UNUSUAL ACID ISOMERS IN THE PHOSPHOLIPIDS OF *Erysimum*

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The seed oils of of the majority of plants of the family Cruciferae contain, apart from the commonly known acids, fatty acids with more than 18 carbon atoms; with 20, 22 and 24 carbon atoms, among which the 22:1 acid (erucic acid) is characteristic for this family. The erucic acid content in the neutral lipids of various species of this family varies from 1 to 82% [1, 2]. The other acids mentioned above are minor components [2-6].

Erucic acid is present in considerably smaller proportions in the total phospholipids than in the neutral lipids [7, 8], and sometimes it is completely absent [9].

We have investigated the fatty-acid composition of the total phospholipids of seeds of an *Erysimum* sp. *(sylvestris?)* collected in the Bostanlykskii region of the Tashkent oblast [10]. The fatty acids of the total phospholipids were split out by alkaline hydrolysis, and their methyl esters were freed from impurities by column chromatography on silica gel. The purity of the fatty acid methyl esters was checked in a thin layer of silica gel and Silufol plates in system 1. The purified mixture of methyl esters contained the following components (%, GLC): 14:0 (0.5); 16:0 (10.6); 16:1 (1.0); 18:0 (0.6); 18:1 (14.7); 18:2 (21.8); 18:3 (32.0); 20:1 (7.4); 20:2 (traces); 22:0 (3.0); 22:1 (6.0); 24:0 (1.1); 24:1 (1.3).

The fatty-acid composition of the total phospholipids of this plant differed greatly from the total neutral lipids both in the quantitative set fatty acids and in their amounts [11].

In order to determine more accurately the structure of the fatty acids of the total methyl esters, the latter were separated on a "silver column" [12]. This gave fractions of saturated, monoenic, dienic, and trienic acids which, after rechromatography on TLC/$AgNO_3$, were analyzed by chromatographic (TLC, GLC), spectroscopic (UV, IR, mass), and certain chemical methods. On a silver-impregnated layer of silica gel in system 1 the saturated-acid fraction appeared in the form of a single spot with R_f 0.85, and GLC showed peaks of the following acids (%): 14:0 (2.1); 16:0 (87.8); 18:0 (3.1); 22:0 (4.1); 24:0 (2.9).

The following acids were identified in the monoenic fraction (%): 16:1 (1.3); 18:1 (50.0); 20:1 (19.0); 22:1 (28.0); 24:1 (1.7). To determine the configurations of the double bonds we recorded the IR spectrum of this fraction. The absence from the IR spectrum of a bond in the 900-100 cm^{-1} region showed the cis configurations of the olefinic bonds of the acid.

According to the mass spectrum, the monoenic fraction was the sum of the methyl esters of fatty acids with molecular weights M^+ 268, 296, 324, 352, and 380. To determine the positions of the double bonds the monoenic fatty acids were subjected to periodate-permanganate oxidation [13].

The sum of the oxidation fragments after methylation with diazomethane was analyzed by GLC at 198°C (dicarboxylic acids from C_4 upwards and high molecular-weight monocarboxylic acids) and 125°C (low-molecular-weight monocarboxylic acids). Under these conditions, by comparison of the monocarboxylic acids, we identified mainly pelargonic (9:0) and a very small amount of heptanoic (7:0), and among the dicarboxylic acids we identified manily azelaic (9-di-) and very small amounts of the 11-di- and 13-di-, and traces of the 15-diacids.

It follows from the results given that the monoenic acids of the total phospholipids of the *E. sylvestris* seeds had the cis configuration of the olefinic bonds and, apart from the 16:1 acid, the same length of the hydrocarbon chain from the methyl end: ω7 — 16:1; ω9 — 18:1; ω9 — 20:1; ω9 — 22.1; ω9 — 24:1.

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On GLC, the dienic fraction was shown to consist of two acids: 18:2 (98.8%) and 20:2 (1.2%). On TLC/$AgNO_3$, three spots appeared with R_f 0.6 (traces), 0.55 (18:2), and 0.45 (20:2; traces). The GLC results on the presence of a very small amount of 20:2 acid in the dienic acid fraction was confirmed by its mass spectrum, which contained the peaks of the molecular ions M^+ 294 and 322.

The IR and UV spectra of the dienic fraction did not show the presence of conjugated or trans-ethylenic bonds [14].

To determine the positions of the double bonds in the acids of the dienic fraction the latter was also subjected to permanganate–periodate oxidation. Part of the oxidation products in the form of methyl esters was analyzed by GLC as described for the monoenic fraction, and the remainder, in the form of the ammonium salts, was investigated by TLC/cellulose in systems 2 and 3.

On GLC we identified adipic (6-di), azelaic (9-di), pimelic (7-di), caproic (6:0), capric (10:0), and pelargonic (9:0) acids (see below).

By comparison with standards on TLC/cellulose in system 2 we identified the 6:0 (R_f 0.8) and 9:0+10:0 (R_f 0.9-0.95) acids, and in system 3 the spot of the 3-dicarboxylic acid (R_f 0.3).

The destructive oxidation of ordinary linolenic acid ($\Delta^{9,12}$ – 18:2) would be expected to lead to the formation of the dicarboxylic acids azelaic and malonic and the monocarboxylic acid caproic.

The detection by GLC of considerable amounts of 6-dicarboxylic acid and 9-monocarboxylic acids with a small amount of the 20:2 acid in the dienic fraction and the absence in the mass spectra of the latter of molecular ions other than M^+ 294 and 322, and also the presence on Ag plates of a spot with R_f 0.6 suggested to us that this fraction also contained another position-isomer of linoleic acid with a double bond in the $\Delta^{6,9}$ or $\Delta^{3,9}$ position. Consequently, the 10:0 and C_7-dicarboxylic acids are fragments from the degradation of the 20:2 acid the double bonds in which may be present at $\Delta^{7,10}$ or $\Delta^{3,10}$.

On TLC /cellulose, among the dicarboxylic acids we detected a spot corresponding to diammonium malonate. Neither free dicarboxylic acids nor their dimethyl eters appeared in system 3.

In view of the fact that the methyl esters of the fatty acids were oxidized and we did not have samples of the monomethyl esters of the dicarboxylic acids for comparison, it may be assumed on the basis of the GLC results that the adipic, pimelic, and azelaic acids in the mixture were present in the form of the monomethyl derivatives, the R_f values of the ammonium salts of which do not coincide with those of their diammonium salts, and the malonic acid in the oxidation products was present in the free form, which excludes the possible $\Delta^{3,9}$ and $\Delta^{3,10}$ structures of the 18:2 and 20:2 acids, respectively. The $\Delta^{6,9}$ – 18:2 (petroslinoleic) acid was first detected among higher plants in the neutral lipids of Petroselinum [15], and the $\Delta^{7,10}$ – 20:2 (eicosadienic) acid has previously been detected in the lipids of animal tissue [16]. This is the first time that these isomeric 18:2 and 20:2 acids have been found in the phospholipids of higher plants.

The amount of the $\Delta^{9,12}$–18:2 and $\Delta^{6,9}$–18:2 isomers in the dienic fraction was determined from the results of an anlysis of the oxidation products [17].

In the oxidation of the $\Delta^{9,12}$–18:2 acid one of the fragments is the 6:0 acid, and the $\Delta^{6,9}$ isomer yields the 9:0 acid in amounts of 68.8 and 26.1%, respectively. The molar ratio of these acids will be

$$\frac{68{,}8}{116{,}15} : \frac{26{,}1}{158{,}23} = 59{,}23 : 16{,}49.$$

Since the molecular weights of these isomers are the same, their weight ratio is 59.23: 16.49. On the basis of the amount of 18:2 acids in the total fatty acids (21.8%) we obtain the amounts of these isomeric 18:2 acids from the formula

$$\%\Delta^{6,9} - 18:2 = \frac{a \cdot b}{a + c},$$

where a and c are the molar precentages of the 9:0 and 6:0 acids, respectively, and b is the total amount of 18:2 acids in the combined fatty acids. Hence, $\%\Delta^{6,9}$–18:2 = 4.75, and $\%\Delta^{9,12}$–18:2 = 17.05. Consequently, the single peak of an 18:2 acid that appears in GLC represents a mixture of two isomers: cis-9, cis-12 and cis-6, cis-9.

The trienic fraction of the acids gave a single peak of linolenic acid on GLC, while TLC/$AgNO_3$ showed a single spot with R_f 0.15 and the mass spectrum contained a single peak of a molecular ion with M^+ 292. On the basis of the UV and IR spectra the presence of conjugated systems and of a trans configuration of the olefinic bonds in the trienic fraction is excluded. Among the methylated acids from the products of periodate–permanganate oxidation of the trienic fraction the main product identified by GLC was the diester of azelaic acid, with small amounts of succinic and pimelic acids.

In the analysis of the ammonium salts of the degradation fragments in a thin layer of cellulose, in addition to the acids identified by GLC the spots of propionic and malonic acids were detected (systems 2 and 3, respectively). Below we give the results of the GLC analysis of the products of the degradation of the mono-, di-, and trienic fractions of the fatty acids of the total phospholipids of the seeds of *Erysimum* sp. *(sylvestris?)*:

Fraction of acids	Monocarboxylic acids, %				Dicarboxylic acids, %						
	C_5	C_7	C_9	C_{10}	C_4	C_6	C_7	C_9	C_{11}	C_{13}	C_{15}
Monoenic	—	3.0	97.0	—	—	—	—	95,4	3,2	1,4	Tr.
Dienic	68,8	—	26,1	5,1	—	13,9	6,0	80,1	—	—	—
Trienic	—	—	—	—	4,8	—	9,5	85,7	—	—	—

Azelaic acid can be formed by the oxidation of ordinary linolenic acid — $\Delta^{9,12,15}$–18:3. Pimelic and succinic acids could be formed either from another, isomeric 18:3 acid which, judging from the amount of acids obtained, is present in small amount, or they could be fragments of the oxidized products of the 18:3 acid, which is more likely, since the mass spectrum of the trienic fraction was practically identical with that of the $\Delta^{9,12,15}$ 18:3 acid.

Thus, a combination of the GLC, TLC/$AgNO_3$, and TLC/cellulose method and mass-spectral analysis, and also the results of a study of the products of periodate–permanganate oxidation of narrow fractions of the fatty acids has enabled us to detect and calculate amounts of isomeric 18:2 and 20:2 acids that are unusual for the phospholipids of higher plants.

EXPERIMENTAL

The mixture of fatty acids was isolated by saponifying the total phospholipids with 5% KOH at room temperature. As the eluate for the column chromatography of the methyl esters of fatty acids we used hexane–ether (9:1). For chromatography we used type KSK silica gel with a particle size for TLC of up to 125 μ with 5% of gypsum in 20% $AgNO_3$ solution, and for column chromatography 160 μ.

The combined fatty acid methyl esters were separated on a column of silica gel containing 23% of silver nitrate [12].

To identify the ammonium salts of the low-molecular-weight mono- and dicarboxylic acids we used plates with dimensions of 7 × 12 cm coated with a fixed layer of cellulose without the addition of gypsum [13]. The salts of the acids were revealed with a 0.05% solution of Bromophenol Blue in acetone–water (9:1) in the form of blue spots on a yellow background. The following systems were used: 1) petroleum ether–diethyl ether (9:1); 2) tert-butanol–ammonia–water (25:3:5); and 3) isopropanol–ammonia–water (25:3:5).

The products of the periodate–permanganate oxidation of the unsaturated fatty acids were identified in the form of methyl esters of mono- and dicarboxylic acids by GLC at 125 and 198°C. The chromatograms were recorded on a UKh-2 instrument using a column with 18% of polyethylene glycol succinate on Celite-545.

The IR spectra were recorded on a UR-20 instrument in the form of films, the UV spectra on a Hitachi spectrometer, and the mass spectra on a MKh-1303 instrument with direct introduction of the sample at 150°C and with an ionizing voltage of 40 V.

Periodate–Permanganate Oxidation of the Unsaturated Fatty Acids of the Total Phospholipids. With stirring, 20 mg of the monoenic (or 18 mg of the dienic or 15 mg of the trienic) fraction of fatty acids in 40 ml of dioxane was added to a reaction flask containing 0.021 g of $KMnO_4$, 2.26 g of KIO_4, and 0.07 g of K_2CO_3 in 60 ml of water fitted with reflux condenser and stirrer. With constant stirring the mixture was kept on the boiling water bath for 30 min, after which the reaction was stopped by cooling. Then the mixture was acidified and the excess of oxidizing agent was destroyed with sodium bisulfite. The mixture was made alkaline with KOH, the dioxane was distilled off, and the residue was acidified with 15% H_2SO_4. For

better isolation of the acids, the acid solution was saturated with NaCl and extracted with diethyl ether. The ethereal solution was analyzed by the GLC and TLC methods.

SUMMARY

The great diversity of the fatty acids of the total phospholipids and of the neutral lipids of the seeds *Erysimum* sp. *(sylvestris?)* has been shown. By combination of the methods of UV, IR, and mass spectrometry, and also by TLC and GLC analyses with chemical methods of analysis with the fatty acids the presence in the phospholipids of the seeds of this plant of isomers of linoleic and eicosadienic acids that are unusual for the phospholipids of higher plants has been shown.

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