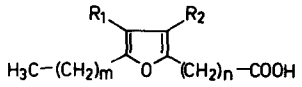
The Common Occurrence of Furan Fatty Acids in Plants

Kerstin Hannemann, Volker Puchta, Ernst Simon, Herta Ziegler, Gunter Ziegler and **Gerhard Spiteller***
Lehrstuhl fur Organische Chemie I der Universitat Bayreuth, D-8580 Bayreuth, Federal Republic of Germany

The observation that F-acids (1) occur in rat chow initiated a search for F-acids in human diet. We observed that the amount of F-acids with a pentyl side chain in α-position taken up with a one-day diet correlates well with the amount of excreted degradation products, the pentyl urofuran acids (2), (3) and (4). Therefore it can be concluded that F-acids with a pentyl side chain are not produced in the human body but are introduced through the diet. The origin of F-acids carrying an α-propyl side chain is less clear. The amount of propyl-urofuran acids (2) and (3) excreted in urine was found in one case out of three to be five times higher than the amount of F-acids carrying a propyl group in α-position taken up by the diet. Therefore, it can presently not be excluded that a portion of the propyl F-acids is produced by the body.

F-acids found in human food are mainly introduced into the body by vegetables and fruits. F-acids were found also in birch leaves in considerable amounts, as well as in grasses, dandelion and clover leaves. Thus, we can conclude that F-acids are common constituents of plants. ***Lipids 24,*** **296-298 (1989).**

F-acids (1), first shown by Glass and Schlenk (1,2) to occur in fish, were later found in soft corals (3) and crayfish (4). Recently, Watanabe showed that they are also present in amphibians and reptiles (5). Our research detected F-acids in mammals (6), including man (7). F-acids in plants have been reported only once: Hasma et al. (8) detected an F-acid in *Hevea brasiliensis.*



1

		m	n	R_1	R_2
a:	F_0	4	6	CH_3	CH_3
b:	F_1	2	8	CH_3	CH_3
c:	F_2	4	8	H	CH_3
d:	F_3	4	8	CH_3	CH_3
e:	F_4	2	10	CH_3	CH_3
f:	F_5	4	10	H	CH_3
g:	F_6	4	10	CH_3	CH_3
h:	[a]	3	10	CH_3	CH_3

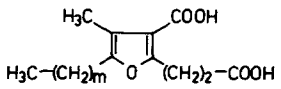
[a]Synthetically produced furan fatty acid.

FORMULA 1

Nevertheless, it was tacitly assumed that F-acids with either 3 or 5 carbons in the aliphatic side chain are produced by animals or man. Biosynthetic experiments with a linoleic acid analog as precursor, in which the side chain had been extended by one carbon atom, were carried out in our laboratory. These experiments failed, because the "labeled" compound was not incorporated into F-acids (9). In the course of these investigations we found that F-acids were introduced into the rats through the diet (9), although previous investigations had shown the absence of F-acids in the rat food (10). This could be explained by the fact that F-acids behave like fatty acids in chromatography and therefore escape detection. If a mixture of fatty acids and F-acids is hydrogenated the resulting tetrahydrofuran acids show a different behavior which allows their separation from fatty acids, even if the latter are present in large quantities (7).

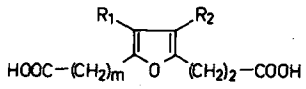
The observation that F-acids are introduced into rats through the diet led us to reexamine whether these compounds may also be part of the human diet. Since it was not known which portion of the diet would contain the F-acids, the whole diet eaten in one day was collected and after extraction an aliquot of the lipids was analyzed for F-acids.

During this experiment we also measured the excretion of the degradation products of F-acids (1), the urofuran acids (2), (3) and (4) (11,12).



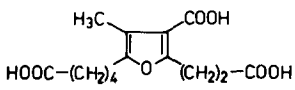
2 m = 2, 4

FORMULA 2



3 m = 2, 4
$R_1 = CH_3$, H
$R_2 = CH_3$, H

FORMULA 3



4

FORMULA 4

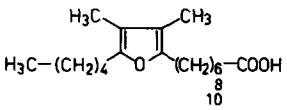
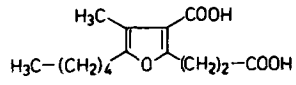
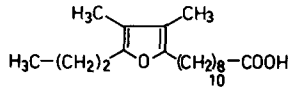
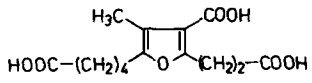
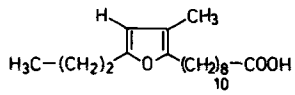
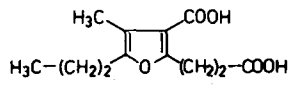
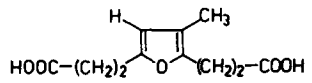
RESULTS

Three people each eating a different diet collected for analysis identical quantities of all foods they consumed over the period of the experiment. These food samples were analyzed for lipids as described in the Materials and Methods section of this paper. During the food collection period, urine samples from the test subjects were collected and analyzed for urofuran acids. Each lipid fraction of the dietary samples was hydrolyzed and hydrogenated to tetrahydrofuran acids (7) which, after methylation, can be more easily separated from fatty acids than the corresponding F-acids (7) by thin-layer chromatography (TLC).

*To whom correspondence should be addressed at Universitätsstrasse 30, Postfach 10 12 51, D-8580 Bayreuth, Federal Republic of Germany.

Abbreviations: FID, flame ionization detector; GC, gas chromatography; MS, mass spectrometry; TLC, thin-layer chromatography; WCOT, wall-coated open tubular.

TABLE 1

F-Acids Determined in Food (μmol/day[a])			Urofuran-Acids Expected in the Urine (μmol/day[a])		
	I	1) 7.01 ± 1.64 2) 7.29 ± 2.29 3) 14.21 ± 4.22		II	1) 6.11 ± 0.15 2) 6.07 ± 0.09 3) 9.87 ± 0.11
					and
	IV	1) 0.66 ± 0.17 2) 3.64 ± 0.87 3) 2.32 ± 0.65		III	1) 0.84 ± 0.07 2) 1.02 ± 0.04 3) 1.86 ± 0.11
	VI	1) 4.21 ± 1.01 2) 0.94 ± 0.25 3) 1.81 ± 0.73		V	1) 2.28 ± 0.02 2) 3.71 ± 0.04 3) 3.18 ± 0.03
				VII	1) 3.14 ± 0.13 2) 0.42 ± 0.06 3) 0.84 ± 0.06

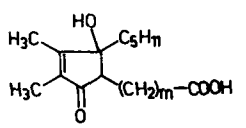
[a]The values were determined by GC. Peak areas of the F-acids were compared with the area of the synthetic F-acid (**1h**) added at an amount of 100 μg as an internal standard. Each sample was lmeasured three times.

The methyl esters of the tetrahydrofuran acids were separated by gas chromatography (GC) and identified by mass spectrometry (MS). Table 1 gives the results of these measurements.

DISCUSSION

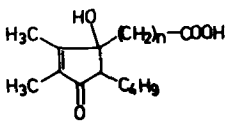
The data in Table 1 show that the amount of F-acids with a pentyl side chain in the α-position introduced into the body through the diet corresponds well with the amount of excreted urofuran acids. The results obtained with α-propyl substituted F-acids do not allow the same clear conclusion to be drawn. Considering the facts that propyl- and pentyl-urofuran acids show a different tendency for adsorption by albumin (13) and that propyl-urofuran acid is excreted in a circadian rhythm while pentyl-urofuran acid does not follow such a rhythm (14), it might be concluded that some of the propyl F-acids are produced in the body.

One source of F-acids may be meat or fish; however, the amounts of F-acids introduced into the body by normal daily fish and meat consumption are low when compared with the excreted amounts of urofuran acids (2), (3) and (4) (11). In looking for other sources of F-acids, we detected these compounds in oranges. Further, F-acids degradation products, the cyclopentenolons (5) and (6) (15), were found in soy oil, but only in low amounts.



5 m = 7, 9

FORMULA 6



6 n = 8, 10

FORMULA 7

These findings, together with Hasma's earlier observation (8) on the occurrence of ab F-acid in the latex of *Hevea brasiliensis*, prompted us to draw the conclusion that F-acids may occur frequently in plants.

Subsequently, a thorough investigation of plant material revealed that F-acids occur in the roots and blades of grasses (*Poaceae spec.*), in clover (*Trifolium pratense*), and also in certain vegetables (e.g. in chive [*Allium sativum*] and cabbages [*Brassica oleracea spec.*]); in potato (*Solanum tuberosum*), wheat (*Triticum aestivum*) and rice (*Oryza sativa*); in some fruits (in lemon [*Citrus limon*], strawberries [*Fragaria spec.*] and orange [*Citrus sinensis osbeck*]); in algae (*Chlorophyta spec.*), in the trunk of birch (*Betula pendula*), and in dandelion (*Taraxacum officinale*). F-acids occur in comparatively high amounts in the green parts of plants (Table 2). Only small amounts (typically 1/100 to 1/1000 of that found in the green parts) occur in the trunks, roots and seeds (Table 2).

We also found the F-acid (**1d**) in mushroom (*Agaricus bisporus*), and traces of the F-acids (**1d**) and (**1g**) in yeast (*Saccharomyces cerevisiae*). In the mushroom the F-acid (**1d**) predominates, while in plants (**1g**) is the main F-acid. In comparison with the amounts found in vegetables (with the exception of potato) and fruits the amounts of (**1g**) found in the blades and leaves of grasses, in dandelion, and in birch are very high. Interestingly, among the land plants and other species so far investigated, F-acids with a propyl side chain occur only in traces. The algae *Chlorophyta spec.* were the only species treated in which high amounts of an F-acid with a propyl side chain was detected (Table 2).

MATERIALS AND METHODS

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). GC was carried out with a

TABLE 2

Occurrence of F-Acids in Plants

		F-acids determined in μg per g of dried plant material[a]				
		(1c)	(1d)	(1e)	(1f)	(1g)
1	Grasses (blade)	<1	50	2	—	189
2	Grasses (root)	—	2	—	—	>1
3	Dandelion (leaf)	<1	2	—	—	82
4	Dandelion (root)	—	—	—	—	<1
5	Dandelion (seed)	—	—	—	—	<1
6	Clover	—	<1	2	<1	18
7	Birch (leaf)	—	6	2	3	183
8	Birch (trunk)	—	<1	—	—	<1
9	Chive	—	—	—	—	16
10	Wheat	—	<1	<1	—	33
11	Rice	—	4	—	—	<1
12	Potato (leaf)	<1	11	4	—	355
13	Potato (fruit)	—	2	—	—	3
14	Cabbage	—	—	—	—	2
15	Orange	2	—	—	—	—
16	Lemon	—	—	—	—	28
17	Strawberry (leaf)	—	—	—	—	6
18	Strawberry (fruit)	—	—	—	—	2
19	Mushroom	—	166	—	—	<1
20	Yeast	—	1	—	—	<1
21	Algae	—	3	145	—	9

[a]The values were determined by comparing the GC peak areas of the identified F-acids with the area of the synthetic F-acid (1h) added to each sample as an internal standard.

Packard model 438S from United Technologies equipped with a flame ionization detector (FID), on a wall-coated open tubular (WCOT)-glass capillary (30 m × 0.3 mm) OV-101 column, temperature programmed from 100°C to 240°C at 2°C min^{-1}. The temperatures of the injector and detector were kept at 270°C and 290°C, respectively. Peak area integration was done by a Shimadzu C-R3A integrator. The carrier gas was hydrogen. The split ratio was 1:10.

GC-MS was performed on a Finnigan MAT 312 GC-MS system with a MAT SS 300 data system. Electron impact mass spectra were recorded with an ionizing energy of 70 eV. The GC column was a 25-m × 0.3-mm i.d. OV-101 WCOT glass capillary column. The carrier gas was helium (2 ml min^{-1}), and the temperature program was the same as used for GC.

Nutrition experiment. Over a period of 5 days, the urine excreted in the 24 hr between 8 a.m. of one day and 8 a.m. of the next day was collected individually from three persons (two male, one female). The urine samples were stored at −20°C.

Quantification of urofuranic acids (2), (3) and (4) in urine. One-ninety sixth (corresponding to 15 min) of a 24-hr urine sample was acidified with conc. HCl, and diluted with water to 20 ml. Ten μg 3-carboxy-4-methyl-5-pentyl-furan-2-acetic acid was added as an internal standard. The extraction of organic compounds and the quantifaication of urofuranic acids (2), (3) and (4) were done as previously described (9), using Chromabond-C_{18} and Chromabond-Si solid phase extraction columns (Macherey & Nagel; D-5160 Düren, Federal Republic of Germany). The amount of urofuranic acids (2) and (3) was calculated by peak area integration, by comparison with the internal standard.

Quantification of F-acids (1) in human food and plants. To each sample 100 μg of (1h) were added as an internal standard. The extraction of the lipids was performed according to the method of Bligh and Dyer (16), and the crude lipid extract was subjected to a "Folch wash" with 0.88% potassium chloride solution (17).

After partition, the chloroform layer was evaporated to dryness under reduced pressure and the residue was saponified with 200 ml 1-N potassium hydroxide solution (methanol/water, 9:1, v/v) under an atmosphere of nitrogen, for 14 hr, which was necessary to hydrolize amid fatty acid bonds, too (18). After cooling and acidification with conc. HCl the potassium chloride was filtered off and 100 ml chloroform and 100 ml water were added. The chloroform layer was separated, the solvent removed under reduced pressure and the residue esterified with ethereal diazomethane. Column chromatography, thin layer chromatography and hydrogenation were done as previously described (7).

The furan fatty acids (1) were quantified as to their tetrahydrofuran methyl esters by peak area integration, using the internal standard (1h) for comparison (7).

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REFERENCES

1. Glass, R.L., Krick, T.P., and Eckhardt, A.E. (1974) *Lipids 9,* 1004-1008.
2. Glass, R.L., Krick, T.P., Sand, D.M., Rahn, C.H., and Schlenk, H. (1975) *Lipids 10,* 694-702.
3. Groweiss, A., and Kashman, Y. (1978) *Experientia 34,* 299.
4. Okajima, H., Ishii, K., and Watanabe, H. (1984) *Chem. Pharm. Bull. 32,* 3281-3286.
5. Ishii, K., Okajima, H., Koyamatsu, T., Okada, Y., and Watanabe, H. (1988) *Lipids 23,* 694-700.
6. Schödel, R., and Spiteller, G. (1987) *Liebigs Ann. Chem.,* 459—462.
7. Puchta, V., Spiteller, G., and Weidinger, H. (1988) *Liebigs Ann. Chem.,* 25-28.
8. Hasma, H., and Subramaniam, A. (1978) *Lipids 13,* 905-907.
9. Gorst-Allman, C.-P., Puchta, V., and Spiteller, G. (1988) *Lipids 23,* 694-700.
10. Sand, D.S., Schlenk, H., Thoma, H., and Spiteller, G. (1983) Biochim. Biophys. Acta 751, 455-462.
11. Schödel, R., Dietel, P., and Spiteller, G. (1986) *Liebigs Ann. Chem.,* 127-131.
12. Schödel, R. (1986) DoctoralThesis, University of Bayreuth, 48-61.
13. Collies, R., Lindup, W.E., Liebich, H.M., and Spiteller, G. (1985) *Brit. J. Clin. Pharmacol. 21,* 610-611.
14. Dietel, P., Hölzel, C., Schneider, K., and Spiteller, G. (1987) *J. Chromatogr. 422,* 1-12.
15. Schödel, R., and Spiteller, G. (1985) *Helv. Chim. Acta 68,* 1624-1634.
16. Bligh, E.G., and Dyer, W.J. (1959) *Can. J. Biochem. Physiol. 37,* 911-917.
17. Folch, J., Lees, M., and Stanley, G.H.S. (1957) *J. Biol. Chem. 226,* 497-509.
18. Karlsson, K.-A. (1986) *Acta Chem. Scand. 22,* 3050-3053.

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