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Furan Fatty Acids of Some Brackish Invertebrates From the Caspian Sea

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ABSTRACT. Eleven furan (F) fatty acids were detected in total lipids from three invertebrates, crab *Rhithropanopeus harrisii*, crayfish *Astacus leptodactylus eichwaldi* and bivalvia *Mytilaster lineatus*, collected in the Caspian Sea. The major component of F-acids was isolated and identified using Ag^+-TLC (silver ion chromatography), preparative HPLC (high-performance liquid chromatography), NMR (nuclear magnetic resonance), HR-MS (high resolution-mass spectrometry), and GC-MS (gas-chromatography-mass spectrometry). The predominance of F_3, F_4 and F_6 acids was demonstrated. COMP BIOCHEM PHYSIOL 114B;3:317–320, 1996.

KEY WORDS. Furan fatty acids, brackish invertebrates, bivalvia, crab, crayfish, Caspian Sea

INTRODUCTION

Furan (F) acids comprise an interesting group of organic compounds (1,18). The mentioned acids have been previously isolated from twenty plant species, the algal *Chlorella* sp., the fungal species *Agaricus bisporus*, the yeast *Sacharamyces cerevisidae* (14) from two marine algae species *Isochrysis* sp. and *Phaeodactylum tricornutum* (2) and from the seed oils of other plants (15,16,23).

Within the animal kingdom, F-acids have been found in the liver of some freshwater fishes (8–10,12,25–28,34) in the crayfish *Procambarus clarii* (17,24), in the marine sponge species *Dictyonella incisa* (6) and *Hymeniacidon hauraki* (30) and in the soft corals *Sarcophyton glaucum* and S. *gemimatum* (11).

During recent years, both metabolism (33) and biogenesis (2,3,32) of these unusual fatty acids were studied, but their biological role, if any, remained unknown. The inhibition of bacterial urease by autoxidation of F-acids was noted (31), and in some studies the active lipoxygenases that produce F-acids were observed (4,5,35). The different medical aspects some F-acids, for instance, such as 3-carboxy-4-methyl-5-propyl-2-furan propanoic acid have been reported (19–22,29).

During the study of the lipids of some Caspian invertebrates (7), we discovered a number of F-acids, which are reported in this work.

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Abbreviations–GC, gas chromatography; MS, mass spectrometry; TLC, thin-layer chromatography.

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MATERIALS AND METHODS

Materials

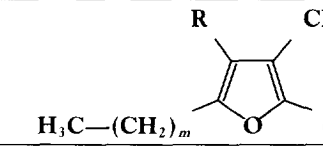
All invertebrates, crab *Rhithropanopeus harrisii* Gold, crayfish *Astacus leptodactylus eichwaldi* Bott. and bivalvia *Mytilaster lineatus* Gmel. (= *Mytilus minilus* Poli. = *Mytilus lineatus* Gmel.) were sampled during September 1991 in the site located 70 km south of Krasnovodsk on the western coast of the Caspian Sea (Republic of Turkmenistan) from 2 to 3 m depths; the water temperature was 20°C. The animals were worked-up immediately after sampling as described by Dembitsky *et al.* (7).

Separation and Detection of F-Acids

The enriched fraction of F-acids esters were prepared (with minor modification) according to Scrimgeour (34). The total methyl esters of fatty acids were chromatographed by Ag^+-thin-layer chromatography (TLC) on 20 × 20-cm plates coated with 0.5-mm layer of silica gel G impregnated with 10% $AgNO_3$. The methyl esters were separated by TLC with hexane-diethyl ether (90:10) and after visualization under ultraviolet light, the silica gel between monoenes and saturates was scraped off the plate and then extracted with ether. The extract was dissolved in 3 ml methanol, 500 mg urea was added and dissolved by warming and the solution was crystalized at −15°C for 3 hr. After filtration, the filtrate was acidified and extracted by hexane.

Methyl esters were identified and quantified (by total ion current, with methyl acid as internal standard) in a Shimadzu QP-1000 apparatus (Shimadzu, Kyoto, Japan) equipped with a fused silica capillary column (60 m × 0.32 mm ID × 0.25 μm/Supelcowax-10; Supelco, Gland, Switzerland); splitless injection, carrier gas hydrogen. Oven temperature was programmed from 100 to 240°C at a rate of

TABLE 1. Furan fatty acid (as methylesters) from Caspian invertebrates



Structure F-acids	n	m	R	ECL	CI* QM	EI† M+	MS M-Alkyl	M-Alkyl-Ester	Furan Ring
F1	8	2	Me	20.66	309	308 (18.5)	279 (12.3)	151	123 (12.3)
F2	8	4	H	21.55	323	322 (17.3)	265 (8.7)	165	109 (16.3)
F3	8	4	Me	22.08	337	336 (21.0)	279 (23.4)	179	123 (19.4)
F4	10	2	Me	22.67	337	336 (19.2)	307 (25.8)	151	123 (15.9)
F5	10	4	H	23.57	351	350 (23.2)	293 (9.9)	165	109 (16.1)
F6	10	4	Me	24.10	365	364 (24.8)	307 (35.3)	179	123 (17.8)
F7	12	2	Me	24.08	365	364 (21.5)	335 (29.0)	151	123 (16.4)
F8	12	4	H	25.59	379	378 (26.9)	321 (11.0)	165	109 (19.2)
F9	12	4	Me	26.11	393	392 (27.3)	335 (34.6)	179	123 (21.4)
F10	14	4	H	27.61	407	406 (26.7)	349 (12.7)	165	109 (18.7)
F11	14	4	Me	28.12	421	420 (29.5)	363 (38.9)	179	123 (23.9)

*Quasimolecular ion (M + 1), i.e., base peak for all F esters.
†Values in parentheses are intensities as percentage of base peak.
n = 8,10,12,14; m = 2,4; R = Me = CH_3, or H; ECL = equivalent chain length; QM = quasimolecular ion; M+ = characteristic ions in the mass spectra of Furan fatty acid methyl esters.

4°C/min. Ionization energy was 70 eV, and electron multiplier voltage was 2.5 kV.

Separation of the Main Components

The hexane extract was evaporated, dissolved in acetonitrile and separated by reverse phase high-performance liquid chromatography-10. The semipreparative Gradient LC System G-1 (Shimadzu) with two LC-6A pumps (2 ml min^{-1}), an SCL-6A system controller, an SPD ultraviolet detector (226 nm), an SIL-1A sample injector and a C-R3A data processor, with an analytical column 250 mm × 4 mm ID, packed with SGX-C18 with 5-mm particles was used (Tessek, Prague). Elution was performed with 90:10 acetonitrile-water; the effluent (single peaks) were collected manually. The F esters F_6 had retention times of 24.5 min and 92% purity (checked by gas chromatography mass spectroscopy [GC-MS]).

The main component of all species examined in this work had the following spectra:

^{13}C-NMR ($CDCl_3$, 75 MHz, ppm, Brucker AM 300): 173.5 ($COOCH_3$), 149.5, 146.1, 115.7, 114.9 (the furan ring carbone atoms), 51.6 (OCH_3), 33.1, 31.5, 28.4-26.5, 21.8 (both aliphtic chains), 14.1 (terminal CH_3), 8.4 and 8.3 (the CH_3 groups on furan ring).

^{1}H-NMR ($CDCl_3$, 300 MHz, ppm): 0.87t (J = 7.2 Hz, H-20), 1.17–1.29m (H-4, H-9, H-18, H-19), 1.57m (H-3), 1.68m (H-17), 1.85s and 1.86s ($C_{13,14}$-CH_3), 2.12t (J=7.6 Hz, H-2), 2.59t and 2.60t (J = 7.4 Hz, H-11, H-16), 3.42s (OCH_3).

High resolution MS was determined on the Finnigan MAT 90 mass spectrometer with EI mode: 364.5735 (M^+, $C_{23}H_{40}O_3$, 34%), 333.5390 ($C_{22}H_{37}O_2$, M-CH_3O, 9%), 307.4571 ($C_{19}H_{31}O_3$, M-C_4H_9, 42%), 179.2846 ($C_{12}H_{19}O$, M-$C_9H_{18}COOCH_3$, 100%), 123.1762 ($C_8H_{11}O$, M-C_4H_9, -$C_9H_{18}COOCH_3$, 22%). According to these spectra the main component was F_6.

RESULTS AND DISCUSSION

The isolated methyl esters of F-acids were used for the determination of the physicochemical characteristics of F-acids. GC-MS analysis of the mixture of F-acids (after urea crystalization) is listed in Table 1. Complete identification of the major F-acid found in the samples indicated that it was F6 (see Material and Methods).

The distribution of F-acids isolated from invertebrates is presented in Table 2. The crab species *Rhithropanopeus harrisii* contained 45.2% of the main F-acid component F_6, which occurred in the largest amounts among the F-acids, followed by 26.2% of F_4 and 16.7% F_3. The highest level of F-acid found in the crayfish *Astacus leptodactylus eichwaldi* was also of F_6 at 67.9%. We identified in the mollusc *Mytilaster lineatus*, apart from six F-acids, five acids ranging from F_7 to F_{11} and a small amount (3.7%) of an unidentified F-acid (Table 2). Okajima *et al.* (24) reported that the F-acids in crustaceans occur abundantly only in the freshwater species and not in marine species.

Other invertebrates, including soft corals (11), marine sponges (6,30) and the freshwater crayfish *Procambarus clarkii* (24), were also investigated. The analysis of crayfish yielded seven F-acids (as is usual in fishes), which consisted predominantly of F_3, F_4 and F_6-acids, 43.2%). Thirty kinds of F-acids, which accounted for 28.49% of the total sterol esters of fatty acids measured, were isolated and identified from the hepatopancreas of the crayfish *Procambarus clarkii*

TABLE 2. Distribution of furan fatty acids in Caspian inverterbrates

	Crab *Rh. harrisii*		Crayfish *A. leptodactylus*		Mollusc *M. lineatus*	
Structure F Acids	**1 a**	**1 b**	**2 a**	**2 b**	**3 a**	**3 b**
F1	—	—	1.3	0.01	2.2	0.02
F2	7.1	0.03	6.4	0.05	7.5	0.07
F3	16.7	0.07	11.5	0.09	11.4	0.11
F4	26.2	0.11	10.3	0.08	13.1	0.13
F5	4.8	0.02	2.6	0.02	4.3	0.04
F6	45.2	0.19	67.9	0.53	40.5	0.38
F7	—	—	—	—	5.4	0.05
F8	—	—	—	—	7.4	0.07
F9	—	—	—	—	1.1	0.01
F10	—	—	—	—	1.1	0.01
F11	—	—	—	—	2.2	0.02
Unidentified c	—	—	—	—	3.7	0.03

a, % Composition in F esters mixture; b, % of F esters in whole methylesters; c, probably F6 with additional double bond and three others unidentified F-acids.

(17). The major F-acids found were F_6 (11.1%), F_3 (6.78%) and F_4 (3.45%). A homologue of a shorter chain length was also isolated from the soft coral species *S. glaucum* and *S. gemmatum*. Its structure was determined to be 3,4-dimethyl-5-pentylfuryl-proioic acid (11). The C_{20} and C_{26} long-chain F-acids were isolated from two marine sponges *Dictyonella incisa* and *Hymeniacidon hauraki* (6,30).

According to our analysis, two new F-acids, F_{10} and F_{11}, were isolated from bivalvia *Mytilaster lineatus* at levels of 1.1 and 2.2%, respectively.

The first description of the isolation of a natural fatty acid containing a furan ring system, extracted from the seed oil of *Endocarpus cupressiformis*, was reported by Morris *et al.*(23). The structures of these unusual fatty acids were shown to be 9,12-epoxyoctadeca-9,11-dienoic acid and 5-hexyl-furyl-capryl acid. However, the biosynthesis and the biological role of these acids still remain to be determined (32). Crundwell and Cripps (6a) were able to demonstrate the ease of formation of F-acids from hydroxy-enynoic fatty acids on treatment of the latter with a strong base or during silver nitrate- siligic acid adsorption chromatography. This observation cast doubts on the authenticity of the F-acids in the seedlings (wyerone and its derivatives) containing substituents of shorter chain-lengths (15).

The latex of *Hevea brasiliensis* (16) has been found to contain 90% of a 3-methyl-5-butyl-furyl-pelargonic acid, a triacylglycerol. Some plants, especially mushrooms, yeast and algae, were analyzed by GC-MS (23). In *Agaricus bisporus*, F_3 was found to be predominant; in *Saccharamyces cerevisiae*, only traces of both F_3 and F_6 were detected, whereas in *Chlorella* sp. an F-acid with a propyl side chain ($n = 3$) at F_6 was the main one. In plant oils and in butter (13), a series of F-acids, predominantly F_2, and F_6, were found.

Glass *et al.* (8–10) discovered a series of triand tetra-substituted F-acids in the lipid extracts of freshwater fishes. Eight isomers (F_1–F_8) were identified by GC-MS and are believed to be biosynthetically related. Some of the most interesting results were reported by Gunstone *et al.* (12), which noted a higher content of F-acids in the liver lipids than in the body oils of various species. The content of F-acids found in the liver lipids were roach, male 13%, female 5%; powan, male 1%, female 2%; pike, male 13%; salmon, female 2%; ice fish, male 4%; dogfish, mixed sample 0.6%; cod, female 0.5%, mixed sample, 1%; and in the freshwater mackerel 0.2%.

F-acids were found to be present mainly in the neutral lipids, particularly, in triacylglycerols (80%), which contained very little cholesterol ester (1%). They were practically absent from phospholipids (12). Among animal organs, such as the liver of fishes or the hepatopancreas of invertebrates, accumulations of F-acids were found (8,10, 17,24).

Recently, Spiteller (35) indicated a special group of lipoxygenases, which are continually active in plant cells and which produce F-acids. Only recently was it shown that F-acids are not produced in fish but originate from food sources, ultimately from algae (35).

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