correlated with an increase in oleic acid for all six algae except *S. quadricauda* where the increase in oleic acid was correlated with a decrease in linoleic acid. The results suggest that heterotrophic growth conditions inhibit the synthesis of polyunsaturated fatty acids from monounsaturated fatty acids and have little effect on the ratio of saturated to unsaturated fatty acids.

Algae grown autotrophically have more total sterol and polyunsaturated fatty acids than algae grown heterotrophically. Biosynthesis of chloroplast membranes or availability of a photosynthetic product such as oxygen or fixed carbon could account for the stimulation of sterol and polyunsaturated fatty acid biosynthesis in autotrophically grown algae. Neither of these two explanations is discounted by the results of this investigation. The possibility that the availability of a photosynthetic product such as oxygen could be limiting in the biosynthesis of sterols or polyunsaturated fatty acids is a particularly interesting hypothesis, since oxygen is required for sterol biosynthesis [17] and the synthesis of polyunsaturated fatty acids [18, 19]. However, measurements of actual oxygen availability were not determined for this investigation. In future investigations involving sterol or polyunsaturated fatty acid biosynthesis, the availability of oxygen or other photosynthetic products should be monitored.

Table 1. Lipid composition of six green algae grown autotrophically, photoheterotrophically and heterotrophically*

Species	GC†	% Lipid	% Sterol	%Fatty acid
Ankistrodesmus braunii	A	27.5	0.23	4.0
	P	13.9	0.21	3.1
	H	17.7	0.01	4.2
Bracteococcus cinnabarinus	A	18.7	0.20	3.7
	P	23.9	0.14	12.2
	H	27.8	0.08	9.6
Chlorella ellipsoidea	A	9.1	0.15	2.1
	P	6.6	0.05	1.4
	H	6.0	0.01	2.0
Chlorella emersonii	A	21.2	0.31	7.3
	P	21.0	0.20	9.5
	H	17.2	0.13	3.6
Oocystis marsonii	A‡	7.7	0.09	0.9
	P	13.5	0.32	3.5
	H‡	8.2	0.08	2.2
Scenedesmus quadricauda	A	15.5	0.22	2.8
	P	14.0	0.16	3.5
	H	12.4	0.10	2.2

*Average percentage of dry wt of three samples expressed as % of dry wt.

†GC = growth conditions, A = autotrophic, H = heterotrophic, P = photoheterotrophic.

‡Failed to grow well under these conditions.

Table 2. Sterol composition of six green algae grown autotrophically, photoheterotrophically and heterotrophically*

Species	Sterol	A†	P	H
Ankistrodesmus braunii	Unidentified	—	0.1	—
	Ergost-7-enol	20.7	19.2	20.7
	Chondrillasterol	55.0	52.4	54.8
	Chondrillast-7-enol	23.0	28.4	24.2
	Unidentified	0.4	—	—
Bracteococcus cinnabarinus	Unidentified	0.1	0.3	0.2
	Ergost-7-enol	14.2	17.2	13.0
	Chondrillasterol	70.1	75.1	81.5
	Chondrillast-7-enol	15.8	7.4	5.1
	Unidentified	—	—	0.5
Oocystis marsonii	Unidentified	—	0.7	—
	Ergost-5-enol	26.5	29.6	30.7
	Portiferasterol	68.8	64.4	65.0
	Clionasterol	3.9	5.3	4.4
Scendesmus quadricauda	Unidentified	0.5	1.6	0.8
	Ergost-7-enol	18.7	22.6	24.0
	Chondrillasterol	75.9	66.7	72.6
	Chondrillast-7-enol	4.7	10.0	2.6
	Unidentified	0.2	—	—
Chlorella ellipsoidea	Cholesterol	4.8	3.1	22.9
	Barassicasterol	2.6	4.5	3.2
	Ergost-5-enol	26.9	26.4	22.8
	Poriferasterol	56.0	54.6	64.1
	Clionasterol	7.6	7.8	3.3
	Unidentified	2.2	3.5	2.3
Chlorella emersonii	Ergosta-7,22-dienol	1.6	1.6	1.8
	Ergost-7-enol	18.5	17.5	15.9
	Chondrillasterol	69.1	66.6	75.4
	Chondrillast-7-enol	10.8	14.3	7.0

*Average percentages based on three determinations expressed as % of total sterol.

†A = Autotrophic conditions, P = photoheterotrophic conditions, H = heterotrophic conditions.

EXPERIMENTAL

Six green algae were grown under 3 different growth conditions: continuous light with inorganic medium, continuous light with glucose supplemented medium, and continuous darkness with glucose supplemented medium. These growth conditions are referred to as autotrophic, photoheterotrophic, and heterotrophic growth conditions, respectively. Algal species chosen for investigation were *Ankis-*

Table 3. Fatty acid composition of six green algae grown autotrophically, photoheterotrophically and heterotrophically*

Species	GC†	14:2	15:0	16:0	16:1	16:2	16:3	16:4	17:0	18:0	18:1	18:2	18:3	s/u
Ankistrodesmus	A	4.2	tr	27.1	tr	5.6	4.5	—	tr	tr	23.6	17.6	10.2	0.45
braunii	P	3.8	tr	30.4	tr	2.4	tr	—	tr	2.6	27.0	11.4	16.7	0.53
	H	2.9	tr	23.1	tr	4.6	2.0	—	tr	2.9	39.9	15.0	9.0	0.37
Bracteococcus	A	tr	tr	19.2	tr	16.0	tr	—	2.0	tr	20.0	18.0	20.8	0.27
cinnabarinus	P	tr	—	16.1	2.9	5.3	—	—	tr	3.9	32.7	27.3	9.2	0.26
	H	tr	tr	16.9	tr	3.1	—	—	tr	2.4	52.0	15.2	2.1	0.26
Chlorella	A	—	2.7	27.9	tr	tr	5.1	—	tr	tr	13.5	22.0	20.1	0.55
ellipsoidea	P	—	tr	38.4	—	tr	tr	—	3.6	tr	17.2	22.4	15.6	0.72
	H	—	tr	21.7	—	tr	—	—	—	3.5	30.3	33.2	9.2	0.36
Chlorella	A	—	4.7	31.9	tr	tr	5.3	10.1	2.8	—	12.2	5.1	25.3	0.65
emersonii	P	—	—	27.4	tr	tr	2.3	tr	tr	2.0	47.0	6.9	9.7	0.42
	H	—	tr	33.6	2.2	2.1	9.0	tr	2.4	tr	25.2	8.6	13.5	0.56
Ocystis	A	tr	tr	19.6	—	tr	—	—	tr	tr	19.7	24.8	30.5	0.27
marsonii	P	tr	tr	41.4	tr	tr	—	—	tr	tr	31.6	11.4	8.1	0.83
	H‡	—	tr	37.2	tr	2.9	—	—	tr	12.9	35.8	5.6	2.6	1.04
Scenedesmus	A	6.5	tr	19.6	5.7	8.8	2.8	—	tr	tr	26.6	18.4	8.7	0.27
quadricauda	P	4.3	tr	34.7	4.6	4.6	tr	—	tr	tr	27.9	11.9	8.1	0.60
	H	2.2	tr	29.2	3.9	2.4	3.2	—	tr	2.1	36.2	5.9	13.1	0.46

*Average percentages based on three determinations, expressed as % of total fatty acid.

†GC = Growth conditions, A = autotrophic, P = photoheterotropic, H = heterotrophic, s/u = total saturated fatty acid divided by total unsaturated fatty acid.

‡Failed to grow well under these conditions.

trodesmus braunii (naeg.) Collins (ICC#245), *Bracteococcus cinnabinus* (Kol et F. Chad) staff (ICC #56), *Chlorella ellipsoidea* Gerneck (ICC #247), *Chlorella emersonii* Shihira et Krauss (MCC #2), *Oocystis marsonii* Lemm. (ICC #287), and *Scenedesmus quadricauda* (Turp.) Breb (ICC #77). *Chlorella* species were grown in 500 ml glass tubes each containing 350 ml autoclaved medium. Other algal species were grown in 20 l. glass carboys containing 15 l. autoclaved medium. The medium contained 10 mM KNO_3, 2 mM K_2HPO_4, 5 mM KH_2PO_4, 1 mM $MgSO_4$, and 7 mM MoO_3; 5 μg/ml Fe, and 1 μg/ml of Ca, Mn, Co, Zn, and Cu chelated with EDTA were also added to the medium. Heterotrophic and photoheterotrophic growth medium also contained 0.5% glucose. The algal cultures were incubated at 27° and constantly bubbled with 1% CO_2 in air. Light was supplied by General Electric F48PG17°CW power grove cool white lamps at an intensity of 8000 lx. When algal cultures reached $A > 0.6$ as measured in an 18×150 mm tube in a spectrophotometer, algae were harvested by centrifugation and immediately frozen. Each sample of frozen cells was freeze-dried. Lipids were extracted by refluxing $CHCl_3$-MeOH (2:1) through the sample in a Soxhlet apparatus. Crude lipid extract obtained was then flash-evapd, resuspended in $CHCl_3$ and filtered. $CHCl_3$ was evapd and the total sample lipid weight determined. Total lipid was saponified with KOH in 70% EtOH, extracted with Et_2O in a liquid–liquid extractor for 24 hr, dried, and refluxed with 10% BCl_3 in MeOH for 5 min to methylate free fatty acids in the sample. The methylated lipid sample was partitioned into hexane. Sterols were separated from fatty acids on an alumina column and purified further by digitonin precipitation [12]. Fatty acids and sterols were tentatively identified and quantitated by GLC. Identification of fatty acids was achieved by a combination of argentation TLC [14] and GLC data.

REFERENCES

1. Dickson, L., Galloway, R. and Patterson, G. (1969) *Plant Physiol.* **44**, 1413.
2. Nichols, B. (1965) *Biochim. Biophys. Acta* **106**, 274.
3. Nichols, B., James, A. and Bruer, J. (1967) *Biochem. J.* **104**, 486.
4. Reitz, R. and Hamilton, J. (1967) *Lipids* **2**, 381.
5. Wolf, F., Coguilio, J. and Brides, R. (1966) in *Biochemistry of Chloroplasts* (Goodwin, T. W. ed.), pp. 187–194. Academic Press, New York.
6. Hitchcock, C. and Nichols, B. (1971) *Plant Lipid Biochemistry*, pp. 31–32, 76. Academic Press, New York.
7. Tremolieres, A. (1970) *Annee Biol.* **9**, 113.
8. Wood, B. (1974) in *Algal Physiology and Biochemistry*, (Stewart, W. D. P. ed.) pp. 236–265. University of California Press, Los Angeles.
9. Grunwald, C. (1975) *Annu. Rev. Plant Physiol.* **26**, 209.
10. Duperon, P. and Duperon, R. (1969) *C. R. Acad. Sci. Paris* **268**, 306.
11. Duperon, P. (1968) *C. R. Acad. Sci. Paris* **266**, 1658.
12. Brandt, R. D. and Benveniste, P. (1972) *Biochim. Biophys. Acta* **282**, 85.
13. Brandt, R. D., Ourisson, G. and Pryce, R. (1969) *Biochem. Biophys. Res. Commun.* **37**, 399.
14. Anding, C., Brandt, R. and Ourisson, G. (1971) *Eur. J. Biochem.* **25**, 259.
15. Stern, A., Schiff, J. and Klein, H. (1960) *J. Protozool.* **7**, 52.
16. Shihira, I. and Krauss, R. (1965) *Chlorella*, pp. 25, 30, 34. Port City Press, Baltimore.
17. Tchen, T., and Bloch, K. (1957) *J. Biol. Chem.* **266**, 921.
18. Yuan, C. and Bloch, K. (1961) *J. Biol. Chem.* **236**, 1277.
19. Harris, P. and James, A. (1969) *Biochim. Biophys. Acta* **187**, 13.