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LIPIDS OF THE TUBEROUS ROOTS OF *Mandragora turcomanica*

D. T. Asilbekova[a], S. D. Gusakova[a], Yu. M. Murdakhaev[b], and A. I. Glushenkova[a]

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The lipids of the tuberous roots of Mandragora turcomanica *Mizger (fam. Solanaceae) have been characterized for the first time. By using CC, TLC, Ag^+-TLC, UV and IR spectroscopies and mass spectrometry, 20 classes of lipids and lipophilic substances have been characterized. The main components of the acyl-containing lipids that were isolated were found to be triacylglycerols and glycolipids, and among the lipophilic compounds they were alkanols and sterols; in the mixture of fatty acids of the acyl-containing lipids the 18:2, 18:1, and 16:0 acids predominated.*

The lipids of the hypogeal organs of medicinal plants have been studied for only a few representatives of plant families. The basic spectrum of all three groups of lipids (neutral lipids and glyco- and phospholipids), differing in amount and in the levels of certain components, has been detected in the roots of *Panax ginseng* L. (ginseng, fam. Araliaceae) [1], *Gossypium hirsutum* L. (cotton, fam. Malvaceae) [2], and *Zea mays* (maize, fam. Poaceae) and in the tubers of *Solanum tuberosum* L. (potato, fam. Solanaceae) [3]. In the roots of *P. ginseng* and *G. hirsutum* the neutral lipids (NLs) predominate, and the level of glycolipids (GLs) exceeds that of the phospholipids; in *Z. mays* roots and *S. tuberosum* tubers the opposite relationship of these components exists. Unusual components have been found in the lipids of the roots of some medicinal plants: high-molecular-mass saturated and branched acids and hydroxyketones [4-7]. In the NLs of the roots of *Solanum ducamara* various sterols and their esters have been identified [8].

Species of *Mandragora* (fam. Solanaceae) have long been known as medicinal plants used as aphrodisiac, hallucigenic, sedative, and anesthetic agents [9]. Of the 5-6 species that exist, only *Mandragora turcomanica* Mizger. has been found in Central Asia [10]. The individual organs of *Mandragora* have scarcely been investigated, and only the presence of tropane alkaloids [11, 12] and of docosanol and triacontanol in the roots [13] has been reported.

We have investigated the lipids of the tuberous roots of *M. turcomanica*, which were extracted from the air-dried and comminuted mass with a mixture of chloroform and methanol. The yield of extractive substances was 55 mg/g of absolutely dry matter (a.d.m.) (5.5%) of the tuberous roots. The lipids were fractionated with the aid of CC, and the fractions isolated were rechromatographed by preparative TLC. Of the polar lipids, the GLs were separated into classes by preparative TLC in system 4, while the PLs, in view of their minor amount, were analyzed only qualitatively by TLC in system 5. From their characteristics (chromatographic mobilities, absence of detection by specific reagents), some of the substances eluted from the column were assigned to the nonlipid compounds. Among them, alkaloids were detected by means of qualitative reactions.

The amount of true lipids was 36 mg/g a.d.m. of the tuberous roots. Their composition is given in Table 1. Almost 2/3 of the weight of the lipids from the tuberous roots of *M. turcomanica* was represented by the NLs, and the GLs predominated among the polar lipids.

In the GLs there was a larger amount of monogalactosyldiacylglycerols (MGDGs) than of digalactosyldiacylglycerols (DGDGs), as in the roots [2] and seeds [14] of *G. hirsutum* and the roots of *Z. mays* [3] studied previously. On the other hand, no MGDGs have been detected in the roots of *P. ginseng* [1]. The amount of steryl glycosides in the roots of *M. turcomanica* was somewhat smaller than that of their esters (SGEs), as also in the tubers of *S. tuberosum* [3].

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a) Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 89 14 75; and b) F. N. Rusanov Botanical Garden, Academy of Sciences of the Republic of Uzbekistan, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 615-623, September-October, 1994. Original article submitted January 23, 1994.

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TABLE 1. Composition of the Lipids of *M. turcomanica*

Class of lipids	Amount	
	mg/g a.d.m.	% on the weight of lipids
Neutral lipids	23.32	64.7
1. Hydrocarbons	1.95	5.4
2. Esters of fatty acids with alkanols, triterpenols, and sterols	1.39	3.9
3. Triacylglycerols	7.44	20.7
4. Oxoacyldiacylglycerols	0.06	0.1
5. Free fatty acids	3.19	8.9
6. High-molecular-mass alcohols	4.89	13.5
7. Hydroxyacyldiacylglycerols	0.05	0.1
8. 1,2(1,3)-Diacylglycerols	0.22	0.6
9. Triterpenols		
10. Methylsterols	3.64	10.1
11. Sterols		
12. Monoacylglycerols	0.49	1.4
Glycolipids	10.10	28.1
13. Steryl glycoside esters	2.03	5.7
14. Monogalactosyldiacylglycerols	4.28	11.9
15. Steryl glycosides	1.48	4.1
16. Digalactosyldiacylglycerols	2.31	6.4
Phospholipids	2.58	7.2
17—20. Phosphatidylethanolamine, phosphatidyl choline, phosphatidylinositol, phosphatidic acid		
Σ_{lipids}	36.0	100.0

In the PLs we qualitatively identified four classes, of which phosphatidylethanolamine was a minor component. Similar components, apart from phosphatidic acid, were present in the other hypogeal plant organs studied previously [1-3, 15, 16]. An exception was the roots of *P. ginseng*, in which, instead of phosphatidylinositol, phosphatidylglycerol was detected, this having also been found in the roots of *Hibiscus cannabinus* (kenaf, fam. Malvaceae) [16] and the tubers of *S. tuberosum* [3]. According to other authors [15], *S. tuberosum* tubers and *Z. mays* roots [3] contain not phosphatidylglycerol but diphosphatidylglycerol and phosphatidic acid. Without excluding the presence of native phosphatidic acid in the tuberous roots of *M. turcomanica* and the tubers of *S. tuberosum*, it must be mentioned that, under the conditions used for the extraction of lipids from samples without first inactivating enzymes, phosphatidic acid could also be formed as the result of the breakdown of other PLs by endogenous phospholipase D.

In the NLs we identified six acyl-containing classes (Table 1), with triacylglycerols making up 32% of the weight of the NLs. A fairly high level of TAGs has been revealed in the lipids of the roots of *G. hirsutum* [2] and *Z. mays* [3]. Although it is not known what role the TAGs play in the roots of higher plants, it may be assumed that these lipids, like seed lipids, accumulate as reserve components in the root tissues. Also in favor of this hypothesis is the high content of TAGs in the tuberous roots of *M. turcomanica*, which is capable of multiplying not only by seeds but also vegetatively with the aid of the tuberous roots [10].

TABLE 2. Fatty-acid Composition of the Acyl-Containing Lipids of the Tuberous Roots of *M. turcomanica* (%, GLC)

Acid	Class of lipids								
	FASEs*	TAGs	FFAs	DAGs	MAGs	SGEs	MADGs	DGDGs	PLs
14 : 0	1.3	0.3	0.4	1.0	1.0	1.4	1.3	2.9	0.4
15 : 0	–	0.2	0.2	Tr.	0.9	2.1	Tr.	Tr.	Tr.
16 : 0	35.6	8.6	46.2	28.3	23.4	44.0	32.1	37.8	26.3
16 : 1	3.3	1.3	1.8	1.8	1.8	3.9	3.7	2.3	Tr.
17 : 0	Tr.	Tr.	1.7	0.3	0.4	Tr.	Tr.	1.2	Tr.
17 : 1	–	0.5	0.9	0.7	1.4	0.8	2.7	2.7	–
18 : 0	7.2	4.4	8.8	10.6	10.2	5.5	11.8	8.9	4.8
18 : 1	37.6	44.0	16.2	35.1	33.6	32.3	12.0	12.9	19.7
18 : 2	4.4	33.6	19.3	20.0	22.7	4.5	31.6	27.8	46.9
18 : 3	–	5.6	3.1	1.2	4.6	Tr.	4.8	3.5	1.9
20 : 0	5.7	1.5	1.4	1.0	Tr.	5.5	Tr.	Tr.	–
$\Sigma_{sat.}$	54.7	15.0	58.9	41.4	35.9	58.5	45.2	50.8	31.5
$\Sigma_{unsat.}$	45.3	85.0	41.1	58.6	64.1	41.5	54.8	49.2	68.5

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*Additionally, 21:0 — 1.9%; 22:0 — 3.0% in the FASEs, and 19:0 in trace amounts in the TAGs and FFAs (GLC, mass spectra).

We may note the presence of monoacyl- and diacylglycerols (MAGs and DAGs) in the NLs of *M. turcomanica*. Also present in minor amounts are oxidized forms of TAGs — oxoacyl- and hydroxyacyldiacylglycerols, which were identified from qualitative reactions and from the TLC mobilities of the initial oxidized lipids and of the products of their hydrolysis [17].

The composition of the fatty acids (FAs) of the acyl-containing lipids was determined with the aid of GLC. The absence of other isomers, homologs and isologs of the FAs was confirmed by the separation of the sum of the FA methyl esters by preparative Ag^+-TLC, followed by the mass spectrometry and GLC of narrow fractions. The acid compositions shown in Table 2 were obtained from the combined results. It can be seen that the TAGs had the highest degree of unsaturation of the total FAs and contained more of the 18:1 acid than the other classes and the lowest level of the 16:0 acid. The 18:1 acid predominated in the DAGs and MAGs and the level of total unsaturation was lower because of an increase in the proportion of the 16:0 acid. The main acids of the MGDGs and DGDGs were the 16:0 and 18:2 species, while these galactolipids differed from one another by the degree of unsaturation of the FAs, which was somewhat higher in the MGDGs. The PLs were found to have the highest level of the 18:2 acid, which was also dominant among the unsaturated acids of the PLs of the roots of *G. hirsutum* [2] and of *H. cannabinum* [16] studied previously. The 16:0 acid was the main component of the FFAs and the SGEs, which had similar compositions of the other fatty acids, as well, indicating a possible mutual connection of the pathways of the biosynthesis of these classes of lipids.

The FASEs were studied by the mass spectrometry of the initial esters and of the products of their severe hydrolysis [18]. Alkanols, triterpenols, monomethylsterols and sterols, with a predominance of the latter, were detected in the alcoholic part of the hydrolysis products with the aid of TLC in system 2 and qualitative reactions. The mass spectrum of the initial FASEs contained ions of the acyl residues $[RCOOH_2]^+$ and $[RCO]^+$ with *m/z* values corresponding to the saturated 14:0-22:0 acyls and the unsaturated 16:1, 18:1, 18:2, and 18:3 acyls. Alkanols of the even series, $C_{22\text{-}30}H_{45\text{-}61}OH$, were identified from the presence in the mass spectrum of the peaks of $[R - 1]^+$ and $[RCOO]^+$ ions of appreciable intensity, which are characteristic for the fatty-alcohol residues of wax esters.

In the light of the values of M^+ (Experimental part), the wax esters were represented by twelve even homologs of saturated, $C_{38\text{-}52}H_{76\text{-}104}O_2$, and unsaturated, $C_{42}H_{82}O_2$, $C_{44}H_{86}O_2$, $C_{46}H_{90\text{-}88}O_2$, and $C_{48}H_{94\text{-}92}O_2$, esters in which the 18:1 acid was esterified with the C_{24}-C_{30} alcohols and the 18:2 acid with only two, the C_{28} and C_{30}, alcohols.

TABLE 3. Composition of the Sterol and Triterpenol Esters of the Tuberous Roots of *M. turcomanica*

Acyl moiety	M^+ and characteristic ions, *m/z** (%)						
	Alcoholic components of the sterol esters						
	Sitosterol	Campesterol	Cholesterol	Stigmasterol	$[M - \text{side-chain}]^+$	$[M{-}43]^+$	$[M{-}112]^+$
14 : 0	624(0.1)	610(0.9)	–	622(0.1)	483(0.9)	579(2.4)	510(0.1)
16 : 0	652(0.2)	638(2.8)	624	650	511(1.9)	607(0.8)	538(0.1)
16 : 1	650(0.7)	636(0.2)	–	648	509(2.3)	605(0.9)	536(0.1)
18 : 0	680(0.1)	666(1.2)	652	678	539(1.1)	635(0.5)	566(0.1)
18 : 1	678(2.3)	664(3.9)	650	676(2.3)	537(0.8)	633(0.1)	564(0.1)
18 : 2	676(2.7)	662(5.0)	648(0.9)	–	535(3.7)	–	–
18 : 3	674(1.9)	660(2.8)	646(0.1)	–	533(2.3)	–	–
	Alcoholic components of triterpenol esters						
	24-Methylenecycloartanol	Cycloartanol	Cycloartenol	Amyrin	$[RH{+}207]^+$		
16 : 0	678	666	664		445(0.5)		
18 : 0	706(0.8)	694(0.1)	692		471(0.5)		
18 : 1	704(1.9)	692(1.3)	690		469(0.5)		
18 : 2	702(0.1)	690(1.5)	688(1.5)		467(0.3)		
20 : 0	734(0.1)	722(0.1)	720(0.7)	–	–		
22 : 0	762(0.1)	750(0.1)	748(0.4)	–	–		

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*$[M - 43]^+$ and $[M - 112]^+$ — from the breakdown of stigmasterol esters; $[RH + 207]^+$ — from amyrin esters; *m/z* 396 (100%).

In the mass spectrum of the FASEs the ions with the highest intensities were the $[M - RCOO]^+$ and $[M - RCOOH]^+$ ions produced in the breakdown of acylated sterols: β-sitosterol, stigmasterol, campesterol, and cholesterol [19]. On the basis of the features of the fragmentation of this class, and taking into account the fatty-acid composition of the FASEs (Table 2), we identified the most probable set of sterol and triterpenol esters, which is given in Table 3.

In the mass spectrum of this fraction, overhigh intensities were observed for M^+ ions with *m/z* values of 638 and 666-660 from campesterol esters: this showed the presence of esters of lophenol (4α-methyl-5α-cholest-7-en-3β-ol) — which belongs to the class of methylsteroids — having the same composition of the acyl groups (Table 3). Ions with *m/z* 287 (7), 269 (64), 260 (7), 245 (6), 243 (14), and 227 (26) are also characteristic for the breakdown of lophenol. This methylsterol is present in the free form in fairly large amounts in the seeds of several species of the family Solanaceae [20], but esters of lophenol with FAs have not previously been identified in representatives of this family.

The peaks of the $[M - RCOO]^+$ and $[M - RCOOH]^+$ fragments present in the mass spectrum of the FASEs corresponded to ester of triterpenols with M^+ 440, 428, and 426. An ion with *m/z* 300, together with other fragments known for the breakdown of triterpenols [21], witnessed the presence of a 24-methylenecycloartanol residue. The mass numbers of the $[207 + RH]^+$ fragments and of an ion with *m/z* 218 showed the presence of esters of α- or β-amyrin with the 14:0-16:0, 18:1, and 18:2 FAs. Consequently, the M^+ 664, 692, 690, and 618 ions could relate both to esters both of amyrin and of cycloartenol. The M^+ ions having mass numbers 2 units greater than M^+ for cycloartenol esters confirmed the presence of cycloartanol esters, and M^+ ions with *m/z* 14 units greater confirmed the presence of 24-methylenecycloartanol esters.

A comparison of the intensities of the M^+ peaks showed that sterols having saturated side-chains (sito-, campe- and cholesterols) were more specifically esterified with unsaturated FAs. Conversely, there were no esters of stigmasterol (a Δ^{22}-unsaturated sterol) with the 18:2 and 18:3 acids. Likewise, among the acylated triterpenols no esters with the 18:3 and 14:0

TABLE 4. Composition of the Free Demethyl-, Monomethyl-, and Dimethylsterols from the Tuberous Roots of *M. turcomanica*

Components	Ag^+-TLC, R_f of the acetates	GLC	
		RRT	Content, %
Cholesterol	1.00	1.00	4.3
Campesterol	1.00	1.33	9.7
Lophenol	1.18		
Stigmasterol	1.00	1.48	13.5
Cycloartenol	1.32	1.50	
β-Sitosterol	1.00	1.67	15.5
β-Amyrin	1.37	1.78	6.2
Gramisterol (24-methylenelophenol)	0.32	1.80	
Cycloartenol	0.56	2.15	25.8
24-Methylenecycloartanol	0.22	2.25	15.5
Citrostadienol	0.56	2.47	9.5

acids were detected. In contrast to the sterols, the triterpenols were also acylated by acids of higher molecular mass — the 20:0 and 22:0 species. At the same time, the amyrin was not esterified either by the 18:3 acid or by high-molecular-mass FAs.

The lipophilic components of the NLs of the tuberous roots of *M. turcomanica* were hydrocarbons, high-molecular-mass alcohols, and dimethyl-, monomethyl- and demethylsterols, with a predominance of the high-molecular-mass alcohols.

According to the results of TLC, UV spectrophotometry, and a mass spectrum,the hydrocarbons consisted mainly of alkylbenzenes having the general formula C_nH_{2n-6} with C_{21}-C_{33} chains. They were accompanied by straight-chain C_nH_{2n-4} trienes and C_nH_{2n}-2 dienes and by only odd-membered paraffins C_nH_{2n+2} of the C_{27}-C_{33} series.

According to their mass spectrum, the high-molecular-mass alcohols consisted of a mixture of alkanols of the even series $C_{20-30}H_{41-61}OH$ and scarcely differed in composition from the alcoholic components of the wax esters. With the exception of the docosanol and triacontanol mentioned above [13], no aliphatic alcohols have been found in the roots of other plants.

The compositions of the free sterols of the three types were determined from their mass spectra and GLC in the native form [22] and by Ag^+-TLC in system 3 in the form of their acetates [20, 21]. The retention times of the peaks in GLC were calculated in relation to cholesterol, and the R_f values of the acetate derivatives on Ag^+-TLC in relation to cholesterol acetate. β-Amyrin was identified from the presence of a poorly resolved peak in GLC with the RRT 1.78 (in relation to cholesterol). The presence of α-amyrin, as well, was not excluded, but its identification is difficult because the GLC behavior of the α-isomer coincides with that of cycloartenol (RRT 2.15). It can be seen from Table 4 that, in the total cyclic alcohols, dimethylsterols — cycloartenol and 24-methylenecycloartanol — predominate, and, of demethylsterols, β-sitosterol. It must be mentioned that nine components out of the eleven sterols — excluding the methylsterols gramisterol and citrostadienol — have also been detected in the FASEs of the tuberous roots of *M. turcomanica* (Table 3).

In contrast to the roots of *S. dulcamara*, the only ones studied previously [8] for the composition of the free and bound sterols, the tuberous roots of *M. turcomanica* contain triterpenols and monomethylsterols, which are characteristic for the seed lipids of representatives of the Solanaceae [20-22].

EXPERIMENTAL

For general observations, see [18].

CC was conducted on silica gel L 100/160 (Czechoslovakia) previously washed with chloroform—methanol (2:1, *v/v*). The NL fractions were eluted from the column as described in [17], the GLs with acetone, and the PLs with methanol. TLC was performed on Silufol and on silica gel L 5/40 (Czecholslovakia) with the addition of 7% of $CaSO_4$, and Ag^+-TLC on silica gel with 7% of $CaSO_4$ and 20% of $AgNO_3$. The following solvent systems were used: hexane—$(C_2H_5)_2O$—CH_3COOH: 1) 90:10:1; 2) 70:30:1; 3) $C_2H_4Cl_2$-CCl_4 (1:5) (four ascents); 4) $(CH_3)_2CO$-C_6H_6—H_2O (91:30:8); 5) $CHCl_3$—CH_3OH—NH_4OH (25%) (14:6:1) (direction I) and $CHCl_3$—CH_3OH—CH_3COOH—H_2O (20:6:1:1) (direction II). Five substances were detected in I_2 vapor, by 50% H_2SO_4 with heating, and by qualitative reactions with 2,4-dinitrophenylhydrazine for the oxo group [17], with α-naphthol for GLs, and with ninhydrin and the Dragendorff and Vas'kovskii reagents for PLs [23].

The tuberous roots of *M. turcomanica* were obtained from the laboratory of medicinal botany, F. N. Rusanov Botanical Garden, Republic of Uzbekistan.

The lipids were extracted from the air-dry roots by Folch's method, and the elimination of ballast substancs, the mild and severe alkaline hydrolyses of the lipids, the isolation of the FAs, and the acetylation of the steroids were carried out as described in [23]; the FAs were esterified with diazomethane.

The detection of the mixture of alkaloids with the aid of standard tests was done by S. F. Aripova.

Hydrocarbons. R_f 0.96, system 1, pink spot with 50% H_2SO_4 and heating. UV spectrum (λ_{max}^{hexane}, nm): 227, 258, 280. Mass spectrum, M^+, *m/z*: 464-380 (paraffins $C_{27-33}H_{56-68}$), 462-294 (olefins $C_{21-33}H_{42-66}$), 460-292 (dienes $C_{21-33}H_{40-64}$), 458-290 (trienes $C_{21-33}H_{38-62}$) and 456-288, 119 (100%), 105-91 (alkylbenzenes $C_{21-33}H_{36-60}$) [24].

Esters of Fatty Acids with Alkanols, Triterpenols, and Sterols. R_f 0.58-0.80, diffuse spot, system 1 [18]. Mass spectrum of the acyl part, *m/z*: 341-229 (22:0-14:0), 283 (18:1), 281 (18:2), 255 (16:1) $[RCOOH_2]^+$; 323-211, 265, 263, 237 $[RCO]^+$ and 264, 262, 236 $[RCO - 1]^+$; alcoholic component: ions of the type of $[M - RCOO]^+$ and $[M - RCOOH]^+$ with the following *m/z* values (%): for sterol esters — 397 (51), 396 (100), β-sitosterol, 395 (46), 394 (74), stigmasterol, 383 (85), 382 (96), campesterol, 369 (28), 368 (44), cholesterol, 296 (99), 255 (69), 231 (17), 213 (54); for triterpenol esters — 423 (11), 422 (6), 300 (5), 24-methylenecycloartanol, 411 (5), 410 (8), 288 (11), cycloartanol, 409 (17), 408 (19), 284 (32), cycloartenol, 271 (11), 218 (17), 203 (60), 189 (54), 175 (73), 95 (98), 69 (92); ions of the $[M - RH]^+$ and $[M - RCOO]^+$ types for esters of aliphatic alcohols — 465 (0.6), 437 (1.0), 409, 381 (0.8), 353 (0.6), and 420 (1.0), 392 (3.9), 364 (1.8), 336 (0.1), 308 (0.1); the M^+ values of the sterol and triterpenol esters are given in Table 3; M^+ of the esters, *m/z* (%): 760 (0.1), 732 (1.0), 704 (1.9), 702 (0.8), 700 (0.4), 676 (2.7), 674 (1.9), 672 (1.0), 648 (1.9), 646 (0.6), 620 (1.6), 618 (0.7), 592 (1.3), 564 (1.2).

Triacyl-, 1,2(1,3)-Diacyl-, and Monoacylglycerols. R_f 0.70, 0.40, 0.32, 0.10, system 2; after mild alkaline hydrolysis FAs were formed, with R_f 0.55 in the same system.

Hydroxy- and Oxoacyldiacylglycerols. R_f 0.43 and 0.57 (orange spot with 2,4-DNPH), system 2; after saponification, FAs, hydroxy-FAs with R_f 0.30, and oxo-FAs with R_f 0.45 (positive reaction with 2,4-DNPH) were formed [17].

High-Molecular-Mass Alcohols. R_f 0.61, system 2. Mass spectrum, *m/z*: 420-280 $[M - 18]^+$ [24].

Dimethyl-, Monomethyl-, and Demethylsterols. R_f 0.42, 0.36, and 0.32, system 2; giving orange-red, pink, and red colorations under the action of 50% H_2SO_4 with heating [18]; the mass spectrum, and behavior in GLC and TLC corresponded to literature information [20-22].

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