ANALYSIS OF GLYCOSIDES OF THE DAMMARANE SERIES IN NATURAL AND SYNTHETIC METHODS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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The possibility has been shown of using the Milikhrom domestic microcolumn liquid chromatograph for the quantitative determination of glycosides of the dammarane series in commercial forms of ginseng and synthetic mixtures. To calculate the amounts of individual ginsenosides by the internal-standard method it is proposed to use dammar-24-ene-3α,12β,17α,20(S)-tetraol 3,20-di-O-β-D-glucopyranoside as the internal standard.

Various medicinal forms based on ginseng (*Panax ginseng* C. A. Meyer) roots have long been widely used in the traditional medicine of the people of southeast Asia [1-3]. Many biological effects of these preparations are connected with the presence of triterpene glycosides — ginsenosides. In the analysis of these compounds ever more frequent use is being made of high-performance liquid chromatography [4-11]. We have studied the possibility of using the Milikhrom domestic microcolumn liquid chromatograph fitted with a UV detector [12] for such purposes and have developed a method for the quantitative determination of glycosides of the dammarane series in various natural mixtures and commercial preparations. As shown earlier by Sticher and Soldata [7], the use of the method of reversed-phase HPLC enables a UV detector to be employed and gives a satisfactory separation of the glycosides. Using columns filled with the sorbents Silasorb C18 (KAX-2) and Spherisorb ODSi and a mixture of the solvents acetonitrile and water as the mobile phase, we have also succeeded in obtaining a satisfactory separation of the chromatographic peaks of the main ginseng glycosides (Fig. 1). The analysis was performed in the gradient regime of elution which was created in a syringe-pump by a set of definite volumes of the eluent acetonitrile—water (20:8) → acetonitrile—water (60:40). This enabled us to perform the analysis of all the main components of the glycosidic fraction isolated from a ginseng extract in one volume of the syringe-pump (2.5 ml). For the quantitative calculation of the amounts of ginsenosides (R_g, R_e, R_{b1}, R_c, R_{b2}, R_d, and R_f) in the samples analyzed we used the internal-standard method [13]. As the standard compounds we selected a betulafolienetetrol diglycoside [dammar-24-ene-3α-12β,17α,20(s)-tetraol 3,20-di-O-β-D-glucopyranoside] that we had obtained, which is a synthetic analog of the ginseng ginsenosides [14]. This compound has a similar absorption coefficient in the UV region at 204 nm and was a convenient marker for performing the analysis.

We have investigated the dependence of the areas of the chromatographic peaks on the concentrations of glycosides introduced into the column and have found that they are related linearly within the range of concentrations of each component of from 0.5 to 4 μg. The minimum amount of substance that could be detected by the UV detector was 0.25 μg. It must also be mentioned that we have not yet succeeded in achieving the complete resolution of the chromatographic peaks of ginsenosides R_g and R_e but the separation of these glycosides that was achieved made it possible to estimate the amounts of each roughly.

We have used this procedure to evaluate commercial preparations of ginseng that were kindly supplied by the Khabarovsk pharmaceutical chemicals factory (KhFZ) and by the experimental production factory of VILR Minmedbioprom [All-Union Scientific-Research Institute of Medicinal Plants, Ministry of the Medical and Biological Industry]. The results obtained are shown in Table 1.

We have also used the method for evaluating synthetic analogs of the ginseng ginsenosides. The quantitative calculation of these compounds was carried out by a variant of the internal-standard method — the standard-additive method [13]. The use of the RF HPLC method permits the determination of the amounts of glycosides obtained directly in the reaction mixture by using a simple operation for preparing the sample (Fig. 2). Thus, in the performance of the glycosylation reaction on the total triterpenoids of the dammarane series from the unsaponifiable fraction of *Betula pendula* [14] calculation of the amounts

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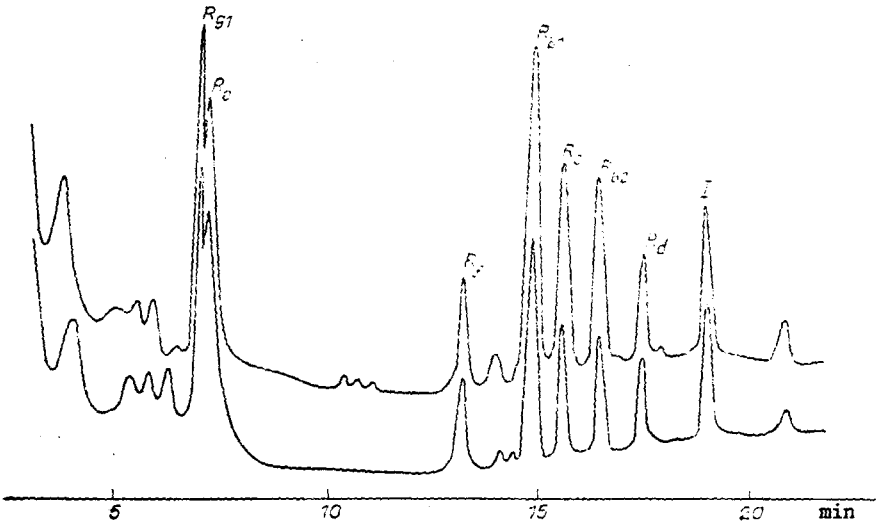

Fig. 1. Reversed-phase chromatography of commercial ginseng preparations: A) tincture of ginseng from the experimental factory of VILR [All-Union Scientific-Research Institute of Medicinal Plants]; B) tincture of ginseng from the Khabarovsk pharmaceutical chemicals factory; I) internal standard.

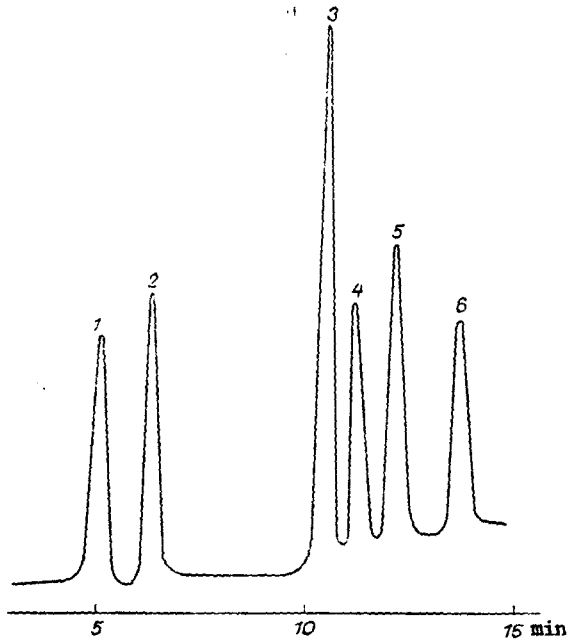

Fig. 2. Reversed-phase chromatogram of a mixture of synthetic analogs of ginseng glycosides: 1) dammar-24-ene-3α,12β,17α,20(s)-tetraol 3,20-di-O-β-D-glusoside; 2) dammar-24-ene-3α,12β,20(S)-triol 3,20-di-O-β-D-glucoside;3)dammar-24-ene-3α,12β,17α,20-(S)-tetraol 3-O-D-glucoside; 4) dammar-24-ene-3α,12β,17α,20(S)-tetraol 12-O-D-glucoside; 5) dammar-24-ene-3α,12β,20(S)-triol 12-O-D-glucoside; 6) dammar-24-ene-3α,12β,-20(S)-triol 3-O-D-glucoside.

TABLE 1. Amounts of Ginsenosides in Commercial Forms of *Panax ginseng*

Preparation	Amounts of ginsenosides, mg						
	$R_{g1}+R_e$	R_f	R_{b1}	R_c	R_{b2}	R_d	Σ
Dry root of ginseng Panax ginseng, 1 g	2.50	0,70	2,40	1,20	0,60	0,3	7,70
Tincture of ginseng from the Khabarovsk KhFZ, 1 ml	0,92	0,25	0,88	0,44	0.36	0.20	2,99
Tincture of ginseng produce by the experimental factory of VILR, Minmedbioprom, 1 ml	1,18	0,28	1,36	0,76	0,60	0,32	4,56

of glycosides obtained made by the RF HPLC method and by the traditional method (column chromatography) gave good agreement.

EXPERIMENTAL

A Milikhrom microcolumn liquid chromatograph and a column (2 × 64 mm) filled with the sorbent Silasorb C18, 4.5 μm, was prepared by the Nauchpribor production combine, Orel. The columns, filled with Spherisorb ODSi 5 μm were prepared in an experimental apparatus in the laboratory. The detection of the eluates was carried out in the single-wave regime at 204 nm. The individual ginsenosides (R_{g1}, R_e, R_f, R_c, R_{b1}, R_{b2}, and R_d) were isolated by column chromatography from *Panax ginseng* roots grown on the plantation of the Zonal Experimental Station of VILR and were identified by ^{13}C and ^1H NMR spectroscopies. Synthetic analogs of the ginsenosides — betulafolienetriol and betualfolienetetraol glycosides — were obtained by Koenigs—Knorr glycosylation [14, 15].

To calculate the standardizing factors and to investigate the dependence of the areas of the chromatographic peaks on the concentrations of the substances introduced into the column, we used model mixtures of glycosides prepared by using standard solutions of the individual compounds in methanol. The concentrations of the glycosides in the standard solutions were 1, 5, and 10 mg/ml.

Samples of ginseng preparations were obtained in the following way. An evaporated methanolic extract of the roots of *Panax ginseng* was dissolved in water and the resulting aqueous solution was treated successively with pentane and with water-saturated butanol. The butanol fraction was used for analysis: it was evaporated to dryness under reduced pressure and the residue was dissolved in a definite volume of methanol in a concentration of 20-30 mg/ml. The volume of the sample analyzed introduced into the column was 2-6 μl.

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