Chemistry of Natural Compounds and Bioorganic Chemistry

Lipids of *Glycyrrhiza glabra* roots

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A petroleum ether extract of *Glycyrrhiza glabra L.* roots was investigated. The extract contains 70 % neutral lipids and 30 % polar lipids. Hydrocarbons, sterols esters, triacylglycerols, free fatty acids, free sterols, and diacylglycerols were identified. The fatty acid contents of all of the acyl-containing lipids were determined. Fourteen fatty acids were identified; the 18 : 2 fatty acid prevails among the unsaturated acids, and the 16 : 0 acid prevails among the saturated acids.

Key words: *Glycyrrhiza glabra*, *Fabaceae*; roots, lipids.

The roots of licorice (*Glycyrrhiza glabra,* fam. *Fabaceae*) are widely used in medicine and the food industry mainly due to the content of glycyrrhizin, which is a mixed K—Mg—Ca salt of glycyrrhizic acid.

Glycyrrhizic acid (triterpene glycoside) is the main biologically active component of licorice roots. The preparations made from them possess a broad spectrum of effects, *i.e.*, deoxycorticosteroid,[1] anti-ulcer, and anti-inflammatory[3,4] activity, and exhibit anti-allergic[2] properties as well.

Flavonoids, coumarins, β-sitosterol, stigmasterol, stilbenes, phenanthrenes, and essential oils have been isolated and identified from licorice roots.[5—9] However, there are still no literature data on the lipids in licorice roots, except a communication, where some numerical characteristics of a so-called liposteroid complex of licorice root were given and some suggestions were made concerning the qualitative make-up of a set of fatty acids.[10]

In the Institute of Organic Chemistry of the Ufa Scientific Center of the Russian Academy of Sciences, a multipurpose treatment of licorice roots and the fabrication of different preparations based on glycyrrhizic acid have been performed. In order to develop methods for more complete and wasteless processing of the raw material, we studied the lipids from licorice roots. According to the preliminary data, these lipids exhibit a pronounced circatrization effect.

We studied the petroleum ether extract from roots of *Glycyrrhiza glabra* collected in Turkmenistan. The lipids were isolated from powdered air-dry licorice roots by extraction with petroleum ether at room temperature. The yield of the lipids was 0.5 % with respect to the weight of the air-dry raw material. The total lipid frac-

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Table 1. Classes of lipids from the roots of *Glycyrrhiza glabra* (% based on total lipids)

Lipid Class	Content
Hydrocarbons	0.5
Sterol esters	27.5
Fatty acid methyl esters	Traces
Triacylglycerols	10.0
Free fatty acids	10.5
Free sterols	11.5
Diacylglycerols	6.2
Glycolipids	19.0
Phospholipids	10.1
Unidentified	4.7

tion was separated into different classes of lipids by column chromatography on silica gel. After repeated purification and chromatography, separate classes were identified, according to TLC data, *i.e.*, by the comparison of their R_f values with the respective values for model samples isolated from natural sources. The data from chemical transformations and physico-chemical analysis were also used (Table 1). As follows from Table 1, the total lipid fraction consists of neutral lipids (NL) and polar lipids (PL) in a 2 : 1 ratio. The NL consist mainly of sterol esters (SE), which account for nearly a half of the NL. Triacylglycerols (TAG), free fatty acids (FFA), and free sterols (FS) are present in almost equal amounts of 10.0, 10.5, and 11.5 %, respectively.

The physiological and food value of the oil, as well as its stability during processing and storage, depend considerably on the types and amounts of the fatty acid constituents.

The fatty acid compositions of all of the acyl-containing lipid fractions (determined by GLC) are listed in Table 2. In the total lipid extract 14 fatty acids were identified, of which about 30 % were saturated and 70 % were unsaturated. The latter are represented mainly by two acids, *viz*, 18 : 2 (linolic acid, 52 %) and 18 : 1 (oleic acid, 12 %). Palmitic acid prevails among the saturated acids. Acids of higher molecular weight (from 20 : 0 to 24 : 0) were also identified.

Table 2 shows that fatty acids are not uniformly distributed within the individual lipid fractions. In the SE and FFA fractions there are 70 % saturated fatty acids. The main fatty acids from the SE fraction are 16 : 0 and 18 : 0 (35.2 and 14.6 %, respectively); the main ones from the FFA fraction are 16 : 0 and 22 : 0 (19.9 and 24.1 %, respectively). The same regularity is observed for the distribution of unsaturated acids in the mentioned lipid classes isolated from the roots and seeds of the cotton plant.[11,12]

The TAG, diacylglycerols (DAG), and glyco- (GL) and phospholipids (PL) do not differ much in saturated and unsaturated acid distribution (on average, 35.1 and 65.3 %, respectively); the predominant component is that of the 18 : 2 acid. In all of the acyl-containing lipid classes, 22 : 0 acid was identified.

Sterols are contained in the licorice root extract in both the free and the bound form (that is, in the form of esters).

In the mass-spectra of the total fraction of the free sterols (**1**) and the sterols obtained by hydrolysis of sterol esters (**2**), there are the peaks of the molecular ions with m/z values of 414, 412, and 398 and those of the characteristic ions at m/z 396, 394, and 380 $[M-H_2O]^+$ and 381, 379, and 365 $[M-H_2O-CH_3]^+$.

Table 2. Fatty acid contents of the roots of *Glycyrrhiza glabra* (% according to GLC analysis)

Fatty acids	Lipid Class						
	Total lipids	SE	TAG	FFA	DAG	GL	PL
12 : 0	1.0	0.7	0.8	—	Traces	0.3	Traces
14 : 0	1.6	1.5	3.6	1.2	1.1	1.4	0.4
15 : 0	0.4	1.3	—	1.1	—	—	Traces
16 : 0	16.6	35.2	13.0	19.9	15.2	20.2	0.4
16 : 1	2.3	2.7	1.4	—	1.7	1.6	1.7
17 : 0	2.3	—	—	—	—	—	—
18 : 0	3.4	14.6	1.7	6.2	6.6	6.8	5.6
18 : 1	12.0	24.3	14.0	15.1	14.8	13.5	11.3
18 : 2	51.4	7.0	44.0	16.9	43.5	43.3	44.5
18 : 3	1.7	—	8.2	1.2	6.7	5.2	4.2
21 : 0	3.4	—	6.0	—	2.5	0.3	—
22 : 0	3.9	9.2	7.3	24.1	7.9	7.4	3.8
23 : 0	Traces	3.5	—	2.2	—	—	—
24 : 0	Traces	—	—	5.6	—	—	—
Saturated total	32.6	66.0	32.4	68.0	33.3	36.4	38.3
Unsaturated total	67.4	34.0	67.6	32.0	66.7	63.6	61.7

Table 3. Contents, compositions, and chromatographic characteristics of free sterols and their esters from the roots of *Glycyrrhiza glabra*

Sterols	Free sterols (%)	Bound sterols (%)	R_f	RR_t
β-Sitosterol	70.9	77.6	0.5	4.9
Stigmasterol	25.1	17.9	0.5	4.5
Brassicasterol	4.0	4.5	0.5	4.1

In the rest part of the spectra there are peaks typical of β-sitosterol, stigmasterol, and brassicasterol.[13] Also, in the MS of sample **2** there are weak peaks of $[M]^+$ 428 and 426.

The ^1H NMR spectra of samples **1** and **2** are in close resemblance and they indicate the presence of β-sitosterol and stigmasterol in a 10 : 1 ratio.[13]

GLC analysis (Table 3) revealed the presence of three components. β-Sitosterol prevails in samples **1** and **2** (70.9 and 77.6 %, respectively). Stigmasterol and brassicasterol make up 25.1 and 17.9 and 4.0 and 4.5 %, respectively. The results obtained are in accordance with the literature data on the individual lipid contents of the lipids of higher plants.[14] Table 3 shows the differences in the contents of different sterols in the free and bound form: β-sitosterol is contained mainly in the bound form, stigmasterol is encountered mainly in the free form (the amount of a free form is 1.5 times higher than that of a bound one), whereas the contents of free and bound brassicasterol are nearly equal. Analogous results, except those for brassicasterol, have been obtained earlier in a study of the sterol fraction of soy lipids.[15] In the lipids of higher plant seeds, brassicasterol is either absent or present in trace amounts, except for the lipids of the seeds of the fam. *Crucifera*, where the brassicasterol content of the total sterol fraction amounts to as much as 19 %.[14] Brassicasterol accounts for ~4 % of the sterols of *Glycyrrhiza glabra* roots.

Experimental

^1H NMR spectra were recorded on a Bruker AM-300 instrument with tetramethylsilane as the internal standard. Mass-spectra were obtained on a MX-1306 spectrometer. GLC analysis of fatty acid methyl esters was carried out on a Chrom-5 chromatograph (FID, a 1.2 m × 3 mm column, 5 % PDEHS on Chromaton N-AW-DMCS, 170 °C, He as the carrier gas, 40 mL min^{-1}).

GLC analysis of sterols was performed using a Chrom-5 chromatograph (FID, a 1.2 m × 3 mm column, 5 % SE-30 on Inerton-Super, temperature programming from 200 to 300 °C, He as the carrier gas, 40 mL min^{-1}). β-Sito- and stigmasterols were used as the model samples.

The licorice roots used were collected in Turkmenistan, in the flood plain of Amudarya (Chardjou) in September-October 1991.

Lipids were extracted from the air-dried ground licorice roots with petroleum ether (b.p. 40—60 °C) by triple infusion (1 : 4) at ~20 °C with stirring.

The total of lipids was fractionated by column chromatography using Chemapol L 100/160 μm silica gel (a 1×0.02 m column; a 1 : 50 lipids to sorbent ratio). The NL were subjected to gradual chromatography with elution with benzene—ether mixture from 99 : 1 to 1 : 99. The GL were eluted with acetone, and the PL with methanol.

Analytical TLC was performed on Silufol plates using the following eluents: benzene for hydrocarbons; benzene—ether 9.9 : 0.1 or 9.5 : 0.5 for SE; benzene—ether 8 : 2 for methyl esters; benzene—ether 7 : 3 for TAG, FFA, and DAG; and benzene—ether 5 : 5 for FS. Preparative TLC on silica gel was performed using the above-listed eluents.

The lipids were discovered either by spraying the plates with 50 % aqueous H_2SO_4 with subsequent heating or by using specific reagents[16,17] and were identified as described earlier.[18—20]

Fatty acids were isolated from TAG, DAG, GL and PL by hydrolysis with a 10 % KOH methanolic solution (heating for 30 min) or from SE by hydrolysis with a 20 % KOH methanolic solution (boiling for 3 h) as described previously.[11]

The sterols were recrystallized from methanol.

References

1. T. Hayashi, *Inzen Igakkai Zassi*, 1981, **90**, 282.
2. A. I. Muravjev and V. D. Ponomarev, *Zh. Med. Prom. SSSR* [*Journal of the Medicine Industry of the USSR*], 1962, 11 (in Russian).
3. Kh. M. Nasyrov and D. N. Lazareva, *Farmakol. i Toksikol.* [*Pharmacology and Toxicology*], 1980, **43**, 399 (in Russian).
4. L. A. Baltina, V. A. Davydova, T. G. Tolstikova, F. S. Zarudii, Yu. I. Murinov, A. I. Bondarev, and G. A. Tolstikov, *Khim.-Farm. Zh.* [*Chemical-Pharmacological Journal*], 1991, **25**, 45 (in Russian).
5. *Rastitel'nye Resursy* [*Plant Resources*], Nauka, Moscow, 1987, 111, 136 (in Russian).
6. T. Fukai, Q. Wang, and T. Nomura, *Phytochemistry*, 1991, **30**, 1245.
7. C. L. Wang, R. Y. Zhang, Y. S. Han, X. G. Dong, and W. B. Liu, *Yaoxue Xuebao*, 1991, **26**, 147.
8. B. M. Lawrence and R. J. Reynolds, *Perfum. Flavor*, 1987, **11**, 39.
9. J. H. Liu, S. S. Yang, Y. Q. Fu, C. L. Yuan, and B. Liu, *Yaoxue Xuebao*, 1990, **25**, 689.
10. N. F. Kononikhina, V. D. Ponomarev, and I. A. Muravjev, in *Voprosy izuchenija solodki v SSSR* [*Problems of the Study of Licorice in the USSR*], Nauka, Moscow, 1966, 159 (in Russian).
11. S. G. Yunusova, S. D. Gusakova, N. N. Stepanichenko, Sh. Kholmuradova, and A. I. Glushenkova, *Khim. Prir. Soedin.*, 1987, 57 [*Chem. Nat. Compd.*, 1987, 57 (Engl. Transl.)].
12. S. G. Yunusova, S. D. Gusakova, Kh. T. Mirzaazimova, and A. I. Glushenkova, *Khim Prir. Soedin.* 1989, 59 [*Chem. Nat. Compd.*, 1989, 59 (Engl. Transl.)].
13. R. C. Heupel, Y. Sauvaire, H. Le Phu, E. J. Parish, and W. D. Nes, *Lipids*, 1986, **21**, 69.
14. S. P. Kochar, *Prog. Lipid Res.*, 1983, **22**, 161.
15. A. Johansson and I. J. Hoffman, *J. Am. Oil Chem. Soc.*, 1979, **56**, 886.

16. A. N. Siakatos and G. J. Rouser, *J. Am. Oil Chem. Soc.*, 1965, **42**, 913.
17. V. E. Vaskovskii and E. Y. Kostetskii, *J. Lipid Res.*, 1968, **9**, 396.
18. S. G. Yunusova, I. P. Nazarova, S. D. Gusakova, and A. I. Glushenkova, *Khim Prir. Soedin.*, 1980, 319 [*Chem. Nat. Compd.*, 1980, 319 (Engl. Transl.)].
19. S. G. Yunusova and S. D. Gusakova, *Khim Prir. Soedin.*, 1982, 40 [*Chem. Nat. Compd.*, 1982, 40 (Engl. Transl.)].
20. D. T. Asilbekova, S. D. Gusakova, and A. I. Glushenkova, *Khim Prir. Soedin.*, 1985, 760 [*Chem. Nat. Compd.*, 1985, 760 (Engl. Transl.).

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