

## FATTY ACID COMPOSITION OF SOME ALGAE FROM THE BLACK SEA

K. STEFANOV, M. KONAKLIEVA, E. Y. BRECHANY\* and W. W. CHRISTIE\*†

Institute of Organic Chemistry, Centre of Phytochemistry, Bulgarian Academy of Sciences, 1040 Sofia, Bulgaria, \*The Hannah Research Institute, Ayr, Scotland KA6 5HL, U.K.

(Received in revised form 21 March 1988)

**Key Word Index**—Rhodophyceae; Phaeophyceae, Chlorophyceae, marine algae, fatty acids, HPLC, GC/MS

**Abstract**—The fatty acid compositions were determined of two marine algae of the Rhodophyceae, one of the Phaeophyceae and four of the Chlorophyceae, from the Black Sea in Bulgaria. The results may be of value for taxonomic purposes.

### INTRODUCTION

As with all organisms, fatty acids are important constituents of algae, where they are located principally in the cellular membranes. They have many other functions, and it has been reported, for example, that the unsaturated fatty acids from a red alga, *Porphyra* sp., have pronounced antifungal activity [1]. It is evident that different species have very different fatty acid compositions, which may have value for taxonomic purposes. The fatty acid compositions of algae in general [2] and marine algae in particular have been reviewed [3]. Data on algal species from the Black Sea in Bulgaria are sparse and information on species from the Rhodophyceae, Phaeophyceae and Chlorophyceae is presented here.

### RESULTS AND DISCUSSION

The formation of fatty acids in algae is controlled by environmental factors, such as the growth conditions (e.g. light, temperature and the availability of nutrients), and is dependent on the growth phase and age. In places with more light, production of unsaturated  $C_{16}$  and  $C_{18}$  fatty acids increases [4–7]. The temperature optimum for the biosynthesis of polyunsaturated fatty acids is ca 15° [8]. The concentration of  $Mn^{2+}$  [9] and of nitrogen [10, 11] can also have an effect. None of these factors can be quantified with samples harvested in the wild, so caution must be exercised in comparing relative proportions of individual components in related organisms from different sources. However, the nature of the fatty acids found should not change appreciably. In this study, fatty acids were identified by mass spectrometric procedures that permitted unequivocal identifications even of positional isomers, and not simply by GC  $R_f$  values as in many comparable studies. In brief, the Me ester derivatives of fatty acids were quantified by capillary GC; then they were separated according to degree of unsaturation by high-performance liquid chromatography (HPLC) with a silver ion column, and the fractions were converted to the

picolinyl ester derivatives for identification by GC-MS [12]. The data obtained are listed in Table 1.

Two species of Rhodophyceae were investigated, viz. *Corallina granifera* and *Phyllophora nervosa*. Red algae in general are known to contain appreciable amounts of  $C_{20}$  polyunsaturated fatty acids, mainly 20:4 ( $n-6$ ) and 20:5 ( $n-3$ ), with relatively small amounts of  $C_{16}$  and  $C_{18}$  polyunsaturated components [3, 11, 13, 14]. This pattern was seen in the species examined here, except that 20:5 ( $n-3$ ) was the most abundant fatty acid in *Corallina granifera*, while 20:4 ( $n-6$ ) (more often associated with animal lipids as a precursor of the prostaglandins) was by far the most abundant component of *Phyllophora nervosa*. There was much more 20:5 ( $n-3$ ) in the lipids of *C. officinalis* from the Firth of Clyde (Scotland) [15], *C. pilulifera* from the Sea of Japan [11], and *C. tenella* from the Red Sea [14]. In the species examined here, a number of minor components were identified, including odd-chain and branched-chain fatty acids and positional isomers of the unsaturated constituents.

In the sample of *Corallina granifera*, appreciable amounts of plasticisers were found (which eluted with the saturated fraction from the silver ion column). There are numerous reports of phthalate esters in natural products [16], and di-(2-methyl-hexyl)-phthalate made up a significant part of the lipid fraction in *Ceramium rubrum* (also from the Rhodophyceae) from New Hampshire (U.S.A.) [17]. None was present in any of the other samples, so accidental laboratory contamination appears improbable.

The fatty acids of one species of the Phaeophyceae, *Cystoseira barbata*, which is the most widespread brown algae in the Black Sea, were identified. They comprised appreciable amounts of  $C_{16}$  and  $C_{18}$  saturated and unsaturated components, together with 20:4 ( $n-6$ ) and 20:5 ( $n-3$ ). In general, the Phaeophyceae have high concentrations of  $C_{18}$  (18:3, 18:4),  $C_{20}$  (20:4, 20:5) and  $C_{22}$  (22:5, 22:6) fatty acids (the last were not detected here), but the compositions appear to be rather variable [3, 11, 14, 15, 18, 19]. *Cystoseira myrica* from the Red Sea contained more saturated fatty acids, but otherwise the composition was similar to that reported here [14].

The fatty acid compositions of many different macroscopic marine green algae have been determined. In this

†Author to whom correspondence should be addressed.

Table 1 Fatty acid composition of some algae from the Black Sea (wt% of total)

| Fatty acid            | Rhodophyceae               |                            | Phaeophyceae              |                    | Chlorophyceae        |                           |                 |
|-----------------------|----------------------------|----------------------------|---------------------------|--------------------|----------------------|---------------------------|-----------------|
|                       | <i>Corallina granifera</i> | <i>Phyllophora nervosa</i> | <i>Cystoseira barbata</i> | <i>Ulva rigida</i> | <i>Cladophora</i> sp | <i>Bryopsis hypnoides</i> | <i>Chara</i> sp |
| 14:0                  | 4.5                        | 2.2                        | 5.4                       | 0.6                | 9.3                  | 0.6                       | 0.8             |
| 14:1 (n-7)            | --                         | --                         | --                        | --                 | --                   | --                        | 0.1             |
| 4,8,12-trimethyl-13:0 | 0.5                        | --                         | --                        | --                 | --                   | 0.2                       | --              |
| iso 14:0              | 0.5                        | 0.8                        | --                        | --                 | 0.1                  | 0.1                       | --              |
| 15:0                  | 0.8                        | 0.3                        | --                        | --                 | 0.1                  | --                        | 0.1             |
| 16:0                  | 38.6                       | 26.2                       | 15.3                      | 4.9                | 17.2                 | 15.0                      | 9.7             |
| 16:1 (n-9)            | 0.3                        | 0.1                        | --                        | 0.4                | 0.8                  | 0.8                       | 0.1             |
| 16:1 (n-7)            | 3.0                        | 1.9                        | 2.7                       | 2.3                | 8.6                  | 1.4                       | 5.0             |
| 16:1 (n-5)            | 0.4                        | 0.4                        | --                        | --                 | --                   | --                        | 0.1             |
| 16:2 (n-6)            | 0.1                        | 0.2                        | --                        | --                 | 0.6                  | 0.6                       | 1.5             |
| 16:2 (n-4)            | 0.2                        | --                         | 0.1                       | 0.6                | 0.6                  | 0.1                       | 0.4             |
| 16:3 (n-3)            | 0.5                        | 0.3                        | 0.1                       | 11.1               | --                   | 14.7                      | 8.5             |
| 16:4 (n-3)            | 0.6                        | --                         | --                        | 1.7                | 9.2                  | --                        | --              |
| iso 16:0              | --                         | 0.1                        | --                        | --                 | 1.1                  | 1.5                       | --              |
| 10-methyl 16:0        | --                         | 0.1                        | 0.1                       | --                 | --                   | --                        | --              |
| 17:0                  | --                         | 0.7                        | --                        | --                 | --                   | 0.1                       | 0.1             |
| 17:1 (n-8)            | 0.1                        | 0.3                        | --                        | --                 | --                   | --                        | --              |
| 18:0                  | 0.6                        | 1.1                        | --                        | 0.3                | 0.1                  | 0.5                       | 0.5             |
| 18:1 (n-9)            | 6.8                        | 7.0                        | 20.8                      | 1.4                | 10.6                 | 2.6                       | 2.2             |
| 18:1 (n-7)            | 2.5                        | 2.1                        | 0.3                       | 3.0                | 3.6                  | 6.9                       | 0.6             |
| 18:2 (n-8)            | 0.1                        | --                         | --                        | --                 | 0.5                  | --                        | --              |
| 18:2 (n-6)            | 1.2                        | 0.9                        | 6.1                       | 7.5                | 6.3                  | 6.2                       | 10.2            |
| 18:3 (n-6)            | 0.2                        | 0.3                        | 0.8                       | 1.7                | --                   | --                        | 1.7             |
| 18:3 (n-3)            | 0.9                        | 2.1                        | 15.2                      | 42.6               | 17.3                 | 34.9                      | 29.3            |
| 18:4 (n-3)            | 1.0                        | 1.4                        | 8.4                       | 10.5               | 0.8                  | 2.1                       | 1.2             |
| 19:1 (n-8)            | 0.1                        | --                         | --                        | --                 | --                   | --                        | --              |
| 20:1 (n-11)           | 0.1                        | --                         | --                        | --                 | --                   | --                        | --              |
| 20:1 (n-9)            | 0.2                        | --                         | --                        | --                 | --                   | 0.2                       | --              |
| 20:2 (n-6)            | --                         | --                         | 0.1                       | --                 | --                   | --                        | 1.6             |
| 20:3 (n-9)            | --                         | --                         | 0.4                       | --                 | --                   | --                        | --              |
| 20:3 (n-6)            | 0.4                        | 0.3                        | 1.5                       | 0.4                | --                   | --                        | 2.3             |
| 20:3 (n-3)            | --                         | --                         | 0.1                       | --                 | --                   | --                        | 1.2             |
| 20:4 (n-6)            | 4.2                        | 44.3                       | 15.5                      | 2.2                | 0.8                  | 3.0                       | 9.9             |
| 20:4 (n-3)            | 0.2                        | --                         | 0.8                       | --                 | 0.5                  | 0.5                       | 1.5             |
| 20:5 (n-3)            | 30.3                       | 5.6                        | 5.6                       | 3.3                | 6.2                  | 3.9                       | 9.6             |
| 22:5 (n-3)            | --                         | --                         | --                        | 1.4                | 1.3                  | 0.1                       | --              |
| 22:6 (n-3)            | --                         | --                         | --                        | --                 | 0.3                  | 0.3                       | --              |

study, species of two common families of Chlorophyceae, viz. *Ulva rigida* and *Cladophora* sp. (Bul), *Bryopsis plumosa*, a unicellular macroalga which appears near the Bulgarian coast of the Black Sea in Spring primarily, and a *Chara* sp. (Bul) were investigated. In the lipids of *U. rigida*, 16:3 (n-3), 18:3 (n-3) and 18:4 (n-3) were the most abundant fatty acids and there were small amounts only of saturated and C<sub>20</sub> fatty acids. Comparable fatty acids were found in *U. lactuca* (Firth of Clyde, Scotland) [15], *U. fasciata* (Bay of Naples) [3], and *U. lactuca* and *U. reticulata* from the Red Sea [14], although there were higher relative proportions of saturated and tetraenoic fatty acids in these species. The fatty acids of the *Cladophora* sp. were mainly C<sub>16</sub> and C<sub>18</sub>, and resembled those reported for *C. rupestris* and *C. albidula* [15]. There was much less 18:4 (n-3) than in *C. heteronema* from the Red Sea [14]. The composition of the fatty acids of *Bryopsis hypnoides* resembled that of *Ulva rigida* in that 18:3 (n-3) was the most abundant fatty acid. In contrast, the fatty acids of the *Chara* sp. also contained a high proportion of

C<sub>20</sub> fatty acids, including 20:4 (n-6) a component not normally associated with green photosynthetic tissues.

Insufficient data are available for different genera for definitive conclusions to be drawn on the value of fatty acid analyses for taxonomic purposes, because environmental factors are superimposed on comparisons of algae from different parts of the world. On the other hand, there are strong similarities in the nature of the fatty acids found within genera although the relative proportions vary somewhat. In particular, the presence of arachidonic acid as an abundant component of particular species appears to be significant.

#### EXPERIMENTAL

All samples were collected in the summer of 1986 from the shores of the Black Sea near Sosopol in Bulgaria, except for *Bryopsis hypnoides* which was collected in the Spring. Lipids were extracted by homogenization with CHCl<sub>3</sub>-MeOH (2:1) and hydrolysed at room temp overnight with 1 M KOH in 95%

EtOH The non-saponifiable lipids were extracted with hexane-Et<sub>2</sub>O (1:1), the soln acidified, the free fatty acids extracted and methylated with MeOH containing 1% H<sub>2</sub>SO<sub>4</sub> [20]. They were stored in the presence of 2,6-di-*tert*-butyl-*p*-cresol (0.02%) to minimise oxidation. Samples extracted and methylated in Bulgaria were sealed under vacuum and sent to Scotland for analysis. They were purified on a small column of Florisil™, eluted with hexane-Et<sub>2</sub>O (9:1).

Analysis of the Me ester derivatives was carried out using split/splitless inj and a fused silica capillary column (25 m × 0.25 mm id) coated with Silar 5CP [12]. After holding the temp. at 155° for 3 min, it was temp. prog. at 4°/min to 195°, then held at this temp. for a further 17 min. H<sub>2</sub> was carrier gas. Components were quantified by electronic integration. Samples were analysed in duplicate.

For seps by HPLC, an ACS Model 750/14 mass detector (Applied Chromatography Systems) was used. A stream-splitter (ca 10:1) was inserted between the column and the detector. A column (4.6 × 250 mm) of Nucleosil™ SSA (HPLC Technology) was utilized in the Ag<sup>+</sup> form as described elsewhere [21]. Me esters (1–2 mg) were applied to the column in 10 µl of CH<sub>2</sub>Cl<sub>2</sub>. The column was eluted with a gradient of MeOH to MeOH-MeCN (9:1) over 40 min at a flow-rate of 0.75 ml/min [12].

After analysis of fractions by GC, they were hydrolysed to the free acids before conversion to the picolinyl ester derivatives as described in ref. [22], in brief, the mixed anhydride of each fatty acid with trifluoroacetic acid was reacted with 3-(hydroxymethyl)-pyridine (10-fold molar excess) in the presence of 4-dimethylaminopyridine (1:2 molar proportion) as catalyst. The derivatives were submitted to GC/MS on a fused-silica capillary column (25 m × 0.2 mm id), coated with a cross-linked (5% phenylmethyl) silicone (Hewlett Packard Ltd, Wokingham, Berks), with He as carrier gas and temp. programming from 60 to 220° at 50°/min then to 250° at 1°/min [23]. The column outlet was connected directly into the ion source of a mass selective detector operated at an ionization energy of 70 eV. Full details of the use of picolinyl esters for location of double bonds, and other structural features in fatty acids, are published in refs [12, 22–27].

## REFERENCES

- Noda, H., Amano, H., Kato, S. and Ohta, F. (1983) *Nippon Suisan Gakkaishi* **49**, 1583.
- Wood, B. J. B. (1974) in *Algal Physiology and Biochemistry* (Bot. Monogr. **10**) (Stewart, W. D. P., ed.) p. 236.
- Pohl, P. and Zurheide, F. (1979) *Marine Algae in Pharmaceutical Science* (Hoppe, H., Levring, T. and Tanaka, Y., eds) p. 473. Walter de Gruyter, Berlin.
- Nichols, B. (1965) *Biochim. Biophys. Acta* **106**, 274.
- Rosenberg, A. and Gouaux, J. (1967) *J. Lipid Res.* **8**, 80.
- Constantopoulos, G. and Bloch, K. (1967) *J. Biol. Chem.* **242**, 3538.
- Pohl, P. and Wagner, H. (1972) *Z. Naturforsch.* **27B**, 53.
- Nichols, B. and Appleby, R. (1969) *Phytochemistry* **8**, 1907.
- Constantopoulos, G. (1970) *Plant Physiol.* **45**, 76.
- Pohl, P., Passig, T. and Wagner, H. (1971) *Phytochemistry* **10**, 1505.
- Khotimchenko, S. and Svetashev, V. (1984) *Biol. Morya (Vladivostok)* **5**, 45.
- Christie, W. W., Brechany, E. Y. and Stefanov, K. (1988) *Chem. Phys. Lipids* **46**, 127.
- Chuecas, L. and Riley, J. (1969) *J. Marine Biol. Assoc. U.K.* **49**, 97.
- El-Sayed, M. M. (1983) *J. Fac. Marine Sci. (King Abdulaziz Univ.)* **3**, 141.
- Jamieson, G. and Reid, E. (1972) *Phytochemistry* **11**, 1423.
- Peakall, D. (1975) *Residue Rev.* **54**, 1.
- Noguchi, T., Ikawa, N., Zebel, J. and Andersen, K. (1979) *Marine Algae in Pharmaceutical Science* (Hoppe, H., Levring, T. and Tanaka, Y., eds), p. 711. Walter de Gruyter, Berlin.
- Smith, K. and Harwood, J. (1984) *Phytochemistry* **23**, 2469.
- Smith, K., Douce, R. and Harwood, J. (1982) *Phytochemistry* **21**, 569.
- Christie, W. W. (1982) *Lipid Analysis* 2nd Edn. Pergamon Press, Oxford.
- Christie, W. W. (1987) *J. High Res. Chromatogr. Chromatogr. Commun.* **10**, 148.
- Christie, W. W. and Stefanov, K. (1987) *J. Chromatogr.* **392**, 259.
- Christie, W. W., Brechany, E. Y., Johnson, S. B. and Holman, R. T. (1986) *Lipids* **21**, 657.
- Harvey, D. J. (1982) *Biomed. Mass Spectrom.* **9**, 33.
- Harvey, D. J. (1982) *Biomed. Mass Spectrom.* **11**, 187.
- Harvey, D. J. (1984) *Biomed. Mass Spectrom.* **11**, 340.
- Christie, W. W., Brechany, E. Y. and Holman, R. T. (1987) *Lipids* **22**, 224.