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AN UNUSUAL FATTY ACID COMPOSITION FOR A FRESH-WATER MUSSEL, *UNIO TUMIDUS*, FROM BULGARIA

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ABSTRACT.—A combination of hplc in the silver ion mode and gc-ms of picolinyl ester derivatives was used to identify the fatty acids in a fresh-water mussel, *Unio tumidus*, from the Danube in Bulgaria. A number of novel fatty acids were found, including 14-methylpentadec-6-enoic and 17-methyloctadec-8-enoic acids. Eicos-7-enoic acid was a major component.

It is well known that marine invertebrates contain a distinctive range of fatty acid components, including many positional isomers of monoenes, non-methylene-interrupted dienes, and branched-chain components (1). Some of these must arise by distinctive metabolic processes in the organisms, and others may come from the food chain. Much less is known of the composition of fatty acids in related species from fresh water. We have recently investigated the composition of a mussel species, *Mytilus galloprovincialis*, from the Bulgarian coast of the Black Sea (2). New methodologies, including silver ion hplc of the fatty acid methyl esters to simplify the complex mixtures and gc-ms of the picolinyl ester derivatives, were employed to obtain definitive structural information on all the fatty acids present. Similar procedures have now been employed to characterize the fatty acids of a fresh-water mussel, *Unio tumidus* Retzius (family Unionidae, class Bivalvia), from the Danube adjacent to the Black Sea. The water in which it was found is now heavily polluted.

The composition of the fatty acids in the total lipid extract is listed in Table 1. In addition to the usual fatty acids expected in aquatic organisms, such as 16:0, 18:0, 9-18:1, and 9,12-18:2, and polyunsaturated compounds, such as 18:3 (n-3), 20:4 (n-6), 20:5 (n-3), and 22:5 (n-3), one of the most abundant components was eicos-7-enoic acid (7-20:1). The mass spectrum of the picolinyl ester derivative shows an appreciable molecular ion ($m/z = 401$), and the double bond is located by a gap of 26 amu from fragmentations on either side of it, i.e., between $m/z = 206$ and 232, and especially by a characteristic doublet at $m/z = 246$ and 260 (3). From its behavior on silver ion hplc [e.g., it cochromatographs with other cis esters and long after those of trans configuration (4)], it is the 7*Z* isomer. This acid has previously been reported at low levels only in a mussel of marine origin, although it was a major component of a sea snail (*Rapana thomasiana*) from the same area (2). Small amounts of 7-19:1, 7-21:1, and 7-22:1 were detected in *U. tumidus*. The most abundant monoenes were the 9-16:1 and 9-18:1, and 7-20:1 has nothing in common with these fatty acids; a separate biosynthetic pathway is indicated.

Saturated branched-chain fatty acids have not been reported previously from fresh-water molluscs, but this may be due to deficiencies in the analytical methodology. In addition in this study, several branched-chain monoenes were found in small amounts and some of these may not have been described elsewhere, other than in a sponge from the Black Sea (Stefanov, Seizova, Brechany, and Christie, unpublished). We were unable to find previous reports of 14-methylpentadec-6-enoate and 17-methyloctadec-8-enoate. Presumably the latter is produced by chain elongation of 15-

TABLE 1. The Fatty Acid Composition of the Total Fatty Acids of the Mussel *Unio tumidus*.

Fatty acid[a]	Abundance (Wt %)
14:0	0.7
4,8,12-trimethyl-13:0	1.3
13-methyl-6-14:1	0.1
15:0	0.5
i-15:0	0.2
14-methyl-6-15:1	0.1
16:0	14.8
7-16:1	0.1
9-16:1	10.1
11-16:1	0.1
15-methyl-6-16:1	0.4
15-methyl-9-16:1	0.1
i-16:0	0.5
ai-16:0	0.3
17:0	1.2
phytanic acid	0.1
9-17:1	0.4
18:0	21.4
5-18:1	0.3
9-18:1	6.0
11-18:1	2.8
13-18:1	0.1
17-methyl-8-18:1	0.1
9,12-18:2	3.5
11,14-18:2	0.1
19:0	0.1
7-19:1	0.4
9,12,15-18:3	2.7
6,9,12,15-18:4	0.9
20:0	0.1
7-20:1	9.2
11-20:1	0.2
13-20:1	0.1
5,11-20:2	0.1
7,13-20:2	0.7
11,14-20:2	0.6
5,11,14-20:3	0.2
5,8,11,14-20:4	3.5
8,11,14,17-20:4	0.2
5,8,11,14,17-20:5	5.6
7-21:1	0.1
7-22:1	0.2
7,13-22:2	2.4
7,13,16-22:3	0.6
7,10,13,16-22:4	0.5
4,7,10,13,16-22:5	0.6
10,13,16,19-22:4	0.3
7,10,13,16,19-22:5	3.4
4,7,10,13,16,19-22:6	1.0

[a]Plus trace amounts (<0.05%) of i-14:0, ai-14:0, ai-18:0, 20:0, 22:0, 23:0, 24:0, 9-oxo-18:0, 13-oxo-18:0, (6*E*)-18:1, (9*E*)-16:1, (9*E*)-18:1, (11*E*)-18:1, (11*Z*)-17:1, (9*Z*)-22:1 and (13*Z*)-22:1.

methylhexadec-6-enoic acid [fatty acids with double bonds in position 6 are not uncommon in marine sources (5)]. Dienes and trienes with several methylene groups between the double bonds, of the type found here, are also common in marine invertebrates (1,2) and have been reported from a fresh-water bivalve (6).

Although the lipids of *U. tumidus* contained the usual range of polyenoic fatty acids of the (n-6) and (n-3) families, these were present at relatively low concentrations in comparison to other fresh-water mussels that have been examined. For example, 20:4 (n-6) was the most abundant fatty acid in the gill tissue of *Ligumia subrostrata* (7), and it was a major component of other species (6,8). Perhaps surprisingly, the ratio of (n-6) to (n-3) fatty acids in *U. tumidus* was 0.7, similar to that in marine invertebrates (1), where the relatively high proportions of (n-3) fatty acids are presumed to be derived from marine plankton in the diet.

EXPERIMENTAL

SAMPLES.—The fresh-water mussel *U. tumidus* was collected from the River Danube near the town of Rousse in Bulgaria in August 1989. It was classified by Dr. S. Andreev, and a voucher specimen was deposited at the Museum of Natural History, Sofia, Bulgaria. Samples were washed in tap H_2O and immediately immersed in EtOH for transport to the laboratory. The total lipids were extracted with $CHCl_3$-MeOH (2:1), giving 105 mg lipid/gm dry wt, of which 51% was neutral lipid and 49% phospholipid by silica cc. The total lipids were saponified, non-saponifiables were removed by extraction, and the fatty acids were methylated.

SILVER ION HPLC.—The silver ion column, ChromSpher Lipids™, was donated by Dr. Stephan Rose of Chrompack Ltd. (Middelburg, Netherlands). For micro-preparative purposes, 1–2 mg of the total methyl esters were applied to the column and eluted with a binary gradient, with mixtures of CH_2Cl_2-dichloroethane (1:1) (Solvent A) and CH_2Cl_2-dichloroethane-MeOH-MeCN (45:45:5:5) (Solvent B), and a linear gradient from 100% A to 95% A/5% B over 15 min then to 80% A/20% B over a further 25 min and finally to 50% A/50% B over 10 min more at a flow rate of 1 ml/min, with evaporative light-scattering detection (2). Fractions differing in de-

gree of unsaturation were collected via a stream-splitter. They were identified by their chromatographic behavior relative to authentic standards and by gc-ms.

GC-MS.—The fatty acids were converted to the picolinyl ester derivatives (9) and analyzed on a fused-silica capillary column (25 mm × 0.25 mm i.d.) coated with a cross-linked (5% phenylmethyl) silicone (CP-Sil 8^{TM}), with the column outlet connected directly into the ion source of a Hewlett Packard Model 5970 Mass Selective Detector, operated at an ionization energy of 70 eV.

The picolinyl ester of eicos-7-enoic acid showed diagnostic peaks at $m/z = 401$ $[M]^+$ 92, 108, 151, and 164 (ester moiety), and 206, 232, 246, and 260 (double bond). That of 17-methyloctadec-8-enoic acid had ions at $m/z = 387$ $[M]^+$ and at 220, 246, 260, and 274 (indicative of a double bond in position 8), together with a gap of 28 amu between 344 and 372 (isomethyl branch). That of 14-methylpentadec-6-enoic acid had ions at $m/z = 345$ $[M]^+$ and 246 (indicative of a double bond in position 6), together with a gap of 28 amu between 302 and 330 (isomethyl branch).

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