Unusually High Levels of Eicosatetraenoic, Eicosapentaenoic, and Docosahexaenoic Fatty Acids in Palestinian Freshwater Sponges

Valery M. Dembitsky[a,*] and Tomas Rezanka[b]

[a]Natural Products Laboratory, Department of Organic Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel and [b]Institute of Microbiology, Videnska 1083, Prague 142 20, Czech Republic

ABSTRACT: The fatty acid composition of three freshwater sponges—*Ephydatia syriaca, Nudospongilla* sp., and *Cortispongilla barroisi*—were studied. Twenty principal fatty acids, and unusually high levels of eicosatetraenoic (5,8,11,14-20:4 up to 10.1% of the total acid mixture), eicosapentaenoic (5,8,11,14,17-20:5 up to 11.6%), and docosahexaenoic acids (4,7,10,13,16,19-22:6 up to 11.8%) were detected. The only demospongic acid found was 5,9,17-hexacosatrienoic acid (1.8–3.7%).

Lipids 31, 647–650 (1996).

At the present time, a definite opinion has been formed concerning the unique nature of sponge biomembranes (1). Sponges can be regarded as live fossils of the contemporary animal world due to the fact that they have undergone practically no evolutionary development, i.e., their present representatives probably remain at the evolutionary level of their remote precursors (2). Sponge lipids differ markedly in their chemical and biochemical properties from lipids obtained from other living organisms (1). Rare lipids, including fatty acids, have been identified in marine sponges, mostly long-chain fatty acids (C_{24}–C_{36}) of straight, branched, and brominated structure (3). Because these acids were isolated for the first time from marine sponges, they are often called "demospongic acids" (1). The total content of demospongic acids can reach up to 80% in some marine sponge species (3,4), and a major part of them is found in individual phospholipid classes (1,3). There is limited information on whether freshwater sponges contain demospongic acids (5–12).

In this work we report a study of the fatty acid composition of three freshwater sponges, which were collected in an expedition to the Jordan river, Lake Hula, and the Sea of Galilee, Palestine.

MATERIALS AND METHODS

Sampling and lipid analyses. The freshwater sponge *Ephydatia syriaca* was collected in August 1993, in the spring of the Jordan river (Banias, near Mountain Hermon); the freshwater sponge *Nudospongilla* sp. was collected in May 1994, in Lake Hula; and the freshwater sponge *Cortispongilla barroisi* was collected in July 1994, in the Sea of Galilee (Lake Tiberias, Lake Kinneret, Palestine). All sponges were washed in water, carefully cleaned of all debris, and cut into small pieces. Immediate lipid extraction after collection was done as described elsewhere (9).

Gas chromatographic/mass spectrometric analyses of fatty acid methyl esters. High-resolution mass spectrometry was done using a MAT 90 magnetic sector mass spectrometer (Finnigan, San Jose, CA) in the electron ionization mode. Gas chromatography/mass spectrometry of fatty acid mixture was done using a Finnegan 1020 B single-state quadrupole gas chromatography/mass spectrometry instrument in the electron ionization mode. Injection temperature (splitless injection) was 100°C, and a fused-silica capillary column (Supelcowax 10; 60 m × 0.25 mm i.d., 0.25 μm film thickness; Supelco, Bellefonte, PA) was used. The temperature program was as follows: 100°C for 1 min, than increased at 20°C/min to 180°C and at 2°C/min to 280°C, which was maintained for 1 min. The carrier gas was helium at a linear velocity of 60 cm s^{-1}. All spectra were scanned within the range *m/z* 70–450.

RESULTS AND DISCUSSION

The fatty acid compositions of three sponge species are presented in Table 1. Twenty principal fatty acids were detected in the sponge total lipids. All the principal fatty acids identified are "classical" acids which are found in invertebrates (13). Only one demospongic acid, namely 5,9,17-26:3 (from 1.8 to 3.7%) was found in all sponge species. Over the last two decades it has been established that the majority of marine sponges contain trace amounts of both 20:4/20:5 and/or 22:6 fatty acids (3,14–30). Both isomers of eicosatetraenoic acid were found in some freshwater sponges ranging in amount from 1.4 to 11.8% (5–7,10–12) and in marine sponges ranging from 0.2 to 18.3% (16,31–49) (see Table 2). Higher

*To whom correspondence should be addressed at Department of Biotechnology, Moscow Academy of Fine Chemical Technology, 86 Vernadsky Avenue, Moscow 117571, Russia.

TABLE 1
Principal Fatty Acids of the Freshwater Sponge

Fatty acids	*Ephydatia syriaca*	*Nudospongilla* sp.	*Cortispongilla barroisi*
14:0	3.2	1.8	2.3
16:0	8.5	10.6	9.4
18:0	4.5	5.1	3.9
20:0	1.6	1.8	2.1
Saturated	17.8	19.3	17.7
7-16:1	1.1	1.2	1.5
9-16:1	5.5	6.2	7.3
9-18:1	7.7	8.9	9.4
11-18:1	2.1	2.2	2.1
11-20:1	1.4	1.4	1.9
11-22:1	1.5	1.6	1.5
Monoenoic	19.3	21.5	23.7
6,9-18:2	5.7	5.8	6.6
9,12-18:2	5.4	6.4	5.3
12,15-18:2	2.2	2.4	2.5
Dienoic	13.3	14.6	14.4
6,9,12-18:3	3.0	3.6	3.8
9,12,15-18:3	9.7	9.6	10.6
5,9,17-26:3	3.6	3.7	1.8
Trienoic	16.3	16.9	16.2
6,9,12,15-18:4	1.7	0.7	0.6
5,8,11,14-20:4	10.1	9.5	9.5
5,8,11,14,17-20:5	9.7	9.5	11.6
4,7,10,13,16,19-22:6	11.8	8.0	6.3
Sum	33.3	27.0	27.4

amounts were found in *Phakellia aruensis*, 12.5% (40); *Ectyoplasia ferox*, 14.1% (35); and in *Spheciospongi confoederata*, 18.3% (47).

Eicosapentaenoic acid was found in some marine sponges (see Table 2) and larger amounts were detected in freshwater sponges (up to 17.2% (10). Docosahexaenoic acid was the major lipid component in the marine sponge *Microciona prolifera*, varying from 15.4 to 25.4% of total lipids (43,45–47). Freshwater sponges usually contained this acid in amounts which ranged from 0.8 to 4.1% (Table 2). In the present study, unusually high levels of eicosatetraenoic, eicosapentaenoic, and docosahexaenoic acids were found in all freshwater sponges (Table 1). The percentage of total lipids formed by these three fatty acids as detected in *E. syriaca* was 31.5%; in *Nudospongilla* sp., 26.3%; and in *C. barroisi*, 26.8%. Weissmann *et al.* (43) reported that a high level of 22:6 fatty acid was found in two phospholipids of the marine sponge *M. prolifera*, namely in phosphatidylcholine (52%) and in phosphatidylinositol (29%). The authors indicated that the high level of 22:6 acid could be the result of differences in the local environment of the samples collected, and appeared to be related to a role for this fatty acid in stimulus–response coupling in these cells.

More interesting data could be associated with 5,9,17-26:3 fatty acid, which was found to be the major demospongic fatty acid in some Palestinian freshwater sponges. According to Hahn *et al.* (7) and Dembitsky *et al.* (9), who studied the fatty acid total content of the freshwater sponges *E. fluviatilis* and *Euspongilla lacustris*, only 5,9,19-26:3 acid was 14.9%

TABLE 2
Comparative Distribution of Eicosatetraenoic, Eicosapentaenoic and Docosahexaenoic Fatty Acids in Total Lipids of Some Freshwater and Marine Sponge Species (class Demospongiae)

Sponge species	Abundance (wt%) 20:4[a]	20:5[b]	22:6[c]	References
Freshwater species				
Baicalospoggia bacilifera	4.3	9.4	0.8	6
B. bacilifera	2.6	1.8	4.1	11
B. baicalensis	2.7	12.2	0.5	10
B. intermedia	5.9	7.1	4.1	6
B. intermedia	3.2	2.0	2.9	11
B. intermedia (green)	1.4	4.5	0.4	10
B. intermedia (grey)	1.3	5.7	0.3	10
Ephydatia fluviatilis	11.8	—	—	7
Euspongilla lacustris	3.7	—	—	9
Lubomirskia abietina	5.2	7.8	2.0	5
L. baicalensis	3.9	6.9	0.9	5
L. baicalensis	1.9	1.7	2.9	12
L. fusifera	3.5	6.0	1.2	5
Swartschewskia bacilifera	3.4	7.6	0.7	10
S. papyracea	4.6	10.1	3.0	6
S. papyracea	4.9	17.2	1.5	10
Marine species				
Adocia cinerea	3.9	6.9	4.6	31
Agelas sp.	3.3	—	—	32
Amphimedon viridis	3.5[c]	1.4[d]	0.7	49
Aplysia archeri	6.9	—	—	16
Chondrosia remiformis	3.0	—	—	34
Cornacuspongia gen. sp.	1.5	5.9	0.3	31
Dysidea fragilis	4.3	2.9	2.9	38
D. herbacea	—	2.0	1.0	40
Ectyoplasia ferox	14.1	—	—	35
Halichondria panicea	4.0	5.6	4.2	44
Homaxinella subdola	0.4	8.6	1.2	31
Hymeniacidon sanguinea	2.7	3.8	0.1	39
Hymeniacidonidae gen. sp.	9.5[d]	—	—	42
Microciona prolifera	4.7	—	25.4[e]	46
M. prolifera	6.2	—	18.9[f]	46
M. prolifera	2.0[d]	3.9[d]	22.4[d]	43
M. prolifera	4.3	—	15.4	47
M. prolifera	1.2	3.6	15.4	45
Mycale laevis	4.7	—	—	33
Myrmekioderma styx	2.0	—	—	34
Myxilla incrustans	3.8	13.3	5.9	31
Ophithaspongia pennata	3.9	1.5	0.2	31
Petrosia pellasarca	1.0	—	—	36
Petrosia sp.	3.3	—	1.8	37
Phakellia aruensis	12.5[g]	2.4[g]	5.4[g]	40
P. cribrosa	0.2	1.9	2.5	31
Phyllospongia papyracea	—	0.1	1.2	40
Pseudaxinyssa sp.	0.5	0.3	0.3	40
Rhopaloeides odorabile	0.5[g]	—	0.2[g]	40
Reniera sp.	—	—	6.0	48
Spheciospongi confoederata	18.3	—	—	47
Suberites domuncula	3.4	4.2	3.9	31
Tylodesma aff. rosca	3.4	4.8	4.5	31

[a]Two isomers, namely 5,8,11,14-20:4 and 6,9,12,15-20:4. [b]20:5 = 5,8,11,14,17-20:5. [c]22:6 = 4,7,10,13,16,19-22:6. [d]Total phospholipids. [e]Collected in July. [f]Collected in December. [g]Without cyanobacteria.

of the total fatty acid methyl esters (phosphatidylethanolamine, 19.8%; and phosphatidylserine, 14.8%) and 10.3% (phospholipid fraction, 20.9%), respectively, in these two species.

Previous gas chromatography/mass spectrometry analyses of the fatty acid levels in three freshwater sponges from Lake Baikal (11,12) indicated that 5,9,17-26:3 acid is the major demospongic fatty acid. Prior and current data show the principal biosynthetic sequence that seems to be operative in the freshwater sponge species. The biosynthetic pathway of the 5,9,17-26:3 acid is predominant.

The results presented in this work are of interest because they clearly demonstrate that three polyunsaturated fatty acids—eicosatetraenoic, eicosapentaenoic and docosahexaenoic—and the demospongic acid 5,9,17-26:3 are the principal fatty acids in freshwater sponges. Further work is in progress toward elucidating the structure of some unusual minor fatty acids.

REFERENCES

1. Litchfield, C., and Morales, R.W. (1976) Are Demospongiae Membranes Unique Among Living Organisms? in *Aspects of Sponge Biology* (Harrison, F.W., and Cowden, R.K., eds.), Academic Press, New York, pp. 183–200.
2. Bergquist, P.R. (1978) *Sponges*, Hutchinson, London.
3. Djerassi, C., and Lam, W.-K. (1991) Sponge Phospholipids, *Acc. Chem. Res. 24*, 69–75.
4. Bergquist, P.R., Lawson, M.P., Lavis, A., and Cambie, R.C. (1984) Fatty Acid Composition and the Classification of the Porifera, *Biochem. Syst. Ecol. 12*, 63–84.
5. Dembitsky, V.M. (1981) Fatty Acid Composition of Freshwater Sponges of the Class Demospongiae. 1. *Genus lubomirskia, Chem. Natur. Prod. (USSR) 4*, 511–513.
6. Dembitsky, V.M. (1981) Fatty Acid Composition of Freshwater Sponges of the Class Demospongiae. 2. Genera *Swartschewskia* and *Baicalospongia, Chem. Natur. Prod. (USSR) 4*, 513–515.
7. Hahn, S., Lam, W.-K., Wu, I., Silva, C.J., and Djerassi, C. (1989) Unusual Pattern of Fatty Acid Biosynthesis. Evidence for C-19 Desaturase Activity in Freshwater Sponges, *J. Biol. Chem. 264*, 21043–21046.
8. Vysotskii, M.V., Imbs, A.B., Popkov, A.A., Latyshev, N.A., and Svetashev, V.I. (1990) *Trans*-Olefinic Very Long-Chain Fatty Acid (26:3,5*c*,9*c*,19*t*) in Lipids of Freshwater Sponges of Lake Baikal, *Tetrahedron Lett. 31*, 4367–4370.
9. Dembitsky, V.M., Kashin, A.G., and Karaganova, M.V. (1991) Phospholipid and Fatty Acid Composition of the Freshwater Sponge *Euspongilla lacustris, Comp. Biochem. Physiol. 100B*, 185–187.
10. Latyshev, N.A., Zhukova, N.V., Efremova, S.M., Imbs, A.B., and Glysina, O.I. (1992) Effect of Habitat on Participation of Symbionts in Formation of the Fatty Acid Pool of Freshwater Sponges of Lake Baikal, *Comp. Biochem. Physiol. 102B*, 961–965.
11. Dembitsky, V.M., Rezanka, T., and Kashin, A.G. (1993) Comparative Study of the Endemic Freshwater Fauna of Lake Baikal. II. Unusual Lipid Composition of Two Sponge Species *Baicalospongia bacilifera* and *Baicalospongia intermedia* (Family *Lubomirskiidae*, Class Demospongiae), *Comp. Biochem. Physiol. 106B*, 825–831.
12. Dembitsky, V.M., Rezanka, T., and Kashin, A.G. (1994) Comparative Study of the Endemic Freshwater Fauna of Lake Baikal. VI. Unusual Fatty Acid and Lipid Composition of the Endemic Sponge *Lubomirsia baicalensis* and Its Amphipod Crustacean Parasite *Brandtia (Spinacanthus) parasitica, Comp. Biochem. Physiol. 109B*, 415–426.
13. Ackman, R.G. (ed.) (1989) *Marine Biogenetic Lipids, Fats and Oils*, Vols. 1 and 2, CRC Press, Boca Raton.
14. Carballeira, N.M., Maldonado, L., and Porras, B. (1987) Isoprenoid Fatty Acids from Marine Sponges. Are Sponges Selective? *Lipids 22*, 767–769.
15. Carballeira, N.M., and Maldonado, M.E. (1989) On the Isolation of the New Fatty Acid 6,11-Eicosadienoic (20:2) and Related 6,11-Dienoic Acids from the Sponge *Euryspongia rosea, Lipids 24*, 665–668.
16. Carballeira, N.M., Shalabi, F., and Negron, V. (1989) 2-Hydroxy Fatty Acids from Marine Sponges. 2. The Phospholipid Fatty Acids of the Caribbean Sponges *Verongula gigantea, Lipids 24*, 229–232.
17. Carballeira, N.M., and Lopez, M.R. (1989) On the Isolation of 2-Hydroxydocosanoic and 2-Hydroxytricosanoic Acids from the Marine Sponge *Amphimedon compressa, Lipids 24*, 89–91.
18. Carballeira, N.M., Shalabi, F., and Maldonado, M.E. (1990) Identification of the New 18-Hexacosenoic Acid in the Sponge *Thalysias juniperina, Lipids 25*, 235–237.
19. Carballeira, N.M., and Shalabi, F. (1990) Identification of Naturally Occurring *trans,trans* 5,9, Fatty Acids from the Sponge *Plakortis Halichondroides, Lipids 25*, 835–840.
20. Carballeira, N.M., and Rodriguez, J. (1991) Two Novel Phospholipid Fatty Acids from the Caribbean Sponge *Geodia gibberosa, Lipids 26*, 324–326.
21. Carballeira, N.M., Shalabi, F., Cruz, C., Rodriguez, J., and Rodriguez, E. (1991) Comparative Study of the Fatty Acid Composition of Sponges of the Genus *Ircinia*. Identification of the New 23-Methyl-5,9-Tetracosadienoic Acid, *Comp. Biochem. Physiol. 100B*, 489–492.
22. Carballeira, N.M., and Restituyo, J. (1991) Identification of the New 11,15-Icosadienoic Acid and Related Acids in the Sponge *Amphimedon complanata, J. Nat. Prod. 54*, 315–317.
23. Carballeira, N.M., and Negron, V. (1991) Identification and Characterization of Two New Methylicosadienoic Acids from *Erylus formosus, J. Nat. Prod. 54*, 305–309.
24. Carballeira, N.M., and Sepulveda, J.A. (1992) Two Novel Naturally Occurring α-Methoxy Acids from the Phospholipids of Two Caribbean Sponges, *Lipids 27*, 72–74.
25. Barnathan, G., Miralles, J., Gaydou, E.M., Boury-Esnault, N., and Kornprost, J.-M. (1992) New Phospholipid Fatty Acids from the Marine Sponge *Cinachyrella alloclada* Uliczka, *Lipids 27*, 779–784.
26. Barnathan, G., Kornprost, J.-M., Doumenq, P., Miralles, J., and Boury-Esnault, N. (1993) Sponge Fatty Acids. 5. Characterization of Complete Series of 2-Hydroxy Long-Chain Fatty Acids in Phospholipids of Two Senegalese Marine Sponges from the Family Suberitidae: *Pseudosuberites* sp. and *Suberites massa. J. Nat. Prod. 56*, 2104–2113.
27. Duque, C., Cepeda, N., and Martinez, A. (1993) The Steryl Ester and Phospholipid Fatty Acids of the Sponge *Agelas conifera* from the Colombian Caribbean, *Lipids 28*, 767–769.
28. Zimmerman, M.P., Thomas, F.C., Thompson, J.E., Djerassi, C., Streiner, H., Evans, E., and Murphy, P.T. (1989) The Distribution of Lipids and Sterols in Cell Types from the Marine Sponge *Pseudaxinyssa* sp., *Lipids 24*, 210–216.
29. Zimmerman, M.P., Hoberg, M., Ayanoglu, E., and Djerassi, C. (1990) Cell Separation of *Tethya aurantia*, an Analytical Study of Embryonic and Differentiated Sponge Cells, *Lipids 25*, 383–390.
30. Walkup, R.D., Jamieson, G.C., Ratcliff, M.R., and Djerassi, C. (1981) Phospholipid Studies of Marine Organisms: 2. Phospholipids, Phospholipid-Bound Fatty Acids and Free Sterols of the Sponge *Aplysina Fistularis* (Pallas) *Forma Fulva* (Pallas)

(*Verongia Thiona*). Isolation and Structure Elucidation of Unprecedented Branched Fatty Acids, *Lipids 16*, 631–646.
31. Dembitsky, V.M., and Nebylitsyn, B.D. (1980) Lipids of Marine Origin. 2. The Comparative Study of Phospholipid and Fatty Acid Composition of Sea Sponges from the Sea of Japan, *Bioorganic Chem. (USSR) 6*, 1542–1548.
32. Carballeira, N.M., and Emiliano, A. (1993) Novel Brominated Phospholipid Fatty Acids from the Caribbean Sponge *Agelas* sp., *Lipids 28*, 763–766.
33. Carballeira, N.M., Negron, V., and Reyes, E.D. (1992) Novel Monounsaturated Fatty Acids from the Sponges *Amphimedon Compressa* and *Mycale Laevis*, *J. Nat. Prod. 55*, 333–339.
34. Carballeira, N.M., Reyes, E.D., and Shalabi, F. (1993) Identification of Novel *iso/anteiso* Nonacosadienoic Acids from the Phospholipids of the Sponges *Chondrosia remiformis* and *Myrmekioderma styx, J. Nat. Prod. 56*, 1850–1855.
35. Carballeira, N.M., and Maldonado, M.E. (1989) New Phospholipid Fatty Acids from the Caribbean Sponge *Ectyoplasia ferox, Lipids 24*, 371–374.
36. Carballeira, N.M., and Reyes, E. (1990) Novel Very Long-Chain Fatty Acids from the Sponge *Petrosia pellasarca, J. Nat. Prod. 53*, 836–840.
37. Carballeira, N.M., and Shalabi, F. (1993) Novel Brominated Phospholipid Fatty Acids from the Caribbean Sponge *Petrosia* sp., *J. Nat. Prod. 56*, 739–746.
38. Christie, W.W., Brechany, E.Y., Stefanov, K., and Popov, S. (1992) The Fatty Acids of the Sponge *Dysidea fragilis* from the Black Sea, *Lipids 27*, 640–644.
39. Christie, W.W., Brechany, E.Y., Marekov, I.N., Stefanov, K., and Andreev, S.N. (1994) The Fatty Acids of the Sponge *Hymeniacidon sanguinea* from the Black Sea, *Comp. Biochem. Physiol. 109B*, 245–252.
40. Gillan, F.T., Stoilov, I.L., Thompson, J.E., Hogg, R.W., Wilkinson, C.R., and Djerassi, C. (1988) Fatty Acids as Biological Markers for Bacterial Symbionts in Sponges, *Lipids 23*, 1139–1145.
41. Garson, M.J., Zimmerman, M.P., Battershill, C.N., Holden, J.L., and Murphy, P.T. (1994) The Distribution of Brominated Long-Chain Fatty Acids in Sponge and Symbiont Cell Types from the Tropical Marine Sponge *Amphimedon terpenesis, Lipids 29*, 509–516.
42. Lam, W.-K., Hahn, S., Ayanoglu, E., and Djerassi, C. (1989) Phospholipid Studies of Marine Organisms. 22. Structure and Biosynthesis of a Novel Brominated Fatty Acid from a *Hymeniaciddonid* Sponge, *J. Org. Chem. 54*, 3428–3432.
43. Weissmann, G., Riesen, W., Davidson, S., and Waite, M. (1988) Stimulus–Response Coupling in Marine Sponge Cell Aggregation: Lipid Metabolism and Function of Exogenously Added Arachidonic and Docosahexaenoic Acids, *Biochim. Biophys. Acta 960*, 351–364.
44. Dembitsky, V.M., Svetashev, V.I., and Vaskovsky, V.E. (1977) Lipids of Marine Origin. 1. Unusual Lipid from Marine Sponge *Halichondria panicea, Bioorganic Chem. (USSR) 3*, 930–933.
45. Morales, R.W., and Litchfield, C. (1976) Unusual C24, C25, C26 and C27 Polyunsaturated Fatty Acids of the Marine Sponge *Microciona prolifera, Biochim. Biophys. Acta 431*, 206–216.
46. Hahn, S., Stoilov, I., Tam Ha, T.B., Raederstorff, D., Doss, G.A., Li, H., and Djerassi, C. (1988) Biosynthetic Studies of Marine Lipids. 17. The Course of Chain Elongation and Desaturation in Long-Chain Fatty Acids of Marine Sponges, *J. Am. Chem. Soc. 110*, 8117–8124.
47. Ayanoglu, E., Rizzolio, M., Beaulieu, S., Roberts, J., Oz, O., and Djerassi, C. (1990) Covalenty Bound Fatty Acids in Membrane Proteins of Some Sponges, *Comp. Biochem. Physiol. 96B*, 597–603.
48. Lawson, M.P., Thompson, J.E., and Djerassi, C. (1988) Cell Membrane Localization of Long-Chain C24–C30 Fatty Acids in Two Marine Demonsponges, *Lipids 23*, 741–749.
49. Carballeira, N.M., and Shalabi, F. (1994) Unusual Lipids in the Caribbean Sponges *Amphimedon viridis* and *Desmapsamma anchorata, J. Nat. Prod 57*, 1152–1159.

[Received September 27, 1995, and in revised form February 5, 1996; Final revision accepted April 8, 1996]