Chemistry of Natural Compounds, Vol. 33, No. 4, 1997

STRUCTURE AND COMPOSITION OF THE TRIACYLGLYCEROLS OF THE TUBEROUS ROOTS OF *Mandragora turcomanica**

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UDC 547.945:665.3

Using the methods of mass spectrometry and enzymolysis with pancreatic lipase, the structure and position-species composition of the triacylglycerols (TAGs) of the tuberous roots of Mandragora turcomanica *Mizger (fam. Solanaceae) have been determined for the first time. The TAGs include 42 possible species, among which the main ones are triunsaturated and unsymmetrical diunsaturated molecules with oleic acid in the sn-2 and linoleic acid in the sn-1(3) positions of the glycerol molecule.*

Triacylglycerols not only accumulate as essential energy-providing substance in seeds but are also found in other organs of higher plants, including roots, in amounts of from 4 to 10% (on the weight of an extract) [1, 2]. We have found a fairly high level of TAGs (20.7% of the weight of an extract) in the lipids of the tuberous roots of the medicinal plant *Mandragora turcomanica* (fam. Solanaceae) [3].

The structures and compositions of the TAGs of the seeds of representatives of various families have been investigated in detail. This has shown a well-supported rule of esterification by unsaturated acids, particularly 18:2 (9,12) in the sn-2 position of glycerol [4, p. 173]. We have found no information in the literature available to us on the structure and composition of the TAGs of the lipids of the hypogeal organs of plants of the Solanaceae family. At the same time, information of this type would permit us to predict the role of this main lipid component in the growth and development of the roots and also to expand our ideas on the pathways for the biosynthesis of TAGs in various plant organs. In this paper we give the results of a study of the structure and composition of the TAGs of *M. turcomanica* by mass spectrometry and enzymolysis with pancreatic lipase.

To determine the structure and composition of the fatty acids (FAs) more accurately, the methyl esters (MEs) of the acids obtained from the TAGs were analyzed for their degree of unsaturation by the Ag^+-TLC method and were subjected to mass-spectrometric analysis. The compositions of the acids of the TAGs and of the sn-2-monoacylglycerols (sn-2-MAGs) after lipolysis were determined by GLC (Table 1).

The TAGs of the tuberous roots of *M. turcomanica* included seven saturated and six unsaturated FAs of known structure, while the sn-2-MAGs contained only the 16:0 and 18:0 saturated acids and the 16:1, 18:1, and 18:2 unsaturated acids. The 16:1 acid was distributed almost uniformly between the primary and secondary hydroxyls of the glycerol molecule. The other saturated FAs occupied the extreme positions of the TAGs preferentially (75-100%).

The distribution of the unsaturated acids in the TAGs was such that the least unsaturated acid, 18:1, esterified the sn-2 position predominantly (61%), while the 18:2, 18:3, and 17:1 acids were bound mainly in the extreme positions. Because of the small amount of the 16:1 acid in the TAGs, it was difficult to establish its accurate quantitative distribution over the glycerol positions from the results of lipolysis.

*The materials of this paper were presented at the IInd International Symposium on the Chemistry of Natural Compounds (SCNC, Turkey, Eskişehir, October 22-24, 1996).

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 40 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 573-577, July-August, 1997. Original article submitted November 25, 1966.

0009-3130/97/3304-0441$18.00

TABLE 1. Distribution of the Fatty Acids in the Triacylglycerols from the Tuberous Roots of *M. turcomanica*, mole-% (GLC)

Fatty acid	Triacylglycerols	sn-2-Monoacylglycerols
14:0	0.2	–
15:0	0.4	–
16:0	9.3	10.0 (35.8)*
16:1	0.9	Tr.
17:0	0.5	–
17:1	0.2	–
18:0	4.4	3.3(25.0)
18:1	43.7	79.8(61.0)
18:2	33.8	6.9(6.8)
18:3	5.5	–
19:0	Tr.	–
20:0	1.1	–
20:1	Tr.	–
Σ sat	15.9	13.3
Σ unsat	84.1	86.7

*Amount of the acid in the sn-2 position as a fraction of its total amount in the TAGs.

The selectivity factor (SF), reflecting the competition of the unsaturated FAs in the acylation of the secondary hydroxyl of the glycerol molecule was calculated by a known formula [5]. The SF for the 18:1 acid was 1.75 and for the 18:2 acid 0.17. It follows from the results obtained that, with a relatively small difference (10%) in the amounts of the two main unsaturated FAs in the TAGs of the tuberous roots of *M. turcomanica*, the 18:1 acid has a higher affinity than the 18:2 acid for the secondary hydroxyl.

The position-species compositions of the TAGs were calculated from the figures in Table 1, and 39 possible isomeric species were obtained (Table 2). Of them, 22 were represented in amounts of more than 1.0 mole-% and 17 in amounts of 0.9-0.1 mole-%.

The mass spectrum of the TAGs revealed peaks of M^+ ions and of characteristic fragments corresponding in molecular masses and relative intensities [3] to the species shown in Table 2.

On comparing the mass-spectral characteristics of the TAGs with the results of lipolysis it was possible to detect another three minor species. From the M^+ *m/z* values of 898 and 910 and the diagnostic fragments $[M - R_{1(3)}COO]^+$ with *m/z* 619 and 631 and also in the light of the above-mentioned nature of the distribution of the saturated and unsaturated acyls in this lipid, it was assumed that the two TAG species with the 19:0 and 20:1 FAs had the most probable structures 18:2-18:1-19:0 and 18:2-18:1-20:1, respectively. The presence of a third molecular species, 18:2-16:1-18:1 may be assumed on the basis of the presence of the two diagnostic fragments $[R_2CO + 74]^+$ and $[R_2CO + 128]^+$ with the respective *m/z* values of 311 and 365 formed in the breakdown of species with the 16:1 acid in the sn-2 position. Also in favor of this hypothesis is the fact that the M^+ ion with *m/z* 852 was fairly intensive (2.6%), while, according to the results of lipolysis the 18:2-16:0-18:3 isomer corresponding to this mass for M^+ was present in an amount of only 1.0%.

The mass-spectrometric characteristics of the TAGs formed evidence in favor of the presence of 16:1 acid in the central position of the molecule, as has also been reported previously for the TAGs of the seeds of several species of higher plants [4].

It can be seen from the position-species composition of the TAGs (see Table 2), that the amount of one-acid species, 18:1-18:1-18:1 and 18:2-18:2-18:2, did not exceed 8.0 mole-%. The main TAG species were the two-acid unsymmetrical, 18:2-18:1-18:1, and symmetrical, 18:2-18:1-18:2. Among the three-acid species the 18:2-18:1-18:3 and other sn-2-oleoyl- and sn-1(3)-linoleoyl-containing isomers predominated.

Thus, a correlation has been observed between the predomination of the 18:1 and 18:2 acids in the total FAs and the high levels in the TAGs of molecules with the 18:1 acid in the central position and the 18:2 acid in one of the extreme positions.

In the position-type composition of the TAGs (Table 3), deduced from the position-species composition, the triunsaturated type UUU predominated. The amount of the diunsaturated symmetrical, SUS, and unsymmetrical, SSU + USS, species was low (5.4 mole-% in total), and the trisaturated type, SSS, was absent, which is normal when the amount of saturated FAs in TAGs is low.

Thus, the TAGs of the tuberous roots of *M. turcomanica*, like other plant TAGs enriched with unsaturated FAs [3, p. 206], are characterized by a high level of triunsaturated and diunsaturated species. Unlike the majority of seed TAGs, with

TABLE 2. Position-Species Composition of the Triacylglycerols from the Tuberous Roots of *Mandragora turcomanica*

Serial No.	Species sn-1(3)-sn-2-sn-3(1)	Content, mole-%	Mass spectrum M^+ (I, %)
1	18:2 – 18:1 – 18:1	20.5	882(8.7)
2	18:2 –18:1 – 18:2	17.8	880(7.6)
3	18:2 – 18:1 – 16:0	8.0	856(1.9)
4	18:2 – 18:1 – 18:3	6.0	878(4.2)
5	18:2 – 18:1 – 20:0	4.2	884(7.6)
6	18:2 – 18:1 – 16:1	1.4*	912(0.3)
7	18:2 – 18:1 – 17:0	1.3	854(3.4)
8	18:2 – 18:1 – 15:0	0.6	870(0.8)
9	18:2 – 18:1 – 17:1	0.5	842(0.4)
10	18:2 – 18:1 – 14:0	0.3	868(1.6)
11	18:2 – 18:1 – 19:0	0.1	828(0.2)
12	18:2 – 18:1 – 20:0	Tr.	898(0.5)
13	18:2 – 18:1 – 20:1	Tr.	910(0.4)
14	18:1 – 18:1 – 18:1	6.2	884(7.6)
15	18:1 – 18:1 – 16:0	3.8	858(1.2)
16	18:1 – 18:1 – 18:3	2.0	882(8.7)
17	18:1 – 18:1 – 18:0	1.9	886(2.5)
18	18:3 – 18:1 – 16:0	1.4	854(3.4)
19	18:3 – 18:1 – 18:0	0.9	882(8.7)
20	18:3 – 18:1 – 18:3	0.5	876(2.4)
21	16:0 – 18:1 – 16:0	1.4	832(0.1)
22	16:0 – 18:1 – 18:0	1.0	860(0.3)
23	18:1 – 18:2 – 18:2	2.1	880(7.6)
24	18:1 – 18:2 – 18:1	0.7	882(8.7)
25	18:1 – 18:2 – 16:0	0.4	856(1.9)
26	18:2 – 18:2 – 18:2	1.8	878(4.2)
27	18:2 – 18:2 – 16:0	0.8	854(3.4)
28	18:2 – 18:2 – 18:0	0.6	882(8.7)
29	18:2 – 18:2 – 18:3	0.5	876(2.4)
30	18:1 – 18:0 – 18:2	1.5	884(7.6)
31	18:1 – 18:0 – 18:3	0.6	882(8.7)
32	18:2 – 18:0 – 18:2	1.2	882(8.7)
33	18:1 – 16:0 – 18:2	2.6	856(1.9)
34	18:1 – 16:0 – 18:1	0.7	858(1.2)
35	18:1 – 16:0 – 16:0	0.6	832(0.2)
36	18:1 – 16:0 – 18:3	0.5	854(3.4)
37	18:1 – 16:0 – 18:0	0.4	860(0.3)
38	18:2 – 16:0 – 18:2	2.2	854(3.4)
39	18:2 – 16:0 – 16:0	1.2	830(0.1)
40	18:2 – 16:0 – 18:3	1.0	852(2.6)
41	18:2 – 16:0 – 18:0	0.8	858(1.2)
42	18:2 – 16:1 – 18:2	(?)	852(2.6)

*Sum of the 18:2—18:1—16:1 and 18:2—16:1—18:2 species.

TABLE 3. Position-Type Composition of the Triacylglycerols from the Tuberous Roots of *Mandragora turcomanica*

Type	Content, mole-%
UUU*	59.7
SUU+UUS	24.6
USU	10.2
SSU+USS	3.0
SUS	2.5
SSS	–

*U is the sum of the unsaturated, and S the sum of the saturated, fatty acids.

the exception of the seeds of some species of *Lamiaceae* [5], the TAGs of the hypogeal organ of the plant studied show the opposite tendency, with a greater selectivity of the 18:1 acid for the secondary and of the 18:2 acid for one of the primary hydroxyls of the glycerol molecule.

EXPERIMENTAL

Mass spectra were taken on an MKh-1321 mass spectrometer with an ionizing energy of 50 eV, a current of 20-40 μA, and a temperature of the ionization chamber of 170-190°C.

GLC was conducted as in [7].

The conditions for the TLC and Ag^+-TLC of the FAMEs are given in [4]. The enzymolysis of the TAGs with pancreatic lipase was carried out as described in [6].

Triacylglycerols. Mass spectrum, *m/z* (*I*, %): 211(2.1), 225(2.0), 239(9.3), 237(4.2), 253(2.7), 251(1.2), 281(3.1), 295(3.3) $[RCO]^+$; 267(7.2) and 266(8.3), 263(32.7) and 262(60.0), 261(11.7) and 260(17.0), 293(4.9) and 292(2.4) $[RCO]^+$ and $[RCO\text{-}1]^+$; 227(4.2), 241(4.5), 255(2.4), 253(2.4), 269(1.8), 283(2.3), 279(4.3), 277(4.0), 287(0.8), 309(2.0) $R[COO]^+$; 311(5.0), 341(13.0), 339(48.2), 337(14.5) $[R_2CO + 74]^+$; 367(5.1), 365(1.5), 395(6.0), 393(7.6), 391(2.7) $[R_2CO + 128]^+$; 549(2.8) and 548(1.9), 551(5.0) and 550(2.0), 563(2.1) and 562(1.3), 573(3.8), 572(1.9), 575(12.4) and 574(8.3), 577(27.5) and 576(16.6), 579(8.3) and 578(10.1), 589(3.9) and 588(3.0), 591(3.0) and 590(3.6), 597(3.5) and 596(2.7), 599(8.9) and 598(7.0), 601(27.2) and 600(27.0), 603(100) and 602(62.0), 605(39.0) and 604(39.0), 619(3.9) and 618(4.1), 631(2.0) and 630(1.7), 633(3.5) and 632(3.9) $[M-R_{1(3)}COO]^+$ and $[M-R_{1(3)}COO-1]^+$.

Methyl Esters of the Fatty Acids of the Triacylglycerols. Mass spectrum, *m/z*: M^+ 242-326 (14:0-20:0), 268-324 (16:1-20:1), 294 (18:2), 292 (18:3), 74 (100%).

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