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TRIACYLGLYCERINES OF *Ruta graveolens* GROWN *in vivo* AND *in vitro**

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The composition of triacylglycerines (TAG) from photosynthetic tissues of Ruta graveolens *L. (Rutaceae) grown* in vivo *and* in vitro *was studied with respect to chain length and unsaturation. Esterification of sn-2-glycerol by unsaturated acids is less selective under* in vitro *than* in vivo *conditions. TAG of the* in vitro *culture have an elevated content of forms with oleinic acid in the sn-2-position.*

Key words: *Ruta graveolens, in vitro,* triacylglycerines, lipolysis.

We previously investigated neutral [1] and polar [2] lipids of photosynthetic tissues from *R. graveolens* L. grown in soil (*in vivo*, I) and culture obtained via callusogenesis and regeneration (*in vitro*, II). In continuation of the study of lipids from this plant, we analyzed the structures and compositions of triacylglycerines (TAG) from differentiated tissues of I and II.

As a rule, TAG accumulate in the seeds of plants as required energy sources. Their structures have been studied in many species [3]. Data on the structure of TAG from photosynthetic tissues of higher plants are scarce [4]. The fatty-acid distribution in TAG of *R. graveolens* has not been previously studied.

The yields of TAG from tissues of I and II were 0.36 and 0.40%, respectively, calculated on a dry-mass basis. The structures and composition of fatty acids and their distribution in TAG were found using mass spectrometry, enzymolysis by pancreatic lipase, and GLC. The studies determined the specific distribution of fatty acids in the *sn*-2-position of TAG, including the fraction of each acid and the selectivity factor for the distribution of unsaturated acids in this position (Table 1). We obtained 48 possible molecular variations of TAG from I and II. The mass spectra of starting TAG from both samples contained peaks for M^+ ions and characteristic fragments and confirmed the structure of the molecular types given in Table 2.

Table 1 shows that TAG from I and II are identical in fatty-acid composition. Unsaturated acids, mainly linoleic acid, dominate in them. Acids of *sn*-2-monoacylglycerines (*sn*-2-MAG from I and II) obtained from TAG of I and II differ substantially. Whereas the content of linoleic and oleinic acids are equal in the TAGs, *sn*-2-MAG from I is typically [3] enriched in linoleic acid (50.3%); *sn*-2-MAG from II, in linoleic (49.9%) and oleinic (47.9%) acids. Therefore, esterification of *sn*-2-glycerol by unsaturated acids in photosynthetic tissues of *R. graveolens* is less selective under *in vitro* conditions compared with *in vivo* ones. Unsaturated fatty acids esterifying the *sn*-2-position of glycerine are distributed by selectivity factor as follows for TAG from I: 18:2 > 18:3 > 18:1 > 16:1; for TAG from II: 18:2 > 18:1 > 18:3 > 16:1 (Table 1).

Acids of *sn*-2-MAG from I and II include saturated palmitic acid, which occurred in the *sn*-2-position of TAG from II 1.7 times less than in TAG from I. Glycolipids and phospholipids that contain 16:0 acid in the *sn*-2-position are known to be biosynthesized in leaves of higher plants by a mechanism unique to prokaryote cells [5]. It was also noted [4] that a prokaryotic path for glycerolipid formation may also be valid for TAG synthesized in photosynthetic plant tissues. Therefore, the compositions of TAG from I and II of *R. graveolens* indicate that *in vitro* tissue cultures contain less TAG with the prokaryote type of fatty-acid distribution than *in vivo* ones (Table 2, TAG types **1-10**).

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TABLE 1. Fatty-Acid Distribution in TAG of *R. graveolens* Cultures I and II

Acid	TAG, mole %		sn-2-MAG, mole %		Acid fraction in sn-2-MAG, %		Selectivity factor	
	I	II	I	II	I	II	I	II
12:0	0.9	1.2	-	-				
14:0	3.1	3.4	-	-				
15:0	0.5	0.6	-	-				
16:0	16.5	17.6	10.8	6.6	21.8	12.5		
16:1	3.7	2.9	2.3	1.2	20.7	13.8	0.53	0.33
18:0	2.9	3.8	-	-				
18:1	15.6	15.1	10.9	21.7	23.3	47.9	0.60	1.13
18:2	37.3	36.6	56.3	54.8	50.3	49.9	1.29	1.18
18:3	19.5	18.8	19.7	15.7	33.7	27.8	0.86	0.66
Σ_{sat}	23.9	26.6	10.8	6.6				
Σ_{unsat}	76.1	73.4	89.2	93.4				

TABLE 2. Chain Lengths of Fatty Acids in TAG of *R. graveolens* Cultures I and II, mole %

	Type	I	II		Type	I	II
1	18:2-16:0-18:3	2.2	1.1	**25**	18:2-18:2-16:0	6.5	7.3
2	18:2-16:0-18:2	1.5	0.7	**26**	18:2-18:2-16:0	2.2	2.5
3	18:2-16:0-18:1	2.0	0.7	**27**	18:3-18:2-18:1	4.1	2.9
4	18:2-16:0-18:0	Tr.*	Tr.	**28**	18:3-18:2-18:3	2.2	2.5
5	18:2-16:0-16:0	2.2	1.2	**29**	18:3-18:2-18:0	1.1	1.3
6	18:3-16:0-16:0	1.5	0.3	**30**	18:3-18:2-16:0	4.5	5.4
7	18:1-16:0-16:0	1.4	0.9	**31**	18:3-18:2-14:0	1.5	2.0
8	18:0-16:0-16:0	Tr.	Tr.	**32**	18:1-18:2-18:1	1.9	0.8
9	18:2-16:1-18:2	1.3	0.5	**33**	18:1-18:2-16:0	4.1	3.2
10	18:2-16:1-16:0	1.0	0.7	**34**	18:1-18:2-14:0	1.4	1.0
11	18:2-18:1-18:3	1.6	3.0	**35**	16:0-18:2-18:0	1.0	1.4
12	18:2-18:1-18:2	1.2	2.1	**36**	16:0-18:2-16:0	2.2	3.1
13	18:2-18:1-18:1	1.5	1.7	**37**	16:0-18:2-14:0	1.5	2.1
14	18:2-18:1-16:0	1.6	3.5	**38**	18:3-18:2-16:1	1.0	0.8
15	18:2-18:1-14:0	0.5	1.3	**39**	16:0-18:2-16:1	1.0	0.9
16	18:3-18:1-18:1	1.1	1.4	**40**	18:2-18:3-18:3	3.0	2.7
17	18:3-18:1-16:0	1.2	2.5	**41**	18:2-18:3-18:2	2.2	1.7
18	18:1-18:1-16:0	1.0	1.5	**42**	18:2-18:3-18:1	2.8	1.5
19	16:0-18:1-16:0	1.2	1.5	**43**	18:2-18:3-16:0	3.0	3.0
20	18:2-18:2-18:3	6.5	6.5	**44**	18:3-18:3-18:3	1.1	1.0
21	18:2-18:2-18:2	4.7	4.5	**45**	18:3-18:3-18:1	2.0	1.1
22	18:2-18:2-18:1	5.9	3.8	**46**	18:3-18:3-16:0	2.1	2.0
23	18:2-18:2-16:1	1.5	1.1	**47**	16:0-18:3-18:1	2.4	1.2
24	18:2-18:2-18:0	1.5	1.7	**48**	16:0-18:3-16:0	1.1	1.4

*Tr < 0.1%

Most types of TAG from I and II contain different acids. Single-acid types are represented only by trilinoleic (**21**) and trilinolenic (**44**). The amount of these types is small and almost the same in both samples. The compositions of TAG from II differ from those of TAG from I in an elevated content of isomers with oleinic acid in the middle position.

TABLE 3. Saturation of Fatty Acids in TAG of *R. graveolens* Cultures I and II (mole %)

Type	I	II
UUU*	45.6	41.1
SUU	37.6	44.0
SUS	6.0	8.3
USU	5.7	2.8
USS	5.1	3.8
SSS	-	-

*Total acids: **U** - unsaturated; **S** - saturated.

We determined the saturation of TAG from I and II by tabulating the molecular types of TAG according to unsaturated (U) and saturated (S) acyls (Table 3). They consist primarily of three unsaturated (**UUU**) and unsymmetric di-unsaturated (**USU**) types. The former dominates in TAG from I; **SUU** type is greater than **UUU** type in TAG from II. The type **SSS** was absent in samples of both cultures.

We previously observed [2] that the contents of phosphatidylcholines and 18:3 acid were less in *in vitro* culture than in *in vivo* culture. The reduction in 18:3 acid in chloroplast lipids (mono- and digalactosyldiacylglycerines, phosphatidylglycerines) is compensated by an increase of 18:2 and 18:1 acids. On the other hand, acids of phosphatidylcholines have reduced 18:2 acid that esterifies selectively the *sn*-2-position of glycerol.

Thus, the relationship between the fatty-acid composition of phosphatidylcholines and the structure of TAG synthesized in photosynthetic tissues of *R. graveolens* is obvious. Apparently phosphatidylcholines act as a substrate for diacylglycerines and acyls for TAG synthesis, like in plant seeds that accumulate TAG [6].

EXPERIMENTAL

Mass spectra were obtained on an MX-1321 mass spectrometer with ionizing potential 70 eV, 60 μA current, and ionization-chamber temperature 145-190°C.

GLC of methyl esters of fatty acids was performed on a Chrom-4 chromatograph equipped with a flame-ionization detector and a column (2500×4 mm) packed with Reoplex 400 (15%) on Chromaton N-AW at 198°C with N_2 carrier gas.

Column chromatography of the $CHCl_3$—CH_3OH extract obtained from cultures I and II [1] was performed over silica gel L 100/160 (Chemapol, Czech Rep.). TAG from I and II were eluted using hexane—diethylether (19:1) and purified by preparative TLC over silica gel L 5/40 (Chemapol, Czech Rep.) and the same solvents (9:1).

Enzymolysis by pancreatic lipase of TAG from I and II followed the literature method [7]. The same solvents (2:3) isolated *sn*-2-MAG from I and II.

Triacylglycerines from I. Mass spectrum, *m/z* (I_{rel}, %): 884 $[M]^+$ (1.8), 882 (2.1), 880 (3.5), 878 (6.5), 876 (6.1), 874 (5.2), 872 (4.4), 858 (1.3), 856 (2.0), 854 (3.3), 852 (8.9), 850 (8.9), 834 (2.7), 832 (1.3), 830 (2.6), 828 (1.9), 826 (2.1), 824 (2.8), 802 (1.5); $[M - R_{1(3)}COO]^+$ and $[M - R_{1(3)}COOH]^+$ 603 (44.2), 602 (45.2); 601 (24.0), 600 (28.9); 599 (23.1), 598 (25.0); 597 (13.5), 596 (13.5); 595 (13.5), 594 (7.0); 579 (61.5), 578 (51.9); 577 (100.0), 576 (75.0); 575 (65.4), 574 (55.8); 573 (19.2), 572 (15.4); 551 (96.1), 550 (38.4); 549 (38.4), 548 (28.3); 547 (19.2), 546 (8.7), and other characteristic ions [8].

Triacylglycerines from II. Mass spectrum, *m/z* (I_{rel}, %): 884 $[M]^+$ (1.0), 882 (0.4), 880 (0.6), 878 (1.3), 876 (2.3), 874 (2.5), 872 (2.0), 858 (1.0), 856 (0.8), 854 (0.9), 852 (0.7), 850 (1.3), 834 (1.8), 832 (2.0), 830 (3.2), 828 (2.2), 826 (0.3), 824 (0.1), 802 (1.3), 800 (2.2); $[M - R_{1(3)}COO]^+$ and $[M - R_{1(3)}COOH]^+$ 603 (43.5), 602 (34.5); 601 (14.0), 600 (13.5); 599 (5.0), 598 (4.4), 597 (1.3), 596 (2.9); 595 (7.0), 594 (2.4); 579 (40.0), 578 (55.0); 577 (100), 576 (35.0); 575 (43.0), 574 (8.8); 573 (1.3), 572 (1.1); 551 (84.0), 550 (24.4); 549 (43.0), 548 (23.0); 547 (13.0), 546 (7.0), etc. [8].

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