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MONO- AND DIOSES OF *Glycyrrhiza glabra* ROOT

S. B. Denisova, E. G. Galkin, V. T. Danilov, and Yu. I. Murinov

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Simple sugars were isolated from the ethanol extract of Glycyrrhiza glabra *L. root. The component composition of the carbohydrate fraction was established using GC—MS (gas-chromatographic separation and mass-spectrometric analysis).*

Key words: *Glycyrrhiza glabra* L., mono- and dioses, GC—MS.

Licorice (*Glycyrrhiza glabra* L.) root is a medicinal plant raw material that is included in the State Pharmacopoeia and contains biologically active compounds [1, 2] belonging to various classes of natural compounds (lipids, polyphenols including flavonoids, triterpenes, etc.). Research on them has been often reported. However, few investigations of the carbohydrate composition of the root have appeared [3, 4]. Mainly polysaccharides of its various species have been studied [5-7].

The principal components of alcohol extracts of licorice root are flavonoids (their glycosides) and insignificant quantities of glycyrrhizic acid. Fractionation of the ethanol extract has shown that a significant part of it, ~50-56%, consists of sugars (7-8 mass % of the dry raw material). The physiological activity of the isolated sugar fraction was evaluated in the Laboratory of New Medicinal Compounds of the Institute of Organic Chemistry, Ufa Scientific Center, Russian Academy of Sciences. The results have shown that the sugars are nontoxic and possess definite anti-ulcer and spasmolytic activities.

We studied the component composition of the total sugars in the ethanol extract of licorice root.

The licorice sugars were separated and determined as the trimethylsilyl (TMS) ethers using a computerized GC—MS. The advantage of using the TMS ethers as the volatile derivatives for GC of carbohydrates over other methods is the ability to convert sugars of all classes into these derivatives [8, 9].

Table 1 contains the GC—MS data for quanitative determination and qualitative identification of 15 components. Characteristic peaks indicate that the mass spectra contain TMS ethers of hexoses and pentoses [10, 11]. The mass spectra were adjusted to a single intensity scale for convenience of identification [10]. The intensity of peaks with *m*/*z* 147 $(Me_2SiOSiMe_3)^+$ was taken as 100% [10, 11]. These fragment ions are the most characteristic and are always seen in spectra of sugars with several TMS groups whereas the intensity of fragments with *m*/*z* 204, which contain C_2 and C_3 moieties [10], depend on the substituents on C_2 and C_3. The agreement index (Q) (Table 1) is a quantitative probability that the unknown spectrum was correctly idenfitied in the library by the "artificial intelligence" program included in the data processing system of the HP ChemStation. A probability $\geq$90 corresponds to a correct interpretation. Therefore, the spectra of chromatographic peaks Nos. 2, 8, 10, 11, and 14 were interpreted as glucofuranose, D-mannopyranose, β-D-glucopyranose, myo-inositol, and saccharose, respectively. Apparently the spectrum of derivative No. 1 belongs to ribitol TMS ether because peaks with *m*/*z* 422(10) $[M - HOTMS]^+$ and 205(70) $[TMSOCH_2CHOTMS]^+$, a fragment of C_5 and C_6 atoms, were observed only in the spectrum of this compound in addition to the peaks listed in Table 1. Obviously peaks Nos. 3 and 4 gave the spectra of α-D-fructose and β-D-fructose because peaks with *m*/*z* 437(50) and (40) $[M - CH_2OTMS]^+$ and 365(6) and (3) $[M - TMSOCH_2CH_2SiMe_2]^+$ are found in the high-mass region.

Institute of Organic Chemistry, Ufa Scientific Center, Russian Academy of Sciences, 450054, Bashkortostan, Ufa, pr. Oktyabrya, 71, fax (3472) 35 60 66, e-mail: murinov@anrb.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 183-185, May-June, 2003. Original article submitted April 29, 2003.

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TABLE 1. Mass Numbers and Relative Intensities of Characteristic Peaks in Mass Spectra of TMS-Ethers of Sugars from the Ethanol Extract of Licorice Root

Sample No.	Retention time, min	Peak area, %	Mass numbers (relative intensities, %)							Assumed sugar (carbohydrate)
			117	191	204	217	305	361	Q, %	
1	5.951	0.953	30	20	30	160			87	Ribitol
2	6.460	0.426	65	70	130	315			94	Glucofuranose
3	6.546	2.011	20	25	15	235			86	α-D-Fructose
4	6.598	2.563	20	20	15	280			83	β-D-Fructose
5	6.688	4.119	15	15	195	90	15		76	Sorbose
6	6.778	12.837	10	65	30	140	65		45	2-*O*-Hydroxyethylglucose
7	6.834	1.875	35	70	85	225	20		64	β-D-Galactofuranose
8	7.153	9.058	20	185	330	70	10	3	93	D-Mannopyranose
9	7.431	1.326	30	15	85	75			74	D-Mannitol
10	7.666	7.059	20	135	265	80	50		93	β-D-Glucopyranose
11	8.480	0.297	8	68	32	138	127		90	Myo-inositol
12	11.481	2.032	35	30	20	330	3	280		Unidentified
13	12.136	2.619	40	25	15	215		125		Unidentified
14	12.943	46.777		30	20	165	6	370	93	Saccharose
15	14.498	3.485	25	30	25	140	5	120	53	β-D-Mannopyranosyl-D-glucitol

Q is the agreement index; the intensity of *m*/*z* 147 is taken as 100%.

A search of the library for the spectrum of peak No. 5 gave the following TMS ethers in order of decreasing Q: D-xylose, 76; sorbose, 68; β-D-xylopyranose, 59; sorbopyranose, 59; and mannose, 58.

We think that component No. 5 is sorbose TMS ether because peaks with *m*/*z* 437(40) $[M - CH_2OTMS]^+$ and 525(4) $[M - CH_3]^+$ are present only in this MS. Diagnostic fragments, among them peaks with *m*/*z* 248 and 261, were identitfied during the interpretation of spectra of monosubstituted hexoses [10]. Therefore, the appearance of peaks with *m*/*z* 248(90) and 261(30) enables the spectrum of peak No. 6 to be assigned to 2-*O*-hydroxyethylglucose. The spectrum of peak No. 7 belongs most likely to β-D-galactofuranose TMS ether because this spectrum contained, in addition to the peaks given in Table 1, fragments with *m*/*z* 319(20) $[M - TMSOCHCHTMSOH]^+$, which are formed by cleavage of the heterocycle at the $O–C_1$ and $C_3–C_4$ bonds.

Carbohydrate No. 9 is obviously D-mannitol TMS ether. We arrived at this conclusion because not only the Q values of the proposed compounds fall in the order: D-mannitol TMS ether, 74; glucose oxime, 72; and galactose phenyloxime, 50; but also only the spectrum of D-mannitol TMS ether contains peaks with *m*/*z* 599(2) $[M - CH_3]^+$. The spectrum of component No. 15 belongs, in our opinion, to the TMS ether of the disaccharide β-D-mannopyranosyl-D-glucitol because peaks with *m*/*z* 539(1), which correspond to a radical of D-mannopyranosyl TMS ether, and 452(3), 437(40), and 361(110), which are formed by cleavage of glucitol TMS ether, were observed.

It has been found that >50% of the ethanol extract of licorice root consists of mono- and disaccharides (7-8 mass % of the dry raw material). GC—MS found that the principal component is saccharose (46.78%). Significant quantities of mannose (9.06%), glucose (7.49%), and 2-*O*-hydroxyethylglucose (12.84%) and smaller quantities of D-fructose (4.57%) and sorbose (4.12%) were observed among the identified sugars. The sugar alcohols mannopyranosyl-D-glucitol (3.49%), ribitol (0.95%), mannitol (1.33%), and myo-inositol (0.33%) were present in insignificant amounts.

EXPERIMENTAL

Isolation of Sugars. Licorice (*Glycyrrhiza glabra* L.) root was collected in Turkmenia in the bottomlands of the Amudar′ya river. Dried root was crushed in an industrial crusher, ground in a laboratory grinder to an average particle size 1.25-2.00 mm, and extracted. Dust and large pieces were removed before the raw material was used. The fractional composition of the ground raw material was determined using sieves.

For successive liquid—solid phase extraction (LSPE), ground licorice root (700 g) was treated three times with hexane (1:4 root:hexane ratio) and stirred at room temperature. The pulp was filtered off. The solvent was removed in a rotary evaporator. The yield of lipids (licorice oil) was 3.36 g (0.48 mass % of dry raw material). Then the pulp was successively extracted with ethylacetate (3.5 L) and ethanol (3.5 L) with stirring at 40-50°C for 4 h. The solid was filtered off. The filtrate was evaporated to dryness in a HP-1M2 rotary evaporator at temperatures less than 40-50°C to afford the corresponding extracts: ethylacetate (yield 26.6 g, 3.8%) and ethanol (yield ~90 g, 12.8%).

Sugars were isolated from the ethanol extract by treatment twice with water (500 mL) with heating up to 70°C and constant stirring. The water-insoluble part was filtered off. The aqueous solution was extracted twice with *n*-butanol to isolate water-soluble flavonoid glycosides. The aqueous and *n*-butanol solutions were evaporated to dryness in a rotary evaporator. The yields were: water-insoluble compounds, 16.1 g (2.3%); water-soluble, 24.5 g (3.5%); sugars, 49 g (7.0 mass % of dry material, 54.5% of the ethanol extract).

Sugars were recrystallized twice from aqueous alcohol to afford crystalline white and odorless substances that were very soluble in water.

Preparation of TMS Derivatives of Sugars. A weighed sample (0.1 g) was dissolved in pyridine (10 mL), treated with trimethylchlorosilane (10 mL) and hexamethyldisilazane (5 mL), held for 2 h at 35°C on a water bath, left for 12 h at room temperature, and filtered to remove the precipitate. The remaining reagents were removed in vacuum. The resulting sample was used for the analysis.

GC—MS of TMS Derivatives of Sugars. Silylated sugars were analyzed using an HP5890 GC and HP 5972A MSD. Data were processed using the HP ChemStation system containing a library of 138,000 mass spectra. The mixture components were separated in a HP-5MS (30 m × 0.25 mm) quartz capillary column with a bound stationary phase of 5% phenylmethylsilicone. The temperature program was 40-250°C with a rise of 10°C per minute. Mass spectra were obtained using electron-impact ionization and a scan range of 70-700 Da at a rate of 1 spectrum per 2 s. Data processing included analysis of spectra using the search library and interpretation of mass spectra using spectrum—structure correlations.

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