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TRIACYLGLYCEROLS OF THE SEEDS AND FRUIT FLESH OF *Mandragora turcomanica*

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The triacylglycerols (TAGs) of the seeds and fruit flesh of Managora turcomanica *have been investigated for the first time using the methods of mass spectrometry and enzymolysis. It has been found that in the TAGs of the seeds the 18:2 acids undergo specific esterification in the sn-2 position, while in the TAGs of the flesh this position is acylated by 18:1 and 18:2 acids. For both parts of the fruit the main TAG species is sn-1(3)-oleoyldilinoleate, and the main types are represented by triunsaturated and monounsaturated-diunsaturated glycerides.*

Triacylglycerols have been detected previously in the lipids of the tuberous roots of *Mandragora turcomanuica* Mizger (fam. Solanaceae), and their structures and position-species compositions have been determined [1]. The amounts of TAGs in the seeds and fruit flesh of this plant [2] were 188.2 and 12.9 mg/g a.d.w. of the material, respectively. In spite of the substantial differences in their relative amounts, they were the main components of the total lipids not only of the seeds but also of the fruit flesh (85.1 and 64.5% by weight).

We have used enzymolysis with pancreatic lipase in order to elucidate features of the distribution of lipids between the primary and secondary hydroxyls of sn-glycerol and to establish the species and type compositions of the TAGs of the seeds (I) and the flesh (II). The fatty acid (FA) compositions of the triacylglycerols and of the sn-2-monoacylglycerols (sn-2-MAGs) obtained after the lipolysis of I and II were determined by GLC. To confirm the results of lipolysis, the initial TAGs of the flesh were analyzed by mass spectrometry.

Table 1 gives the compositions of the acids of the TAGs and sn-2-MAGs, the proportion of each acid in the sn-2 positions of the TAGs, and the selectivity factors (SFs) for the unsaturated acids [3]. The fatty acids of TAGs I and II of *M. turcomanica* proved to be highly unsaturated with a predominance of the 18:2 acid, which is characteristic for the seed lipids of the Solanaceae family [4, 5]. In the TAGs of the flesh, as compared with those of the seeds, there was a higher proportion of the 16:0, 18:0, and 18:3 acids, while there was a smaller difference in the levels of the two main unsaturated acids — the 18:1 and 18:2 varieties.

The sn-2-MAGs obtained from the TAGs of the seeds and flesh were mainly esterified with the 18:1 and 18:2 unsaturated acids. They contained only a small proportion of the saturated 16:0 acid, while the 18:0 and 16:1 acids were absent.

The proportions of individual acids in the sn-2-MAGs and the SFs of the unsaturated acids show that in the TAGS seeds of *M. turcomanica* the secondary hydroxyl of the sn-2-glycerol is predominantly esterified with the 18:2 acid, as for the TAGs of the seeds of tomatoes, which also belong to the Solanaceae family [5]. These facts also agree with the known characteristics of the distribution of unsaturated acids in the seeds of the majority of representatives of various families [6].

In the TAGs of the flesh the 18:2 and 18:1 acids esterified the secondary hydroxyl of glycerol with almost the same proportions and selectivities. Although it is difficult to judge the distribution of acids present in small amounts [6], we may nevertheless note that the 18:3 acid was detected in the sn-2 position of the flesh TAGs.

The distribution of the other acyl radicals in TAGs I and II were such that the 16:0 acid was largely, and the 18:0 and 16:1 acids completely, bound in the extreme positions.

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TABLE 1. Distribution of the Acids in the TAGs of the Seeds and Fruit Flesh of *Mandragora turcomanica* (mole-% GLC)

Acid	TAGs		sn-2-MAGs		Selectivity factor	
	seeds	flesh	seeds	flesh	seeds	flesh
16:0	7.4	16.3	0.4(1.8)*	1.6 (3.5)		
16:1	0.5	0.6	-	-		
18:0	1.8	4.6	-	-		
18:1	26.1	35.5	16.8 (21.5)	44.3 (41.4)	0.59	1.01
18:2	64.2	42.1	82.8 (43.0)	51.8 (40.6)	1.18	0.99
18:3	Tr.	1.3	-	2.3 (59.0)	-	1.44
Σ_{sat}	9.2	19.9	0.4	1.6		
Σ_{unsat}	90.8	80.1	99.6	98.4		

*Proportion of the acid, % in relation to its total amount in the TAGs.

The results on the composition of the acids in TAGs I and II have been used to calculate the position-species and type compositions of TAGs I and II that are shown in Tables 2 and 3.

Of the numerous possible TAG isomers, 25 position species were realized in the seed lipids and 48 in the flesh lipids (Table 2). Another 13 species of TAGs II, not shown in Table 2, were present in amounts of 1.0—0.1%, making up 5.3% in total.

A comparison of the position-species compositions of the acylglycerols of the specimens studied, shows that the majority of them had a mixed character. The mono-acid species of TAGs I and II were represented by the trilinoleate and trioleate, while the tripalmitate was present only in the fruit flesh in an amount of 0.1%. In the seed TAGs the trilinoleate predominated, while there was more trioleate in the flesh (4.3%) and tuberous root (6.2%) [1]. The main components of TAGs I and II were diacid species.

Among the diacid species in the seed TAGs the highest percentage fell to dilinoleoyl-containing molecules. A comparison of the structures of individual species of seed TAGs shows that in 12 molecular species the sn-2-position of the TAG was acylated by the 18:2 acid, the main species being the unsymmetrical 18:1-18:2-18:2 and the symmetrical 18:2-18:2-18:2 types. The level of these two species in the TAGs of tomato seeds is also high [5]. In the TAGs of the tuberous root [1] the main components were molecules of analogous structure to the seed TAGs (1—5), but with the 18:1 acid in the sn-2 position, among which the unsymmetrical 18:1-18:1-18:2 and the symmetrical 18:2-18:1-18:2 types predominated. Thus, the main components of the TAGs of the seeds and of the tuberous root have similar structures and are isologs. Here again a correlation is observed the amounts of the 18:1 and 18:2 acids in the total FAs of the TAGs and their distribution in the central position.

In the TAGs of the flesh, in addition to the seven main components of the TAGs of the seeds (1—5, 13, and 14) a considerable proportion was due to three other TAG species (15—17, 18.5%) with localization of the 18:1 acid in the sn-2 position. These molecular species were minor components of the seed TAGs but they were present in considerable amount (18%) in the tuberous root of the mandrake [1]. Consequently, it may be considered that the TAGs of the flesh consist mainly of species of TAGs analogous to those of the seeds and tuberous root.

Among the triacid species of TAGs in the seeds there was a larger amount of the 16:0-18:2-18:1 species. In the TAGs of the flesh the proportions of this species and of its 16:0-18:1-18:2 position isomer were almost equal, while in the tuberous root the latter isomer with the 18:1 acid in the central position predominated [1].

In the seeds, there were only two positional species of TAGs with the 16:0 acid in the sn-2 position. Among the 48 species of the flesh TAGs obtained as a result of calculation there were an additional five analogous species, amounting in sum to 1.1%.

In the mass spectrum of the TAGs of the specimens studied we detected the peaks of the M^+ ions and of characteristic fragments [7] corresponding in molecular masses and relative intensities to the species obtained by calculation after the enzymatic hydrolysis of the TAGs.

TABLE 2. Position-species Composition of the TAGs of the Seeds and Fruit Flesh of *M. turcomanica*

TAG species	Content, mole %	
	seeds	flesh
1. 18:1 - 18:2 - 18:2	28.0	11.8
2. 18:2 - 18:2 - 18:2	25.1	7.2
3. 16:0 - 18:2 - 18:2	10.0	9.1
4. 18:1 - 18:2 - 18:1	7.8	4.8
5. 16:0 - 18:2 - 18:1	5.5	7.4
6. 18:0 - 18:2 - 18:2	2.4	2.7
7. 16:1 - 18:2 - 18:2	0.7	0.4
8. 18:0 - 18:2 - 18:1	1.4	2.2
9. 16:1 - 18:2 - 18:1	0.4	0.3
10. 16:0 - 18:2 - 16:0	1.0	2.9
11. 18:0 - 18:2 - 16:0	0.4	1.7
12. 16:1 - 18:2 - 16:0	0.1	0.2
13. 18:1 - 18:1 - 18:2	5.7	10.1
14. 18:2 - 18:1 - 18:2	5.1	6.2
15. 16:0 - 18:1 - 18:2	2.0	7.8
16. 18:1 - 18:1 - 18:1	1.6	4.3
17. 16:0 - 18:1 - 18:1	1.1	6.4
18. 18:0 - 18:1 - 18:2	0.5	2.3
19. 18:0 - 18:1 - 18:1	0.3	1.9
20. 16:1 - 18:1 - 18:2	0.1	0.3
21. 16:1 - 18:1 - 18:2	0.1	0.3
22. 18:0 - 18:1 - 16:0	0.1	1.4
23. 16:0 - 18:1 - 16:0	0.2	0.2
24. 18:1 - 16:0 - 18:2	0.2	0.3
25. 18:2 - 16:0 - 18:2	0.2	0.2
26-48. others	-	5.3 (13 species)

TABLE 3. Position-type Composition of the TAGs of the Seeds and Fruit Flesh of *M. turcomanica*

Type*	Content, mole-%	
	seeds	flesh
UU	74.8	47.7
SUU+UUS	23.3	41.6
USU	0.4	0.8
SUS	1.7	9.2
SSU+USS	-	0.6
SSS	-	0.1

*U — total unsaturated acids; S — total saturated acids.

The results presented in Table 3 show that the TAGs I and II consisted predominantly of the triunsaturated — UUU — and the unsymmetrical diunsaturated — SUU — types, as in many other plant oils having similar sets of FAs [8] In the TAGs of the seeds and the fruit flesh the UUU type predominated. The TAGs of the flesh differed from those of the seeds (Table 3) and of the tuberous root [1] by an increased content of SUU and SUS. The SSS type was realized only in the flesh in the form

of the tripalmitate.

Thus, the acylglycerols of the seeds, fruit flesh, and tuberous root of *M. turcomanica* differ not only in their FA compositions but also in their levels of positional species and types of TAGs. In all cases the species formed by combinations of the 16:0, 18:1, and 18:2 acids predominate. However, in the seeds TAG molecules with the 18:2 acid in the sn-2 positions and the 18:1 and/or the 18:2 acid in the sn-1(3) positions of the glycerol molecule predominate, while in the TAGs of the tuberous root [1] the level of analogous species with the 18:1 acid in the sn-2 position is higher. The substantial differences in the compositions and structures of the TAGs from the different plant organs are probably due to a specificity of their acylation during biosynthesis according to the different roles of the individual molecular species in the occurrence of cell processes.

EXPERIMENTAL

Mass spectra were taken on a MKh-1321 mass spectrometer with an ionizing voltage of 70 eV, a current of 60 μA and a temperature of the ionizing chamber of 170—190°C.

GLC was conducted on a Chrom-4 chromatograph with a flame-ionization detector and a column (4 mm × 2.5 m) filled with 17% of Reoplex 400 on Chromaton N-AW-DMCS at a column temperature of 198°C with helium as the carrier gas.

Preparative TLC was carried out on silica gel L 5/40 (Czech Republic) with 10% of $CaSO_4$ in the hexane—diethyl ether (2:3, v/v) system. Enzymolysis with pancreatic lipase was performed as described in [6], the sn-2-MAGs being isolated from the lipolysis products by preparative TLC.

The acids were isolated from the TAGs and sn-2-MAGs after mild alkaline hydrolysis by extraction with diethyl ether, and they were methylated with diazomethane. The methyl esters obtained were analyzed by GLC.

The Triacylglycerols of the Seeds. Mass spectrum *m/z* (*I*, %): M^+ 886 (0.5), 884 (1.9), 882 (4.8), 880 (6.7), 878 (6.0), 876 (0.7), 860 (0.4), 858 (1.6), 856 (3.6), 854 (4.6), 852 (0.6), 832 (1.4), 830 (2.8), 828 (2.6); $[M\text{-}R_{1(3)}COO\text{-}1]^+$ and $[M\text{-}R_{1(3)}COO]^+$ 606 (5.0) and 605 (1.5), 604 (90.3) and 603 (98.3), 602 (94.0) and 601 (96.1), 600 (95.0) and 599 (100), 598 (16.1) and 597 (14.0), 580 (8.0), 579 (1.7), 578 (17.0) and 577 (37.1), 576 (45.1) and 575 (45.0); $[R_2CO+128]^+$ 393 (3.0), 391 (5.8), 389 (2.0), 367 (4.0); $[R_2CO+74]^+$ 339 (5.2), 337 (31.0), 335 (2.0), 313 (6.2) and others [7].

The Triacylglycerols of the Fruit Flesh. Mass spectrum *m/z* (*I*, %): M^+ 888 (1.4), 886 (2.2), 884 (6.6), 882 (11.6), 880 (19.7), 878 (14.7), 876 (3.2), 860 (3.6), 858 (7.5), 856 (22.3), 854 (23.0), 852 (0.4), 832 (4.6), 830 (7.2), 828 (7.2); $[M\text{-}R_{1(3)}COO\text{-}1]^+$ and $[M\text{-}R_{1(3)}COO]^+$ 606 (21.3) and 605 (34.4), 604 (39.3) and 603 (49.2), 602 (45.9) and 601 (45.9), 600 (45.8) and 599 (50.8), 598 (45.9) and 597 (49.2), 578 (62.3) and 577 (100), 576 (85.2) and 575 (85.2), 574 (70.5) and 573 (55.7) and other characteristic ions [7].

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