Identification of the New 11,15-Icosadienoic Acid and Related Acids in the Sponge Amphimedon complanata

Néstor M. Carballeira, and José Restituyo

J. Nat. Prod., **1991**, 54 (1), 315-317• DOI:
10.1021/np50073a043 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 1, pp. 315-317, Jan-Feb 1991

IDENTIFICATION OF THE NEW 11,15-ICOSADIENOIC ACID AND RELATED ACIDS IN THE SPONGE *AMPHIMEDON COMPLANATA*

NÉSTOR M. CARBALLEIRA* and JOSÉ RESTITUYO

Department of Chemistry, University of Puerto Rico, Rio Piedras, Puerto Rico 00931

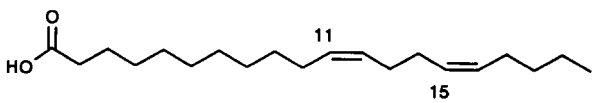
ABSTRACT.—The total phospholipid fatty acids from the Caribbean sponge *Amphimedon complanata* were studied, revealing the presence of the new 11,15-icosadienoic acid [**1**], the rare 7-methyl-6-hexadecenoic acid [**2**], and the recently discovered 6,11-icosadienoic acid. These acids were mainly encountered in phosphatidylethanolamine and phosphatidylcholine.

Sponges have attracted the attention of natural products chemists because of their versatility in using different metabolic pathways that have no counterpart in the terrestrial world. Interesting sponges have been the *Amphimedon* sp., which present metabolites of different origin (1–4), including phospholipid α-hydroxy fatty acids from the Caribbean sponge *Amphimedon compressa* (5). In the present study we have found that the Caribbean sponge *Amphimedon complanata* Duchaffaing (family Nephatidae, order Haplosclerida) contains the new 11,15-icosadienoic acid [**1**], the rare 7-methyl-6-hexadecenoic acid [**2**], and the recently discovered 6,11-icosadienoic acid. Herein we report the results of our investigation.

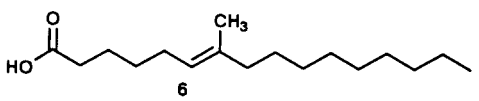
The complete phospholipid fatty acids identified in *A. complanata* are presented in Table 1. The mixture was particularly interesting for the presence of several diunsaturated fatty acids, in particular some rare icosadienoic acids which were noteworthy of study. The characterization of the new structures

TABLE 1. The Phospholipid Fatty Acids from *Amphimedon complanata*.

Fatty Acid	Abundance (%)
Tetradecanoic (14:0)	1.8
Methyltetradecanoic (15:0)	1.5
6-Hexadecenoic (16:1)	1.0
Hexadecanoic (16:0)	5.7
8-Heptadecenoic (17:1)	0.5
7-Methyl-6-hexadecenoic (17:1) [**2**]	2.0
9-Heptadecenoic (17:1)	1.0
Heptadecanoic (17:0)	1.5
Octadecadienoic (18:2)	1.0
9-Octadecenoic (18:1)	3.0
11-Octadecenoic (18:1)	2.0
Octadecanoic (18:0)	9.0
6,11-Icosadienoic (20:2)	0.5
11,15-Icosadienoic (20:2) [**1**]	6.0
5,9-Icosadienoic (20:2)	1.2
11-Icosenoic (20:1)	1.6
Icosanoic (20:0)	3.3
Heneicosanoic (21:0)	4.1
Docosanoic (22:0)	13.7
Tricosanoic (23:0)	3.7
17-Tetracosenoic (24:1)	3.9
19-Tetracosenoic (24:1)	4.2
Tetracosanoic (24:0)	10.0
Pentacosanoic (25:0)	0.8
5,9-Hexacosadienoic (26:2)	1.9
Hexacosanoic (26:0)	0.3
5,9-Octacosadienoic (28:2)	0.7
Octacosanoic (28:0)	0.3
5.9-Nonacosadienoic (29:2)	0.3
5,9,23-Tricontatetraenoic (30:3)	12.8



1



2

was possible by means of gc-ms analysis of several derivatives, such as the pyrrolidine derivatives, which were instrumental for the location of double bonds and branching (6). If an interval of 12 amu, instead of the usual 14, is observed between the most intense peaks of clusters of fragments containing n and $n-1$ carbon atoms in the acid moiety, a double bond is present between carbons n and $n+1$ in the molecule. One novel fatty acid in *A. complanata* presented a mol wt of $[M]^+$ 361 and a base peak at *m/z* 113, confirming it to be the pyrrolidine derivative of an icosadienoic acid. The double bonds in the molecule were readily localized as an interval of 12 amu was observed between fragments at *m/z* 278 (C_{14}) and *m/z* 290 (C_{15}), indicating a Δ^{15} double bond, while a second difference of 12 amu between fragments at *m/z* 224 (C_{10}) and *m/z* 236 (C_{11}) confirmed the position of the second double bond at Δ^{11}. Upon catalytic hydrogenation (PtO_2), the methyl ester derivative of this acid afforded icosanoic acid methyl ester, which co-injected in gc with an authentic sample, thus excluding the possibility of any branching. The lack of branching was also confirmed by nmr as a triplet at 0.8 ppm was observed. To confirm the proposed structure we cleaved the methyl ester of acid **1** with $KMNO_4/NaIO_4$ followed by esterification with HCl/MeOH. The short chain undecanedioic acid dimethyl ester was obtained as one of the fragments, confirming the first double bond in the chain to be at C-11. An Ft-ir spectrum of the methyl esters presented no absorption in the 960–980 cm^{-1} region, indicating cis rather than trans unsaturation. These data indicate that the new acid is 11,15-icosadienoic acid [**1**], which apparently has not been reported before in nature. A second dienoic acid in the phospholipid fatty acid mixture was identified as 6,11-icosadienoic acid by using the same characterization strategy outlined before. This other fatty acid has recently been reported from the sponge *Eurypongia rosea* (7).

The phospholipid fatty acid mixture from *A. complanata* also revealed a mixture of heptadecenoic acids. Two of these acids were readily characterized as the 8- and 9-heptadecenoic acids, but a third was not readily identifiable. The pyrrolidine derivative of this last acid showed a mol wt at $[M]^+$ 321 and a base peak at *m/z* 113. More informative for the characterization was a prominent peak at *m/z* 208 with an abnormal abundance of 10% together with a diminished peak at *m/z* 180. This implies methyl substitution at C-7, which was corroborated by hydrogenating the acid to 7-methylhexadecanoic acid methyl ester. The double bond position was determined to be at C-6 by a difference of 12 amu between fragments at *m/z* 154 (C_5) and at *m/z* 166 (C_6) in the mass spectrum of the corresponding pyrrolidide. The fatty acid is thus the rare 7-methyl-6-hexadecenoic acid [**2**], which has not previously been detected in a sponge or as a component of a phospholipid. It has only been isolated before in whale oils by both Pascal and Ackman (8) and Sano (9).

The phospholipid composition of *A. complanata* was analyzed by tlc. The principal phospholipids in this sponge were phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, and phosphatidylcholine.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The methyl esters were analyzed by electron impact gc-ms using either a Hewlett Packard 5995 A gas chromatograph-mass spectrometer or a Hewlett Packard 59970 MS ChemStation equipped with a 30 m × 0.25 mm nonpolar fused silica column coated with DB-1. Gc/Ft-ir spectra were recorded on a Nicolet 740 FT IR spectrometer.

SPONGE MATERIAL.—*A. complanata* was collected July 7, 1989 near the shelf edge of La Parguera, Puerto Rico at a depth of 80 ft. The sponge was kindly classified by Dr. Vance Vicente. A voucher specimen is on file at the National Museum of Natural History of the Smithsonian Institution.

EXTRACTION AND ISOLATION OF PHOSPHOLIPIDS.—The sponge (500 g) was washed in sea water, carefully cleaned of all nonsponge debris, and cut into small pieces. Immediate extraction with 700 ml of $CHCl_3$-MeOH (1:1) yielded the total lipids. The neutral lipids, glycolipids, and phospholipids (100 mg) were separated by cc on Si gel (60–200 mesh) using a procedure similar to that of Privett *et al.* (10). The phospholipid classes were investigated by preparative tlc using Si gel G and $CHCl_3$-MeOH-H_2O (25:10:1) as solvent and comparing with authentic samples.

PREPARATION OF FATTY ACID DERIVATIVES.—The fatty acyl components of the phospholipids were obtained as their methyl esters by reaction of the phospholipid fraction with methanolic HCl (11) followed by cc purification eluting with *n*-hexane–Et_2O (9:1). Approximately 30 mg of fatty methyl esters were obtained. For the location of double bonds, *N*-acylpyrrolidide derivatives were prepared by direct treatment of the methyl esters with pyrrolidine-HOAc (10:1) in a capped vial (3 h at 100°) followed by ethereal extraction from the acidified solution and purification by preparative tlc. Hydrogenations were carried out in 10 ml of absolute MeOH and catalytic amounts of PtO_2. Mass spectral data of the key fatty acid methyl esters for this discussion follow.

11,15-Icosadienoic acid methyl ester.—Ms *m/z* (rel. int.) $[M]^+$ 322 (6), 292 (13), 290 (3), 250 (4), 248 (3), 229 (3), 208 (4), 199 (2), 194 (2), 185 (3), 177 (2), 171 (2), 164 (4), 157 (3), 150 (7), 143 (8), 137 (10), 129 (10), 123 (17), 121 (12), 95 (64), 87 (40), 81 (74), 79 (43), 74 (57), 69 (58), 67 (80), 55 (100); ir ν max 2980, 2900, 2820, 1725, 1450, 1155 cm^{-1}; ^1H nmr 5.3 (m, 4H), 3.6 (s, 3H), 2.3 (t, 2H), 1.2 (m, 18), 0.8 (t, 3H).

11,15-Icosadienoic acid pyrrolidide.—Ms *m/z* (rel. int.) $[M]^+$ 361 (7), 332 (0.4), 318 (0.7), 304 (0.9), 290 (0.6), 278 (0.9), 264 (2.8), 250 (2.6), 236 (1.2), 224 (1.1), 210 (2.7), 196 (2.2), 182 (3.6), 168 (5.2), 154 (3.1), 140 (6.8), 126 (46), 113 (100), 98 (22), 85 (13), 72 (25), 70 (33), 67 (17), 55 (34).

ACKNOWLEDGMENTS

Dr. Vance Vicente from the United States Department of the Interior, Fish and Wildlife Service, collected and classified the sponge. This work was supported by the National Science Foundation under Grant No. CHE-8715649 and the National Institutes of Health under Grant No. S06 RR08102-17.

LITERATURE CITED

1. S. Hirsch and Y. Kashman, *Tetrahedron*, **45**, 3897 (1989).
2. J.R. Fookes, M. Garson, J.K. MacLeod, B.W. Skelton, and A. White, *J. Chem. Soc., Perkin Trans. 1*, 1003 (1988).
3. M. Garson, *J. Chem. Soc., Chem. Commun.*, 35 (1986).
4. F.J. Schmitz, S.K. Agarwal, S.P. Gunasekera, P.G. Schmidt, and J.N. Shoolery, *J. Am. Chem. Soc.*, **105**, 4835 (1983).
5. N. Carballeira and M.R. Lopez, *Lipids*, **24**, 89 (1989).
6. B.A. Andersson, *Prog. Chem. Fats Other Lipids*, **16**, 279 (1978).
7. N. Carballeira and M.E. Maldonado, *Lipids*, **24**, 665 (1989).
8. J.C. Pascal and R.G. Ackman, *Lipids*, **10**, 478 (1975).
9. Y. Sano, *Yukagaku*, **16**, 605 (1967).
10. O.S. Privett, K.A. Dougherty, W.L. Erdahl, and A. Stolyhwo, *J. Am. Oil Chem. Soc.*, **50**, 516 (1973).
11. J.P. Carreau and J.P. Dubacq, *J. Chromatogr.*, **151**, 384 (1978).

Received 1 August 1990