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IDENTIFICATION OF THE NEW 10,15-EICOSADIENOIC ACID AND RELATED ACIDS IN THE OPISTHOBRANCH *HAMINAEA TEMPLADOI*

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ABSTRACT.—The free fatty acids of the Mediterranean opisthobranch *Haminaea templadoi* were isolated and characterized, revealing the presence of the new 10,15-eicosadienoic acid [**1**] and several unusual cis Δ^6 monoenoic acids, such as (*Z*)-6-tetradecenoic acid, (*Z*)-6-pentadecenoic acid, and (*Z*)-6-heptadecenoic acid. These results reveal some previously unrecognized fatty acids in mollusks.

The fatty acid composition of mollusks has been amply studied by several groups (1–5). The principal fatty acids in these organisms have been recognized to be the saturated acids palmitic (16:0) and octadecanoic (18:0), the monoene 18:1 (n-9), and polyunsaturated fatty acids belonging principally to the n-6 and n-3 series, such as 20:4 (n-6), and 22:5 (n-3). Furthermore, very characteristic of mollusks has also been the presence of two C_{20} non-methylene interrupted dienes (NMID), namely 20:2, $\Delta^{5,11}$ and 20:2, $\Delta^{5,13}$. The major presumptive monoene precursors of these NMID have been established as 20:1, Δ^{11} and 20:1, Δ^{13}, which upon Δ^5 desaturation are transformed into the above NMID (6,7).

Although the lipids from most major classes of mollusks have received particular attention, little is known about the lipids from the subclass Opisthobranchia. This observation prompted us to study the free fatty acids from the Mediterranean opisthobranch *Haminaea templadoi* Turton and Kingston (family Haminoeidae, order Cephalaspidea), a recently described opisthobranch from the Atlantic Iberian coast (8). The opisthobranchs have been particularly interesting for their alarm pheromones (9–11). For example, the Mediterranean opisthobranch *Haminoea navicula* possesses the alarm pheromones haminol A and haminol B (9).

The free fatty acids from the opisthobranch *H. templadoi* are presented in Table 1. The mixture was particularly interesting for the presence of several monounsaturated fatty acids, in particular some rare Δ^6 acids. The principal fatty acids were hexadecanoic acid (16:0), 9- and 11-octadecenoic acids (18:1), octadecanoic acid (18:0), and the hitherto unreported 10,15-eicosadienoic acid [**1**]. The ^{1}H-nmr spectrum of the complete fatty acid mixture displayed a typical fatty acid spectrum, i.e., an intense peak at 1.2 ppm due to the methylenes in the fatty acyl chain, a triplet at 0.85 ppm revealing terminal methyl groups, another triplet at 2.3 ppm due to the methylene groups α to the carbonyl, and the double bond absorptions observed at 5.35 ppm. For further characterization, the total mixture was esterified with HCl/MeOH,

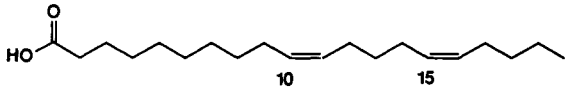

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TABLE 1. The Free Fatty Acids from *Haminaea templadoi*.[a]

Fatty Acid	Abundance (%)	Fatty Acid	Abundance (%)
6-Tetradecenoic (14:1)	0.1	11-Octadecenoic (18:1)	14.0
Tetradecanoic (14:0)	5.0	13-Octadecenoic (18:1)	1.9
6-Pentadecenoic (15:1)	0.1	Octadecanoic (18:0)	13.0
Methyltetradecanoic (15:0)	0.8	11-Nonadecenoic (19:1)	0.4
Pentadecanoic (15:0)	0.1	Methyloctadecanoic (19:0)	0.1
4,8,12-Trimethyl-tridecanoic (16:0)	0.4	Nonadecanoic (19:0)	0.1
6-Hexadecenoic (16:1)	0.2	5,8,11,14-Eicosa-tetraenoic (20:4)	0.7
9-Hexadecenoic (16:1)	8.0	10,15-Eicosadienoic (20:2)	6.0
Hexadecanoic (16:0)	20.0	7-Eicosenoic (20:1)	0.2
Methylhexadecanoic (17:0)	6.0	11-Eicosenoic (20:1)	2.2
6-Heptadecenoic (17:1)	0.1	13-Eicosenoic (20:1)	0.4
8-Heptadecenoic (17:1)	0.1	Eicosanoic (20:0)	0.1
9-Heptadecenoic (17:1)	0.4	13-Docosenoic (22:1)	0.8
11-Heptadecenoic (17:1)	0.4	Tricosanoic (23:0)	0.1
Heptadecanoic (17:0)	1.8	Tetracosanoic (24:0)	0.1
8-Octadecenoic (18:1)	0.1	Pentacosanoic (25:0)	0.1
9-Octadecenoic (18:1)	13.0	Hexacosanoic (26:0)	0.1

[a]Trace amounts of the 22:5 (n-3) and 22:6 (n-3) acids were also detected.

and the corresponding fatty acid methyl esters were prepared as described previously (12).

The most interesting fatty acid from Table 1 turned out to be the NMID 10,15-eicosadienoic acid [**1**]. The mass spectrum of the corresponding fatty acid methyl ester displayed a molecular ion at *m/z* 322, an $[M - MeOH]^+$ peak at *m/z* 290, and a base peak at *m/z* 67, typical of eicosadienoic acids. The pyrrolidide derivatives were instrumental for the location of the double bonds. For example, 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 (13). The pyrrolidide derivative of this acid displayed a mol wt of $[M]^+$ 361 and a base peak at *m/z* 113, confirming it to be the pyrrolidine derivative of an eicosadienoic 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* 210 (C-9) and *m/z* 222 (C-10) confirmed the position of the second double bond at Δ^{10}. To further confirm the double bond positions we cleaved the methyl ester of **1** with $KMnO_4/NaIO_4$ followed by esterification with HCl/MeOH (14). The short chain decanedioic acid dimethyl ester was obtained as one of the fragments, confirming the first double bond in the chain to be at C-10. The other fragments obtained were pentanoic acid methyl ester and the pentanedioic acid (glutaric) dimethyl ester. The identity of the latter compounds was confirmed by gc-coinjection with authentic samples. For example, commercially available decanedioic acid (sebacic) was coinjected in the gc, as the dimethyl ester, with the C-10 fragment obtained from the above reaction. Upon catalytic hydrogenation (PtO_2), the methyl ester derivative of **1** afforded eicosanoic acid methyl ester, which co-injected in gc with an authentic sample, thus exclud-

ing the possibility of any branching. An Ft-ir spectrum of the methyl esters presented no absorption in the 960–980 cm^{-1} region, indicating cis rather than trans unsaturation. This data indicates that the new acid is 10,15-eicosadienoic acid [**1**], which has not been previously recognized to exist in nature.

The monoenes in the mixture, as most of the other acids as well, were characterized by means of gc-ms of methyl ester derivatives, equivalent chain lengths (ECL) values, hydrogenation to the corresponding saturated methyl esters, and derivatization to dimethyl disulfides (15). For example, consider the characterization of the rare (*Z*)-6-heptadecenoic acid and the (*Z*)-8-heptadecenoic acid. The 6- and 8-heptadecenoic acid methyl esters afforded, after hydrogenation in MeOH with PtO_2 as catalyst, methyl heptadecanoate as confirmed by gc co-injection with an authentic sample. This experiment excludes the possibility of any methyl branching. The double bond positions were determined by derivatization to methyl 6,7-bis(methylthio)heptadecanoate and methyl 8,9-bis(methylthio)heptadecanoate. In the former the double bond was found to be at C-6 from the ms fragments at *m/z* 175 $[C_8H_{15}SO_2]^+$ and at *m/z* 201 $[C_{12}H_{25}S]^+$, while in the second isomer the double bond was found to be at C-8 from the favorable fragmentations at *m/z* 203 $[C_{10}H_{19}SO_2]^+$ and at *m/z* 173 $[C_{10}H_{21}S]^+$. The 6-heptadecenoic acid has been detected in the open Mediterranean sea before (16), while 8-heptadecenoic acid is quite rare and was reported in the fin whale *Balaenoptera physalus* (17).

These results revealed some previously unrecognized fatty acids in the opisthobranchia. The identification of the Δ^6 acids (*Z*)-6-tetradecenoic, (*Z*)-6-heptadecenoic acids indicates that these organisms are either able to use Δ^6 desaturases in fatty acid biosynthesis, or that they can accumulate these acids by filter-feeding (18). For example, *cis*-6-hexadecenoic acid (and other Δ^6 acids) have been found in seawater and in marine sediments (19,20). Also, a symbiotic relationship with some microorganisms cannot be ruled out. The identification of the novel 10,15-eicosadienoic acid [**1**] adds a new dimension to the possible NMID in mollusks. Work is in progress studying other opisthobranchs for unusual lipids.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The methyl esters were analyzed by electron impact gc-ms using 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 and nmr spectra on a Varian Gemini 200 MHz spectrometer.

OPISTHOBRANCH MATERIAL.—*H. templadoi* (25 specimens, average length 1 cm) were collected by hand in the intertidal zone of the coast of Huelva, Spain on May 1990. They were stored in Me_2CO at −5° for 1 year. A voucher specimen is on file at the Facultad de Ciencias del Mar, Universidad de Cádiz.

EXTRACTION AND ISOLATION OF FATTY ACIDS.—Filtration and evaporation of the solvent (Me_2CO) led to a residue that was partitioned between H_2O and CH_2Cl_2. The organic layer was dried over Na_2SO_4 and the solvent evaporated. The resulting extract was chromatographed on Si gel column eluting with mixtures of hexane/EtOAc in increasing polarity. The resulting fractions were combined according to their nmr spectra, some of which showed the presence of complex fatty acid mixtures (92 mg).

PREPARATION OF FATTY ACID DERIVATIVES.—The fatty acids were methylated with methanolic HCl followed by cc purification eluting with *n*-hexane–Et_2O (9:1). The fatty acids were characterized as a mixture. 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. Dimethyl disulfide derivatives were prepared by dissolving the esters (2 mg) in dimethyldisulfide (0.2 ml) and adding a solution (0.05 ml) of iodine in Et_2O (60 mg/ml). The solution was heated at 50° for 24 h, and the product was purified as described previously. Hydrogenations were carried out in 10 ml of MeOH and catalytic amounts of PtO_2. Mass spectral data for the key fatty acid methyl ester for this discussion follow.

10,15-Eicosadienoic acid methyl ester.—Ms *m/z* (rel. int.) $[M]^+$ 322 (1.5), 291 (1), 290 (0.7), 251 (0.1), 210 (0.2), 205 (0.2), 192 (0.3), 191 (0.25), 178 (0.4), 165 (0.4), 164 (0.7), 150 (1.4), 149 (1.4), 136 (1.4), 135 (1.8), 123 (2.7), 110 (5), 109 (7.8), 96 (15), 95 (24), 83 (10), 82 (22), 81 (52), 74 (64), 69 (27), 68 (25), 67 (81).

10,15-Eicosadienoic acid pyrrolidide.—Ms *m/z* (rel. int.) $[M]^+$ 361 (9.5), 346 (0.23), 334 (0.22), 332 (0.42), 330 (0.10), 318 (0.4), 306 (0.24), 304 (0.46), 292 (0.34), 290 (0.45), 280 (0.16), 278 (0.94), 276 (0.52), 266 (0.37), 264 (2.0), 262 (0.23), 252 (0.69), 250 (1.60), 248 (0.42), 238 (0.49), 236 (0.77), 224 (0.58), 222 (0.85), 220 (0.37), 212 (0.11), 210 (1.53), 208 (1.1), 196 (1.32), 194 (1.27), 182 (2.0), 180 (1.5), 170 (0.22), 168 (3.5), 166 (1.06), 156 (0.28), 154 (4.74), 152 (1.2), 142 (0.46), 140 (4.7), 138 (0.6), 128 (0.8), 126 (34), 124 (1.2), 113 (100), 98 (43), 85 (23), 81 (22), 67 (50), 56 (30).

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