Unusually High Levels of C_{24}-C_{30} Fatty Acids in Sponges of the Class Demospongiae

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ABSTRACT

Twenty genera of sponges from the class Demospongiae have been examined for fatty acid composition. All contain unusually high levels (34-79%) of C_{24}-C_{30} fatty acids not generally found in other organisms. These characteristic "demospongic acids" are mostly polyunsaturated.

INTRODUCTION

In 1951 Bergmann and Swift (1) first reported the presence of unexpectedly high mol wt fatty acids in two marine sponges, *Spheciospongia vesparia* and *Suberites compacta.* In addition to surveying fatty acid chain lengths by fractional distillation, they isolated 17,20-hexacosadienoic and 9-hexacosenoic acids from the former organism and octacosatrienoic and octacosenoic acids from the latter. More recently, we have identified 5-*cis*,9-*cis*-hexacosadienoic, 5-*cis*,9-*cis*,19-*cis*-hexacosatrienoic, and other C_{24}-C_{27} homologous acids in the sponge *Microciona prolifera* (2-4).

These findings raise the question of whether the C_{24}-C_{28} fatty acids found in these three sponges are isolated occurrences or whether such ultra long chain acids are characteristic of all sponges. To help answer this question, we have examined the chain length distribution of fatty acids from 20 different genera of the Demospongiae, the most numerous of the three taxonomic classes in the phylum Porifera (sponges).

EXPERIMENTAL PROCEDURES

Living sponges were obtained from the following sources: *Cliona, Halicondria,* and *Haliclona,* near Woods Hole, MA, October 1974 (Northeast Marine Specimens Co., Woods Hole, MA); *Isodictya* and *Mycale*, same source, January, 1975; *Microciona*, near Navesink, NJ, June 1974 (4); *Anthosigmella, Chondrilla, Iotrochota,* and *Tedania,* in the Bahia de Jobos near Guayama, Puerto Rico, December 1974; *Spongilla,* in Brachears Creek near Taylorsville, KY, July 1975; *Axinella, Dysidea, Spongia, Stelletta, Xestospongia,* and *Xytopsene,* near Panacea, FL, April 1975 (Gulf Specimen Co., Panacea, FL); *Lissodendoryx,* same source, March 1972.

Each sponge was cleaned very carefully, and the lipids (ca. 0.5-1.5% of wet wt) were extracted with chloroform:methanol (5). Fatty acid methyl esters were prepared by KOH-catalyzed methanolysis (6) and isolated by thin layer chromatography (TLC). A portion of the methyl esters was hydrogenated using a PtO catalyst in methanol (7) and then purified once more by TLC.

Fatty acid methyl esters were analyzed by gas-liquid chromatography (GLC) on a 1.82 m x 2.4 mm (inside diameter) stainless steel column packed with 10% EGSS-X silicone-polyester at 194 C. Peak areas were measured with an electronic integrator, corrected by appropriate calibration factors, and reported as % by wt.

RESULTS AND DISCUSSION

Previous experiments have shown the fatty acid compositions of marine sponges from the class Demospongiae to be extremely complex. In *Microciona,* for example, we identified 95 different fatty acids in amounts of 0.1% or more (4), and many of these had new and unusual structures. For a comprehensive survey of $>C_{22}$ acids in 20 different Demospongiae genera, therefore, a simplified approach was chosen. Each fatty acid mixture was analyzed by GLC both before and after hydrogenation. Quantitation of the chromatogram from the hydrogenated sample accurately identified the fatty acid chain lengths present, while comparison of unhydrogenated and hydrogenated runs allowed us to estimate whether the major $>C_{22}$ components were saturated, monoenoic, or polyunsaturated acids.

The distribution of fatty acid chain lengths in the 20 sponge genera examined are reported in Table I. All samples contained unusually high levels (34-79%) of C_{24}-C_{30} fatty acids. C_{26} was the most prevalent of these ultra long chain lengths. However, 8-17% C_{28} was present in *Haliclona, Xestospongia, Spheciospongia,* and *Suberites;* and C_{30} chains comprised 38% of *Chondrilla* and 11% of *Cliona.* A major amount (3-8%) of an equivalent chain length (ECL) 23.41-23.47 branched chain acid (presumably *iso*-24:0) was present in five of the

TABLE I

Distribution (% by wt) of Fatty Acid Chain Lengths in Sponges of the Class Demospongiae

SUBCLASS[a] / Order / Genus and species	Carbon atoms in fatty acid chain[b]																	
	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Σ(24-30)
CERACTINOMORPHA																		
Poecilosclerida																		
Isodictya deichmannae	3	tr[c]	4	1	5	tr	19	1	6	-	1	2	55	-	3	-	-	61
Lissodendoryx sp.	1	tr	3	1	3	4[d]	15	tr	11[e]	tr	16[f]	3	41	tr	1	-	-	61
Iotrochota birotulata	1	tr	7	tr	7	tr	20	tr	1	-	3	5	55	1	-	-	-	64
Microciona prolifera (4)	1	1	7	3	9	1	7	1	22	1	7	2	38	tr	tr	-	-	47
Mycale fibrexilis	-	-	5	1	4	1	22	1	5	tr	8	1	52	-	-	-	-	61
Tedania ignis	1	1	8	tr	7	tr	14	tr	7	tr	11[g]	4	46	1	-	-	-	62
Haplosclerida																		
Haliclona oculata	1	1	4	tr	6	tr	5	tr	17	-	11[h]	tr	43	3	9	-	-	66
Spongilla lacustrus	1	2	10	2	13	tr	15	tr	1	tr	18[i]	6	30	tr	1	-	-	55
Xestospongia halichondroides	3	tr	7	1	10	tr	5	-	13	-	2	1	39	6	13	-	-	61
Xytopsene sigmatum	1	tr	5	2	9	tr	28	tr	8	-	4	1	42	tr	-	-	-	47
Halichondrina																		
Halichondria panicea	4	1	14	2	18	1	15	1	9	tr	5	2	27	tr	1	-	-	35
Dictyoceratida																		
Dysidea camera	tr	2[j]	17[k]	4[l]	17	tr	10	tr	10	tr	8	11	20	tr	-	-	-	39
Spongia sp.	1	6[j]	15	3	13	1	7	tr	15	1	12	25[m]	1	-	-	-	-	38
TETRACTINOMORPHA																		
Astrophorida																		
Chondrilla nucula	tr	5[j]	25	6[n]	10	tr	11	tr	1	-	1	tr	2	-	1	tr	38	42
Stelletta grubii	tr	5[j]	20	1	20	tr	5	-	4	-	3	2	34	5	1	-	-	45
Hadromerida																		
Anthosigmella varians	3	tr	18	tr	27	tr	11	tr	7	tr	5	1	28	-	-	-	-	34
Cliona celata	1	tr	2	1	2	tr	12	1	1	tr	8[o]	4	54	1	1	-	11	79
Spheciospongia vesparia (1)	2	-	12	-	17	-	7	-	4	-	4	-	39	-	15	-	-	58
Suberites compacta (1)	tr	-	10	-	12	-	9	-	18	-	12	-	22	-	17	-	-	51
Axinellida																		
Axinella polycapella	-	-	1	1	2	1	19	-	1	-	7	2	50	15[p]	1	-	-	75

[a]Taxonomic classification follows Levi (9). *Xestospongia* and *Xytopsene* included in the Poecilosclerida (10).
[b]All branched chain acids are included under their estimated carbon numbers: i.e., C_{16} = *n*-16:0 + *iso*-16:0 + *anteiso*-16:0. Any major (>2%) branched chain components are indicated in footnotes.
[c]tr = 0.1-0.5%.
[d]Mainly a mixture of branched chain structures.
[e]ECL 21.45 = 3%, ECL 22.00 = 8% (ECL = equivalent chain length).
[f]ECL 23.41 = 5%, ECL 24.00 = 11%.
[g]ECL 23.44 = 3%, ECL 24.00 = 8%.
[h]ECL 23.42 = 8%, ECL 24.00 = 3%.
[i]ECL 23.47 = 3%, ECL 24.00 = 15%.
[j]All ECL 14.48-14.61.
[k]ECL 15.50 = 3%, ECL 16.00 = 14%.
[l]Mostly ECL 16.64.
[m]ECL 24.48 = 24%, ECL 25.00 = 1%.
[n]Mainly ECL 16.53.
[o]ECL 23.41 = 4%, ECL 24.00 = 4%.
[p]ECL 26.71 = 4%, ECL 27.00 = 11%.

genera. As far as we know, no other group of organisms regularly contains such high levels of C_{24}-C_{30} chains in the total fatty acids.

Comparison of GLC analyses before and after hydrogenation indicated that almost all of the C_{24}-C_{30} peaks moved more than 0.40 ECL units (8) upon hydrogenation. Thus, only small amounts of saturated or monoenoic $\geq C_{22}$ acids could be present; and we conclude that these ultra long chain acids are mostly polyunsaturated. No consistent pattern of C_{24}-C_{30} peaks was observed for the unhydrogenated samples; so exact identification of these polyunsaturates must await detailed structural analyses. In the four genera where such analyses have already been carried out, however, C_{26}-C_{30} polyunsaturates have been definitely identified: i.e., 26:2 and 26:3 in *Microciona* (2,4), 30:4 in *Cliona* (Litchfield and Noto, unpublished data), 26:2 in *Spheciospongia* (1), and 28:3 in *Suberites* (1).

High levels of C_{24}-C_{30} fatty acids are certainly widespread if not ubiquitous throughout all subdivisions of the class Demospongiae of the phylum Porifera. They are common to both the Tetractinomorpha and Ceractinomorpha subclasses, and are present in all seven orders examined. Since these C_{24}-C_{30} fatty acids are so characteristic of the Demospongiae but are not generally found in nonsponge organisms, we propose the term "demospongic acids" as a convenient nomenclature for referring to these compounds as a group. The biochemical significance of demospongic fatty acids in Demospongiae tissue membranes is discussed in detail elsewhere (3).

Do demospongic acids also occur in the other two classes of sponges in the phylum Porifera, i.e., the Calcarea and the Hexactinellida? Our efforts to answer this question have so far been frustrated by the lack of suitable tissue samples. Calcarea specimens received to date have all been so heavily contaminated with algae that reliable data on the sponge fatty acids could not be obtained. The Hexactinellida grow only in deep sea locations (>1000 m), and we have been unable to obtain any living specimens of these animals. We would appreciate hearing from any reader who could help us in obtaining Calcarea or Hexactinellida samples for analysis.

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