OXYGENATED ACIDS OF THE RESERVE LIPIDS OF Rumex paulsenianus

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The composition of the fatty mono- and dihydroxy acids of the fruit of Rumex paulsenianus Rech. fil. (family Polygonaceae) has been determined by a combination of IRS, UVS, GLC, and mass-spectrometric methods. (22) Monohydroxy acids of the C_{14}–C_{20} series, including isomers and isologues, and four dihydroxy acids from C_{18} to C_{20}, including a new isomeric 9-OH-10, 12-17:2 acid have been detected.

Investigating the lipids of the fruit of Rumex paulsenianus Rech. fil. (family Polygonaceae), we have detected oxygenated lipids (OLs) in them, the combined amount of which was almost 13% of the total weight of the extract [1]. In the present paper we give the results of an analysis of the structure sof the oxygenated fatty acids isolated from the OLS.

According to the preceding investigations, the OLs of the fruit of this dock consisted of five fractions (OLs I-V). Under the conditions of TLC they were all revealed by I_2 vapor, and the OLS-II gave a weak positive reaction with picric acid for the presence of a hydroxypyran ring, while the quantitative reaction of the OLs with 2,4-dinitrophenylhydrazine for a free carbonyl group was negative.

The IR spectrum of OLs-I showed bands of the vibration of OH groups (3300-3600, 1098), of an isolated trans–CH=H bond (965), and of a cis-trans-$(CH{=}ch)_2$ grouping (945, 980 cm^{-1}); in that of the OLs-II, there were additional weak bands of the stretching vibration of a cis and trans-epoxide rings (810, 830, 890 cm^{-1}); the IR spectrum of the OLs-III and V were analogous to the spectrum of the OLs-I with the exception of the intensity of the bands of the vibrations of C–O bonds at 1060 and 1250 and of hydroxy groups at 3250-3600 cm^{-1}, which increased from the Ols-III to the Ols-V.

In the UV spectrum of the Ols, weak absorption of a conjugated dienic chromophore was observed in the region of λ_{max}^{EtOH} 234 nm.

The saponification of the Ols I-V led to the formation of unsubstituted fatty acids (FAs) and of three main types of oxygenated acids having R_f 0.3, 0.25, and 0.21 on TLC in system 1. After the methylation of the acid products, the MEs of unsubstituted FAs (R_f 0.87) and of oxygenated FAs with R_f 0.53 (the main one), 0.45, and 0.42 (traces) (system 1) were obtained. All the spots were revealed with iodine vapor, and the lower part of the spot of the ME with R_f 0.53 gave a positive reaction with picric acid.

On the basis of a comparison with a chromatographic mobility of the known ME of ricinoleic acid (12-OH-9-18:1), the results obtained previously on the behavior of MEs of oxygenated FAs in this system [2], and literature information [3], the spot with R_f 0.53 was assigned to a mixture of the MEs of monohydroxy and hydroxyepoxy acids, and those with R_f 0.45-0.42 to the MEs of dihydroxy acids with vicinal and separated hydroxy groups.

The mass fraction of the oxygenated FAs in relation to the fraction of unsubstituted FAs rose with an increase in the polarity of the OLs from fraction II to V. The alcoholic moieties of the OLs III-V were not studied, but in accordance with the results of analysis we assigned these OLs predominantly the estolide type, and the OLs-I and OLs-II to the triacylglycerols with one acyl residue of an oxygenated FA.

By preparative TLC in system 1, from the products of the alkaline hydrolysis of the OLs-I and OLs-II we isolated the MEs of unsubstituted FAs and two fractions of MEs of oxygenated FAs. The results of the analysis of the unsubstituted FAMEs by GLC are given in Table 1.

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TABLE 1. Composition of the Oxidized Lipids of Rumex paulsenianus

Acid	Amount, %	
	OLs-I	OLs-II
12:0	0,9	4,3
14:0	3,8	4,7
16:0	63,9	56,9
16:1	0,8	0,5
18:0	6,6	7,9
18:1	22,2	24,4
18:2	1,6	1,3
20:0	0,1	Tr.
Σ_{sat}	75,4	73,8
Σ_{unsat}	24,6	26,2

The MEs of the oxygenated acids were converted into their trimethylsilyloxy (TMS) derivatives and were analyzed with the aid of mass spectrometry. The results of the mass-spectrometric analysis of the TMS derivatives of the MEs of the monohydroxy acids are given in Table 2, where A represents the CH_3-containing and B the H_3CCOO-containing fragments. The configurations of the double bonds of the hydroxy acids are shown in those cases where they are known.

In addition to the fragments shown in Table 2, the spectrum contained other known ions formed on the fragmentation under the action of electron impact of the following hydroxy acids: 10-OH-18:0 – with m/z 169 (13) [4]; 9-OH-12-18:1 – with m/z 230 (5); 12-OH-9-18:1 – with m/z 270 (4); and 9-OH-10-18:1 – with m/z 294 (2) [5].

In the mass spectrum of this fraction, the peaks of ions with m/z 398 (M^+), 385 $(M - 15)^+$, and 367 $(M - 31)^+$ corresponded to the TMS ethers of epoxyhydroxyoctadecaenoates, but because of their small amount and the complex composition of the monohydroxy acids neither the accurate structures of the epoxyhydroxy acids nor the structures of the minor C_{14}–C_{16} hydroxy acids were established.

In spite of the relative nature of the quantitative estimation of the TMS ethers from the mass spectrum, it may be concluded, with a certain approximation, that in the mixture

TABLE 2. Compositions and Structures of the Monohydroxy Acids of the Fruit of Rumex paulsenianus

Acid	Mass numbers and intensities of the characteristic fragment of the TMS ethers of the MEs of the hydroxy acids, m/z (%)*					
	M^+	$(M-15)^+$	$(M-31)^+$	$(M-47)^+$	A	B
OH-14:2	326	311	295	Abs.	—	—
OH-15:2	340	325	309	Abs.	—	—
OH-16:0	Abs.	343	327	311	—	—
OH-16:1	355	341	325	309	—	—
OH-16:2	354	339	323	Abs.	—	—
OH-17:0	Abs.	357	341	325	—	
9-OH-12-17:1	370	355	339	Abs. 323	213 (14)	259
12-OH-9-17:1					173 (13)	299
9-OH-10,12-17:2	368	353	337	Abs.	211 (14)	311
9-OH-18:0	Abs.	371	355	339	229 (18)	259
10-OH-18:0					215 ([illegible])	273 (4)
9-OH-12Z-18:1	384	369	353	337	227 (58)	259 (56)
12-OH-9Z-18:1					187 (46)	299 (16)
9(11)-OH-10E(9Z)-18:1					227	285 (17)
10(8)-OH-8E(9Z), 18:1					241 (13)	271 (34)
9-OH-10E,12Z-18:2	382	367	351	Abs.	—	—
13-OH-9Z,11E-18:2					—	—
10-OH-8E,12Z-18:2					—	—
12-OH-9Z,13E-18:2						
OH-20:1	412	397	381	365	—	—

*m/z 73–100%.

TABLE 3. Compositions and Structures of the Dihydroxy Acids of the Fruit of *Rumex paulsenianus*

Acid	Mass numbers and intensities of the characteristic fragments of the TMS ethers of the MEs of the dihydroxy acids, m/z (%)*					
	M^+	$(M-15)^+$	$(M-31)^+$	Rearranged ion.	A	B
9,10-diOH-18:0	Abs.	459	443	332 (5)	215 (30) 317 (2)	259 (75) 361 (7)
9,10-diOH-12-18:1	472	457	441	332	213 (10) 315 (6)	259 361
12,13diOH-9-18:1				270 (1)	173 (42) 275 (35)	299 (10) 401 (0,1)
11,12-diOH-20:0	Abs.	487	471	360 (0,5)	215 317	287 (8) 389 (1)

*m/z 73–100%.

of 22 monohydroxy acids from the fruit of *Rumex paulsenianus* hydroxyoctadecaenoic acids predominated by weight and by the number of isomers (6 isomers), and, among these, ricinoleic and isoricinoleic acids.

Of the two possible isomeric conjugated α-hydroxydecadienoates, according to the ratios of the intensities of the peaks of the A and B ions [5] the proportion of the 9-OH isomer was the higher. This shows a definite position specificity of the action of the lipoxygenase complex in the reserve tissues of *Rumex paulsenianus*, similar to the specificity of the lipoxygenase hydroxylation of some representatives of the *Solanacea* [6].

In an investigation of the mass spectrometry of the TMS derivatives of the MEs of the dihydroxy acids (fraction with R_f 0.45-0.42) four components were reliably identified (Table 3).

The spectrum of this fraction contained the peaks of ions with m/z 147 and 146 that are characteristic of the fragmentation of derivatives of the MEs of dihydroxy acids [4]. The presence of fragments with m/z 199 (10) and 285 (9) showed the possible presence among the dihydroxy acids of the nonvicinal 9,13-diOH-10-18:1 and 9,13-diOH-11-18:1 species [3]. According to these results, the main components were the 9,10-diOH-18:0 and 12,13-diOH-18:1 acids. All the hydroxy acids detected in the fruit of *Rumex paulsenianus* with the exception of the 9-OH-17:2 acid were known natural compounds that had been isolated previously from the reserve and photosynthetic tissues of higher plants [3-5, 7, 8]. The 9-OH-10,12-17:2 acid is a new isomer of the 13-OH-9,11-17:2 acid described by us previously [8].

Thus, oxygenated fatty acids have been detected for the first time in the generative organs of plants of the family *Polygonaceae* growing in Central Asia, and their compositions and structures have been determined.

EXPERIMENTAL

UV and IR spectroscopy, mass spectrometry, GLC analysis, the saponification of the lipids, and the esterification of the acids were carried out under the conditions of [9]; the TMs ethers were obtained as described in [10].

The identification of the TMS ethers from their mass spectra was carried out with the use of literature information [3-5, 11] and the results of our own investigations [2, 8, 10].

For TLC we used silica gel L 5/40 (Czechoslovakia) containing 10% of $CaSO_4$ with preliminary activation of the layer at 120°C for 1 h, and solvent system 1 (hexane–diethyl ether (7:8)).

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INFLUENCE OF INFECTION BY Verticillium dahliae ON THE CUTICULAR LIPIDS OF THE LEAVES OF COTTON PLANTS OF VARIETY 175

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The composition of the cuticular lipids of young leaves of cotton plants of the wilt-resistant variety 175-F has been determined and it has been established that on artificial infection of the plant with cotton wilt (*Verticilliuim dahliae*) the component composition of these lipids did not change but the level of the 18:0, 18:1, and 18:2 acids in esters of fatty acids with alkanol, sterols, and triterpenols fell.

Continuing investigations of the metabolism of lipids in cotton plants infected with *Verticillium dahliae* Kleb., we have made an analysis of the cuticular lipids of young leaves of cotton plants of the wilt-resistant variety 175-F artificially infected with cotton wilt due to this organism.

For analysis we took healthy (I) and wilt-damaged (II) leaves of plants grown under artificial climate conditions. The yield of cuticular lipids (CLs) for samples (I) and (II) were 3.7 and 3.5 mg/g of dry substance (d.s.), respectively. The extracts of the CLs I and II were light brown and green, respectively. In the CLs of II we identified chlorophylls a and b and more polar chlorophyll-like compounds with the aid of TLC in system 1 by comparison with literature information [1], while these compounds were absent from the CLs of I. The presence of chlorophylls in the CLs of II was confirmed by absorption at $\lambda_{max}^{acetone}$ 662 nm in the UV spectrum of this extract and is apparently explained by a destruction of the integrity of the membranes as the result of the penetration of the infection into the photosynthetic tissues. To determine their composition, the CLs were separated by CC on silica gel followed by TLC in systems 2-5. The components were identified by the use of standards and from the results of mass-spectrometric analysis and of chemical transformations; the amount of each component was estimated gravimetrically (Table 1). The chlorophylls were eluted together with the residual lipids. As can be seen from Table 1, with respect both to the set of classes and to their quantitative amounts, the CLs of the two samples were almost identical.

According to mass-spectrometric results, the hydrocarbons of the CLs I and II consisted of a mixture of paraffinic and unsaturated homologues. In the case of the paraffins, the homologues from C_{27} to C_{33} (M^+, m/z 380-464) were identified, the main ones being the C_{29} and C_{31} varieties. The unsaturated hydrocarbons had two, four, and five double bonds

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