Pergamon

PII: S0031-9422(97)00680-8

Phytochemistry, Vol. 47, No. 5, pp. 761–765, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

METHOXY FATTY ACIDS ISOLATED FROM THE RED ALGA, *SCHIZYMENIA DUBYI*

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(*Received in revised form* 9 *June* 1997)

Key Word Index—*Schizymenia dubyi*; Gigartinales; red alga; fatty acids; methoxy fatty acids; hydroxy fatty acids.

Abstract—We investigated the fatty acid composition of the total lipids from the red alga *Schizymenia dubyi* (Gigartinales, Gymnophlaeaceae) harvested in Sicily. Twenty-four fatty acids were identified as methyl esters, including C_{14}–C_{28} saturated acids (77% of total acid mixture) and three hydroxylated acids. Four novel mid-chain methoxylated fatty acids (16% of total acid mixture) were identified by GC-mass spectrometry as 9-methoxypentadecanoic, 9-methoxyheptadecanoic, 13-methoxyheneicosanoic and 15-methoxytricosanoic acids.

INTRODUCTION

Methoxy fatty acids are not very widespread in nature. Marine sponges have provided new α-methoxy acids including long-chain acids [1–4] and several mid-chain methoxy fatty acids have been reported only in cyanobacteria and certain microorganisms. Shallow water species of the marine cyanobacterium, *Lyngbya majuscula*, contained the new 7-methoxytetradec-4-enoic acid and related amides [5–9], and some other natural amide derivatives of this acid were isolated from the digestive gland of the sea hare, *Stylocheilus longicauda* [10–12]. Deep-water species of *L. majuscula* contained two amides of 7-methoxy-9-methylhexadec-4-enoic acid but not the free acid [13], and the new related 7-methoxy-9-methylhexadec-4,8-dienoic acid [14]. A new methoxy fatty acid was identified in a Mediterranean species of *L. majuscula*, namely 7-methoxydodec-4-enoic acid, together with a related amide [15]. Polar lipids of four species of the acid-producing bacterium *Thiobacillus*, contained the unusual 10-methoxyoctadecanoic, 11-methoxyoctadecanoic, 12-methoxyeicosanoic and 13-methoxyeicosanoic acids [16]. 11-Methoxyheptadecanoic and 13-methoxynonadecanoic acids were also identified in the total lipids from *Helicobacter pylori*, a bacterium isolated from human gastric mucosae [17].

Total lipids of the Sicilian red alga, *Schizymenia dubyi* Gigartinales, Gymnophlaeaceae) are examined here, as part of our ongoing studies of the constituents of this species [18–20]. Complete fatty acid composition is reported, including four new mid-chain methoxylated fatty acids identified by GC-mass spectrometry.

RESULTS AND DISCUSSION

As indicated in Table 1, 24 fatty acids were identified from the total lipids of the red alga *S. dubyi*. Most of these acids were identified simply as methyl esters by comparing their GC mobilities with those of commercial standards or other known acid mixtures.

C_{14}–C_{28} saturated fatty acids accounted for ca 77% of the total acid mixture, composed mainly of 14:0, 16:0 and 18:0. No isoprenoid fatty acids and, surprisingly, no polyunsaturated fatty acids were detected. Unsaturated fatty acids were only present as traces. GC-mass spectrometry allowed us to confirm these structures and also to identify three hydroxylated acids and the new methoxylated acids [21].

2-Hydroxydocosanoic and 2-hydroxytetracosanoic acids were easily identified [22]. Both mass spectra showed diagnostic fragment ions at *m/z* 90 (McLafferty rearrangement) and at *m/z* 103, instead of the usual corresponding ions at *m/z* 74 and 87, respectively, for saturated acids. The spectra also displayed the corresponding $[M]^+$ at *m/z* 370 and *m/z* 398, respectively, accompanied by the related ions $[M-MeOH]^+$, at *m/z* 338 and *m/z* 366 respectively, and $[M-COOMe]^+$ at *m/z* 311 and *m/z* 339, respectively. 3-Hydroxyhexadecanoic acid was identified from the

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Table 1. Fatty acid composition of total lipids from the red alga *Schizymenia dubyi*

Fatty acids	% total lipids
14:0	17.8
15:0	2.1
16:0	36.8
17:0	0.8
18:0	14.1
br-20:0	1.0
br-22:0	1.2
24:1	0.5
24:0	2.1
26:0	0.4
28:0	0.2
Hydroxy acids	
3-OH-16:0	3.8
2-OH-22:0	0.3
2-OH-24:0	0.2
Methoxy acids	
9-MeO-15:0	8.3
9-MeO-17:0	7.2
13-MeO-21:0	0.3
15-MeO-23:0	0.2
Unidentified	2.7

br-: The branching is neither *iso* nor *anteiso*. Acids identified as traces (< 0.2%): br-: 19:0, 22:0, 23:0, *ai*-25:0, 25:0 and 27:0.

mass spectrum of its methyl ester, which showed *m/z* 103 as base peak, together with the usual peaks at *m/z* 74 and 87 [22].

Among the fatty acid methyl esters, four GC peaks (Table 1) gave unusual mass spectra, compounds **1** (8.3%) and **2** (7.2%) (Fig. 1), and **3** (0.3%) and **4** (0.2%) (Fig. 2). Compounds **1** and **2** displayed major fragment ions at *m/z* 201, 169 and 137, in addition to the expected low relative intensity ions at *m/z* 74 and 87 (Fig. 1). The spectrum of **1** also showed peaks at *m/z* 129 (base peak) and 97, whereas that of compound **2** showed peaks at *m/z* 157 and 125. These diagnostic fragment ions corresponded to cleavage on both sides of the CH-OCH_3 group (Table 2). Comparison with the mass spectra already reported for 11-methoxy acids [17] and for other mid-chain methoxy acids arising from ring-opening of cyclopropane-containing fatty acids [23, 24], indicated that **1** and **2** are the methyl esters of the new acids, namely 9-methoxypentadecanoic and 9-methoxyheptadecanoic, respectively.

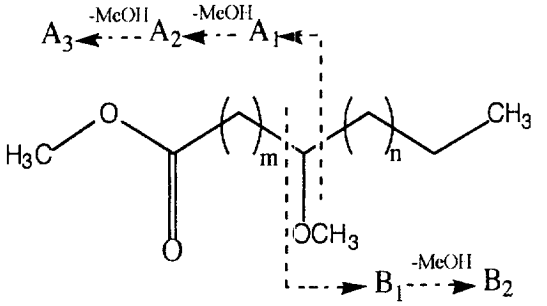
Likewise, the mass spectrum of compound **3** displayed fragment ions at *m/z* 257 as a major peak and the ions at *m/z* 225 and 193, whereas that of compound **4** displayed peaks at *m/z* 285, 253 and 221 (Fig. 2). Both spectra showed fragment ions at *m/z* 157 and 125, in addition to the usual ions at *m/z* 74 and 87. These diagnostic ions corresponded to cleavage on both sides of the CH–OCH_3 group (Table 2). Thus, compounds **3** and **4** were identified as the new 13-methoxyheneicosanoic and 15-methoxytricosanoic acids. These methoxy acids were not products of ring-opening of cyclopropane-containing acids, since they were not accompanied by any expected additional acids usually produced in these conditions. For example, the ring-opening of 7,8-methylenehexadecanoic acid gave a mixture of six fatty acids, namely 9-MeO-17:0, 7-MeO-17:0, 7-MeO-8-Me-16:0, 8-MeO-7-Me-16:0, and two isomeric hexadecanoic acids bearing a CH_2OCH_3 group at C-7 or C-8 [24].

In previous work on fatty acids from the Rhodophyta, including *S. dubyi*, the major acids were found to be 16:0 and 20:5 n-3, together with other polyunsaturated fatty acids [25]. Thus, *S. dubyi* contained almost 50% of 20:5 n-3 in this study [25], whereas our specimens of *S. dubyi* were devoid of polyunsaturated acids. It is known that the formation of fatty acids in marine organisms varies throughout the year. Highly unsaturated acids predominate in the cold season, as reported for marine phytoplankton [26] and gorgonians [27]. Presumably, these variations reflect adaptations to environmental changes. The specimens of *S. dubyi* studied here were collected in shallow waters that were probably warmer than those of the specimens of *S. dubyi* collected in Scotland, for which the growth period was not indicated [25].

The most interesting result of our study was the identification of four related mid-chain methoxy fatty acids showing chain-elongation, three of which were formed successively. The following biosynthetic scheme may be proposed:

(7-MeO–15:0) → 9-MeO–17:0 → (11-MeO–19:0) → 13-MeO–21:0 → 15-MeO–23:0

Table 2. Major diagnostic fragment ions (*m/z*) in the mass spectra of methoxy fatty acids (methyl esters) **1–4** obtained by GC-MS (DB-1; *Rt*/Me16:0: 6.76 min.).



		R_t(min)	*m*	*n*	A_1	A_2	A_3	B_1	B_2
1	9-MeO-15:0	13.57	7	5	201	169	137	129	97
2	9-MeO-17:0	18.62	7	7	201	169	137	157	125
3	13-MeO-21:0	28.15	11	7	257	225	193	157	125
4	15-MeO-23:0	32.69	13	7	285	253	221	157	125

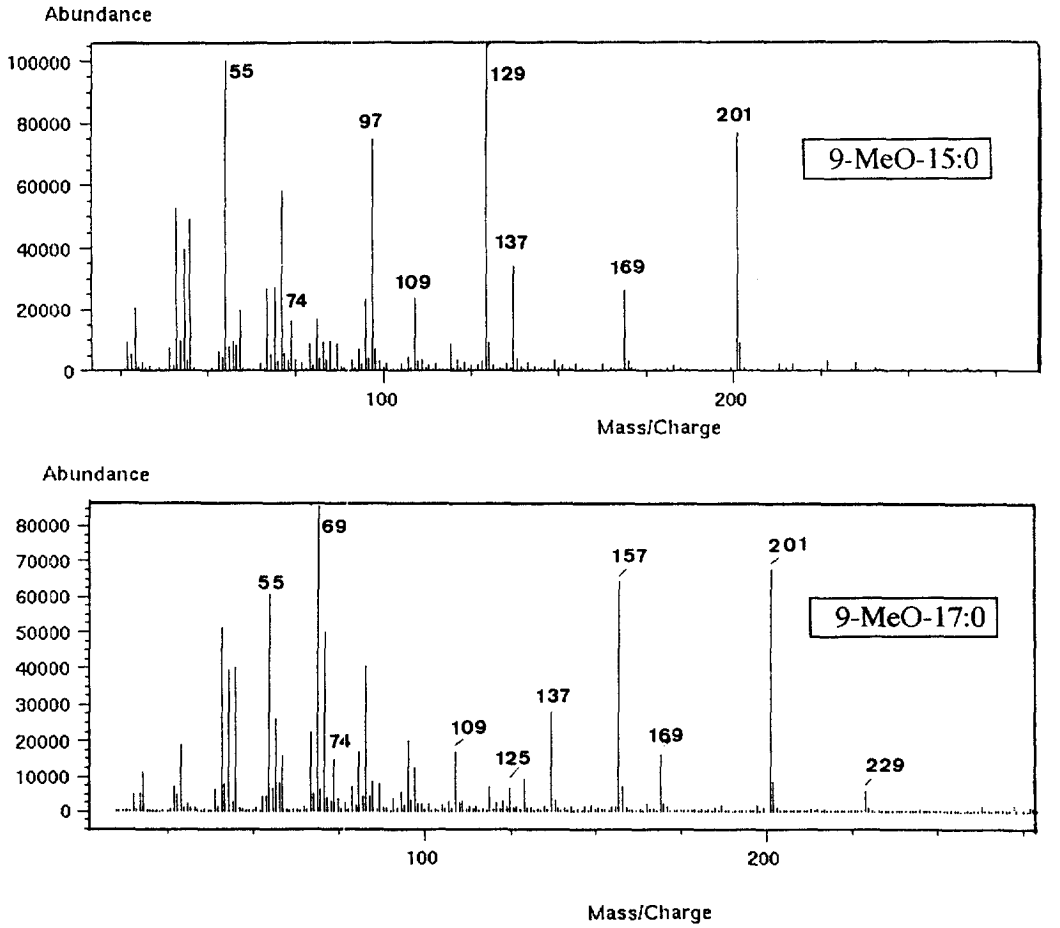


Fig. 1. Mass spectra of fatty acids **1** and **2** (methyl esters).

The intermediate 7-MeO–15:0 acid is still unknown, whereas 11-MeO–19:0 acid has been reported in the bacterium, *Helicobacter pylori* [17]. Since the known mid-chain methoxy fatty acids have been reported from bacteria or cyanobacteria, they could be of symbiotic origin. Cyanobacteria are widespread in marine ecosystems and their fatty acid compositions are different from those of bacteria. Polyunsaturated fatty acids are generally absent in bacteria, whereas high concentrations of 18:3 n-6 and 18:3 n-3 have been found in cyanobacteria and, of 20:5 n-3, in diatoms [28]. Thus, even though the possibility of lipid autoxidation may not be excluded, it can be assumed that the the new methoxy acids arise from symbiotic bacteria, rather than from cyanobacteria or diatoms. In addition, 3-hydroxylated short-chain acids are known to be characteristic of bacteria, so that 3-hydroxyhexadecanoic acid could also be of symbiotic origin. Furthermore, it is noteworthy that antimicrobial activity of methoxylated fatty acids has been reported [6]. Work is in progress in our laboratory to analyse fatty acids from further lipid extracts of *S. dubyi* relative to different areas and times of the year in order to study the related occurrence and unusual methoxy fatty acids as well as polyunsaturated fatty acids.

EXPERIMENTAL

Samples of *S. dubyi* were collected in May 1993 on the east coast of Sicily, Italy. Algal material was washed with dist. H_2O, dried and ground in a Waring blender with chloroform–methanol (1:1). Lipids were extracted continuously with this solvent in a Soxhlet apparatus. Saponification was performed with 2M KOH in EtOH and unsaponifiable matter separated. The remaining aq. fraction was acidified, fatty acids recovered by hexane extractions and converted into Me esters by refluxing with 3% HCl in MeOH. GC-MS was performed on a Hewlett–Packard HP-5890 chromatograph linked to a HP-5989-A mass spectrometer (70 eV) equipped with a HP-9000/345 integrator, using a 30 m × 0.32 mm I.D. fused silica capillary column coated with DB-1 (0.25 μm phase thickness). The carrier gas was He. Column temp. was programmed from 180 to 300° at 3°C min^{-1}.

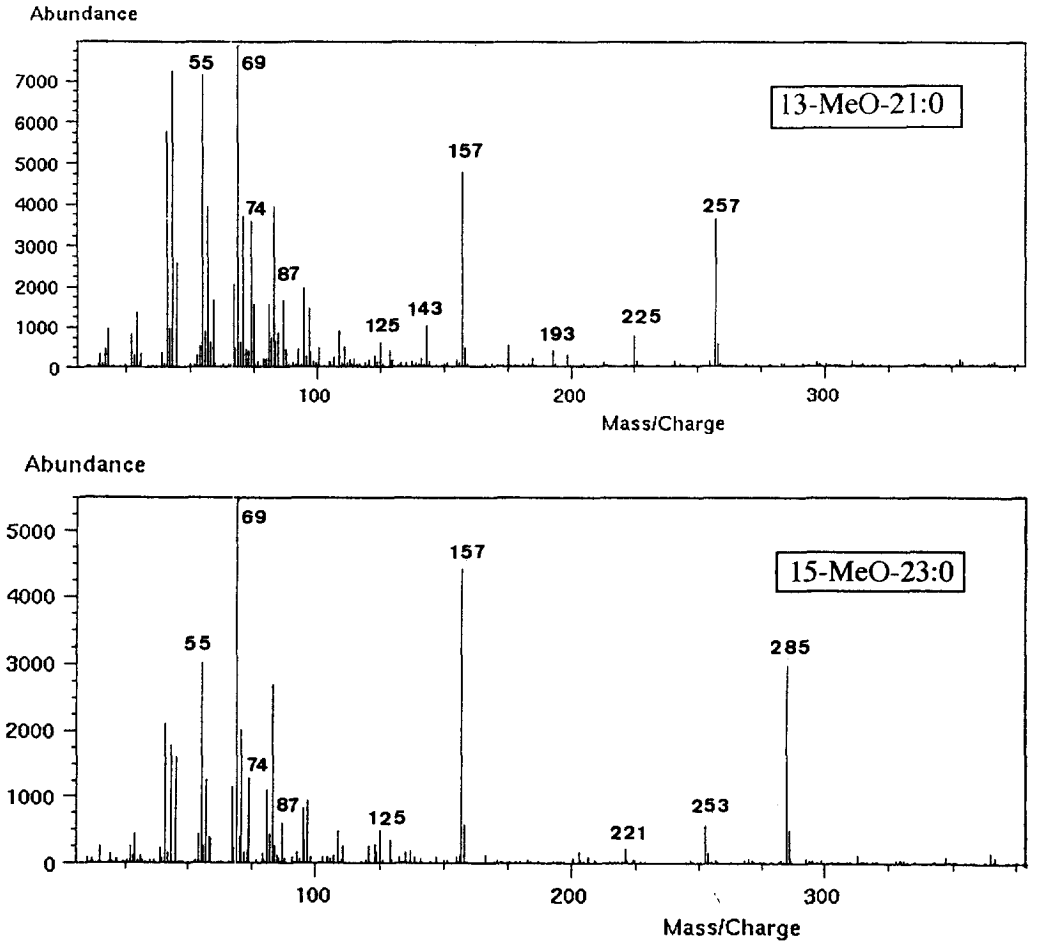


Fig. 2. Mass spectra of fatty acids **3** and **4** (methyl esters).

Acknowledgements—We wish to thank Drs M. Cormaci and G. Furnari from the Istituto e Orto Botanico, Università di Catania, Italy, for collecting the algal material, and G. Nourrisson, Laboratoire de Synthèse Organique, CNRS, Faculté des Sciences de Nantes, France, for GC-MS.

REFERENCES

1. Ayanoglu, E., Kornprobst, J. M., Aboud-Bichara, A. and Djerassi, C., *Tetrahedron Lett.*, 1983, **24**, 1111.
2. Ayanoglu, E., Popov, S., Kornprobst, J. M., Aboud-Bichara, A. and Djerassi, C., *Lipids*, 1983, **18**, 830.
3. Carballeira, N. M., Shalabi, F. and Maldonado, M. E., *Lipids*, 1990, **25**, 235.
4. Carballeira, N. M. and Sepulveda, J. A., *Lipids*, 1992, **27**, 72.
5. Cardellina, J. H., Dalietos, D., Marner, F. J., Mynderse, J. S. and Moore, R. E., *Phytochemistry*, 1978, **17**, 2091.
6. Gerwick, W. H., Reyes, S. and Alvarado, B., *Phytochemistry*, 1987, **26**, 1701.
7. Wright, A. D., Coll, J. C. and Price, I. R., *J. Nat. Prod.*, 1990, **53**, 845.
8. Orjala, J., Nagle, D. and Gerwick, W. H., *J. Nat. Prod.*, 1995, **58**, 764.
9. Ainslie, R. D., Barchi, J. J., Kuniyoshi, M., Moore, R. E. and Mynderse, J. S., *J. Org. Chem.*, 1985, **50**, 2859.
10. Rose, A. F., Scheuer, P. J., Springer, J. P. and Clardy, J., *J. Am. Chem. Soc.*, 1978, **100**, 7665.
11. Paik, S., Carmeli, S., Cullingham, J., Moore, R. E., Patterson, G. M. L. and Tius, M. A., *J. Am. Chem. Soc.*, 1994, **116**, 8116.
12. Todd, J. S. and Gerwick, W. H., *Tetrahedron Lett.*, 1995, **36**, 7837.
13. Mynderse, J. S. and Moore, R. E., *J. Org. Chem.*, 1978, **43**, 4359.
14. Loui, M. S. M. and Moore, R. E., *Phytochemistry*, 1979, **18**, 1733.
15. Praud, A., Valls, R., Piovetti, L. and Banaigs, B., *Tetrahedron Lett.*, 1993, **34**, 5437.

16. Kerger, B. D., Nichols, P. D., Antworth, C. P., Sand, W., Bock, E., Cox, J. J., Langworthy, T. A. and White, D. C. *FEMS Microbiol. Ecol.*, 1986, **38**, 67.
17. Inamoto, Y., Hamanaka, S., Hamanaka, Y., Ariyama, S., Takemoto, T. and Okita, K., *Proc. Japan Acad.*, 1993, **69B**, 65.
18. Bourgougnon, N., Lahaye, M., Quemener, B., Cormaci, M., Furnari, G. and Kornprobst, J. M., *J. Appl. Phycol.*, 1996, **8**, 147.
19. Bourgougno, N., Lahaye, M., Quemener, B., Chermann, J. C., Rimbert, M., Cormacci, M., Furnari, G. and Kornprobst, J. M., *J. Appl. Phycol.*, 1996, **8**, 155.
20. Bourgougnon, N., Lahaye, M., Chermann, J. C. and Kornprobst, J. M., *Bioorg. Med. Chem. Lett.*, 1993, **3**, 1141.
21. Barnathan, G., Kornprobst, J. M., Doumenq, P. and Miralles, J. *Lipids*, 1996, **31**, 193.
22. Barnathan, G., Kornprobst, J. M., Doumenq, P., Miralles, J. and Boury-Esnault, N., *J. Nat. Prod.*, 1993, **56**, 2104.
23. Gerson, T., Patel, J. J. and Nixon, L. N., *Lipids*, 1975, **10**, 134.
24. Lageot, C., Bianchini, J. P., Gaydou, E. M. and Ralaimanarivo, A., *Rev. Fr. Corps Gras*, 1994, **41**, 81.
25. Jamieson, G. R. and Reid, E. H., *Phytochemistry*, 1972, **11**, 1423.
26. Thompson, P. A., Guo, M. X., Harrison, P. J. and Whyte, J. N. C., *J. Phycol.*, 1992, **28**, 488.
27. Miralles, J., Barnathan, G., Galonnier, R., Sall, T., Samb, A., Gaydou, E. M. and Kornprobst, J. M., *Lipids*, 1995, **30**, 459.
28. Kayama, M., Araki, S. and Sato, S., in *Marine Biogenic Lipids, Fats and Oils* ed. R. G. Ackman CRC Press, Boca Raton, Florida, 1989, pp. 3–48.