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POLAR LIPIDS AND ALKALOIDS OF *Ruta graveolens* GROWN *in vivo* AND *in vitro*

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We have studied the polar lipids and alkaloids of the biomass of Ruta graveolens *L. (fam. Rutaceae) grown* in vivo *and* in vitro *and have determined the quantitative compositions of the classes of glyco- and phospholipids, the fatty-acid compositions of their individual acyl-containing components, and their alkaloid contents. The lipids of the biomass of the* in vitro *culture contained smaller amounts of phosphatidylcholine, of linolenic acid in all classes of acylglycerols, and of hexadec-*trans*,3-enoic acid in the phosphatidylglycerol than those of the* in vivo *culture. Graveoline was found among the total alkaloids of both the* in vivo *and* in vitro *cultures and it was isolated from the* in vivo *culture.*

We have previously reported the presence and group composition of the lipids of the epigeal part of *Ruta graveolens* L. (fam. Rutaceae) grown in soil (*in vivo*, I) in comparison with these indices of differentiated (shoots, leaves, II) and callus tissue of the plant obtained by the callusogenesis and regeneration (*in vitro*) method [1].

Continuing investigations of the lipids and secondary metabolites of *R. graveolens*, we have determined the quantitative compositions of the glycolipids (GLs) and phospholipids (PLs), their fatty-acid compositions, and the amounts of alkaloids that they contain.

The GLs were fractionated with the aid of CC, and the PLs and nonhomogeneous fractions of the GLs after CC with the aid of preparative TLC. The amounts of the individual classes of GLs and PLs were evaluated gravimetrically and they were identified from their chromatographic mobilities, the results of specific qualitative reactions, and chemical transformations. The phosphatidylglycerol (PG) fractions of two samples contained traces of a diphosphatidylglycerol, and the steryl glycoside fraction contained an unidentified substance. These minor components were not isolated individually. The FAs were obtained from the acyl-containing components and their compositions were analyzed by GLC. The compositions of the GL and PL classes of biomasses I and II are given in Table 1, and their fatty-acid compositions in Tables 2 and 3.

According to our previous results [1], the sum of the neutral and polar lipids of differentiated tissues of an *in vitro* culture is distinguished from that of an intact plant by a lower PL content. In a quantitative evaluation of the individual classes of tissue PLs (mg/g a.d.w.) these differences were less pronounced, and, in the case of the GLs, even reversed (Table 1). This is explained by different levels of total lipids and the different moisture contents of samples I and II. In biomass II a higher level of GLs and a lower level of PLs was observed than in biomass I.

Results on the composition of the PLs show that samples I and II had the same set of classes. In the GLs galactolipids predominated — mono- and digalactosyldiacylglycerols (MGDGs and DGDGs). The biomass grown *in vitro* differed from that grown *in vivo* by a slight increase in the amounts of all the classes of GLs. At the same time, the ratio of the individual classes in GLs II was the same as in GLs I.

PG predominated in FLs I and II. In the biomass of the *in vitro* plant, the amount of PG and phosphatidylethanolamine (PE) was higher, and that of phosphatidylcholine (PC) lower by a factor of 2, than in the PLs of biomass I.

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TABLE 1. Composition of the Polar Lipids of Biomass I and II from *R. graveolens*

Class	mg/g a.d.w.		% on the weight of the lipids	
	I	II	I	II
Glycolipids:	25.60	27.60	29.4	25.7
Esters of steryl glycosides	1.95	2.07	2.2	1.9
Monogalactosyldiacylglycerols	12.26	13.44	14.3	12.5
Steryl glycosides + unidentified substance	1.46	1.55	1.7	1.4
Digalactosyldiacylglycerols	9.20	9.77	10.4	9.2
Sulfoquinovosyldiacylglycerols	0.73	0.77	0.8	0.7
Phospholipids:	21.40	19.00	24.7	17.7
Phosphatidylglycerol + diphosphatidylglycerol	5.93	6.25	6.8	5.8
Phosphatidylcholine	4.82	2.44	5.6	2.3
Phosphatidylethanolamine	4.94	5.72	5.7	5.3
Phosphatidylinositol	4.09	3.14	4.7	2.9
Phosphatidylserine	0.49	0.40	0.6	0.4
Phosphatidic acid	1.13	1.05	1.3	1.0

TABLE 2. Fatty-acid Composition of the Glycolipids of Biomasses I and II from *R. graveolens*

Acid	Esters of steryl glycosides		Monogalactosyldiacylglycerols		Digalactosyldiacylglycerols	
	I	II	I	II	I	II
14:0	2.4	7.7	Tr.	Tr.	Tr.	0.7
16:0	22.2	24.0	2.3	12.6	12.4	21.3
16:1	Tr.	Tr.	Tr.	Tr.	1.0	Tr.
18:0	7.5	2.5	0.7	4.0	2.4	8.5*
18:1	24.0	6.2	1.0	9.8	0.8	7.0
18:2	21.9	24.2	4.3	19.7	4.0	22.7
18:3	22.0	33.8	91.7	52.2	79.4	39.8
20:0	Tr.	1.6	Tr.	1.2	Tr.	Tr.
$\Sigma_{unsat.}$	67.9	64.2	97.0	81.7	85.2	69.5
$\Sigma_{sat.}$	32.1	35.8	3.0	18.3	14.8	30.5

TABLE 3. Fatty-acid Composition of the Phospholipids of Biomasses I and II from *R. graveolens* % GLC

Acid	Phosphatidylserine + diphosphatdylglycerol		Phosphatidylcholine		Phosphatidylinositol		Phosphatidylethanolamine	
	I	II	I	II	I	II	I	II
14:0	0.7	0.7	0.5	3.9	2.0	0.7	Ca.	1.0
16:0	28.2	29.3	29.8	37.6	28.4	34.8	33.6	41.7
16:1	15.3*	1.5*	3.0	2.0	3.1	1.7	5.8	1.6
18:0	3.0	2.9	3.5	9.0	3.0	2.5	1.6	3.0
18:1	5.0	5.7	3.5	9.9	4.4	4.8	1.8	2.9
18:2	22.9	48.0	36.8	27.1	29.8	39.6	34.8	35.0
18:3	23.8	11.9	22.9	9.5	27.0	15.9	22.4	14.8
20:0	1.1	Tr.	Tr.	Tr.	1.5	Tr.	Tr.	Tr.
$\Sigma_{unsat.}$	67.0	67.1	66.2	48.4	64.3	62.0	64.8	54.3
$\Sigma_{sat.}$	33.0	32.9	33.8	41.6	35.7	38.0	35.2	45.7

*16:1 acid (3-trans).

The qualitative compositions of the FAs of polar lipids I and II were identical, but there were substantial differences in the quantitative ratios of the individual acids. In addition to the FAs given in Tables 2 and 3, the PLs contained trace amounts of the 12:0, 15:0, and 17:0 acids, while in the PC the level of the 15:0 acid was 1.0% and in the phosphatidylinositol (PI) that of the 17:0 acid was 0.8%. In the GLC of the methyl esters of the FAs from PG I a peak was obtained with a relative retention time (in relation to the 16:0 acid) greater (1.18) than the peak of hexadec-*cis*-9-enoic acid (1.10). In accordance with the literature on the GLC behavior of isomeric FA methyl esters [2] and also on the fatty acid composition of the lipids of photosynthetic tissues [3], we assigned this peak to hexadec-*trans*-3-enoic, the 16:1(3-*trans*), acid.

The galactolipids of biomasses I and II, particularly the MGDGs, had the highest unsaturation of the FAs, which is characteristic for the photosynthetic tissues of plants [3]. The unsaturation of the acids of the steryl glycoside esters (SGEs)

of both samples was also high. The degrees of unsaturation of the phosphatidylglycerols and phosphoinositols I and II were the same, while in the other acylglycerols of biomass II this index had a smaller value than those of analogous lipids.

Of the seven saturated acids in the lipids of *R. graveolens*, the 16:0 species was the main one in the PLs of biomasses I and II. The level of the 16:0 acid was higher in all the lipid classes of sample II than of sample I, the sharpest differences in the levels of this acid being characteristic for the MGDGs and the smallest for the PG and the SGEs.

The amounts of the 18:3 acid in the total GLs and, particularly, in the PLs of biomass II were lower than in lipids I. It can be seen from Tables 2 and 3 that this fall (1.5- to 2-fold) was observed in all the individual classes of PLs, except for the SGEs, but was most pronounced in the PCs. In SGEs I, the 16:0, 18:1, and 18:2 acids were present in almost equal amounts, while in the SGEs II the level of the 18:1 acid had fallen by a factor of 4 in comparison with the intact plant and an accumulation of the 14:0 and 18:3 acids was observed. The level of the 18:2 acid in the majority of classes of PLs II was higher than for the PLs I, with the exception of the PC of biomass II (Table 3).

The PG of the intact plant contained the 16:1(3-*trans*) species as the main monoenic FA, while it was present in only small amounts in the PG of the *in vitro* culture. This acid, which usually esterifies the sn-2 position of PG, together with the 18:3 acid of the galactolipids, is responsible for the structural organization of membranes and participates in the regulation of the photosynthetic activity of the chloroplasts [4].

Thus, in the PLs of the biomass of an *in vitro* culture that are characteristic for chloroplasts (MGDGs, DGDGs, PG), with a lower level of the 18:3 and the 16:1(3-*trans*) FAs the general tendency in the composition of the FAs that is observed in the intact plant is preserved.

Among representatives of the Rutaceae family, the species *R. graveolens* is distinguished by the largest set of secondary metabolites [5]. At the present time, more than 25 alkaloids have been isolated from this plant, these being derivatives of furano- and dihydrofuranoquinolines, of 2-phenylquinoline, of 2-alkyl(phenyl)quinolin-4-ones, and of acridone. Many of them possess physiological activity [6]. No alkaloids were detected qualitatively in the neutral lipids of biomasses I and II, and in the PLs of samples I and II they were present in trace amounts.

The investigation of biomasses I and II showed that leaves containing small stems, flower heads and rosettes of biomass I contained 0.5% of total alkaloids on the weight of the raw material, the stems 0.1%, and the differentiated tissues of biomass II 0.1%. From leaf extract I we isolated a crystalline substance with mp 204°C, identified by direct comparison (TLC, IR, mass, and PMR spectra) with a specimen of the 2-phenylquinolin-4-one alkaloid graveoline [7].

Graveoline was also identified by TLC in the total alkaloids isolated from the stems of biomass I and the differentiated tissues of biomass II.

In the mass spectrum of the alkaloids from the leaves I, in addition to M^+ of graveoline, we detected a molecular ion with *m/z* 259 the fragmentation of which under mass-spectrometric conditions (*m/z* (I_{rel}, %)): 259 (M^+, 100), 258 (15), 244 (75), 230 (32), 216 (32), 201 (27), 186 (15), 173 (15), was typical for 4-methoxyfuranoquinoline alkaloids with two methoxy groups in the benzene ring [8]. A comparative analysis of the intensities of the peaks of these ions with those published for skimmianine, kokusaginine, and maculosidine [8] gave grounds for assuming that the mixture of alkaloids from biomass I included at least two isomeric alkaloids of this type.

EXPERIMENTAL

IR spectra were recorded on a UR-20 spectrometer in KBr tablets, PMR spectra on a Tesla BS 567A, 100 MHz, instrument (δ scale, CF_3COOH, 0 — HMDS) and mass spectra on a MKh-1310 spectrometer. The conditions for the GLC of the FA methyl esters and for the identification of the PLs have been described in [1].

The CC of the glycolipids, which contained transformed chlorophyll pigments, was conducted on silica gel of type L 100/160 (Chemapol, Czechoslovakia), using the system of [9].

Preparative TLC was performed on silica gel L 5/40 (Chemapol, Czechoslovakia) with 10% of $CaSO_4$. The SGEs were purified in the $CHCl_3$—$(CH_3)_2CO$—CH_3OH—CH_3COOH (73:25:1.5:0.5) system, the MGDGs and steryl glycosides in $CHCl_3$—$(CH_3)_2CO$—H_2O (15:30:1) [10], and the DGDGs in $CHCl_3$—$(CH_3)_2CO$—MeOH—CH_3COOH—H_2O (65:20:10:10:3) [1]. The FLs were fractionated in the $CHCl_3$—CH_3OH—H_2O (65:25:4) system, and the PG and PE were additionally purified on silica gel impregnated with $(NH_4)_2SO_4$ in the $(CH_3)_2CO$—MeOH—H_2O (91:30:8) system [11]. The lipids were re-extracted from the sorbent by repeated treatment with $CHCl_3$—MeOH (2:1).

The chromatographic monitoring of the alkaloids was effected by TLC (neutral Al_2O_3 LSL 5/40) in ethyl acetate and the solvent system ethyl acetate—toluene—CH_3COOH (5:4:1) [1].

Isolation of the Alkaloids. Leaves with small stems, flower heads, and rosettes (75 g, end of flowering—beginning of fruit-bearing) were extracted with methanol. The evaporated extract was separated by distribution between 5% H_2SO_4 and chloroform. After the acid solution had been alkalinized with ammonia (A), the alkaloids were extracted with chloroform (B). Evaporation of chloroform solutions A and B gave a neutral fraction (15 g) and the total alkaloids (0.091 g), respectively. The neutral fraction was chromatographed on a column of neutral deactivated Al_2O_3 (Brockmann activity II) (1:100). Hexane—ether eluates yielded graveoline (0.3 g), mp 188°C (alcohol—water), 204°C (after drying).

PMR spectrum (100 MHz, CF_3COOH, δ, ppm): 3.83 (3H, s, N−CH_3), 5.67 (2H, s, O−CH_2−O), 6.63-6.60 (3H, m, H−2′5′6′), 6.85 (1H, s, H−3), 7.52 (1H, m, H−5), 7.76 (2H, m, H−6.7), 8.22 (1H, dd, J = 9 and 2.5 Hz, H−8).

Mass spectrum (EI, 70 eV), *m/z* (I_{rel}, %): 279 (M^+, 100) 251 (M^+ − 28, 53).

Analogously, biomasses I (stems, 30 g) and II (3 g) yielded mixtures of alkaloids (0.03 and 0.003 g, respectively).

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