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GLYCOSIDES OF THE EPIGEAL PART OF Panax ginseng

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The qualitative and quantitative compositions of ginsenosides in the epigeal part of ginseng cultivated in the Maritime Territory have been determined. The concentration of ginsenosides in the epigeal part of this plant is fairly high and depends on the growth site and seasonal conditions. A method of alkaline hydrolysis for obtaining ginsenoside R_{g2} from R_e is described. Under these conditions for cleaving the glycosidic bond in the ginsenosides no epimerization at C_{20} is observed.

Ginseng is one of the best known medicinal plants. It is mainly the root of this plant that is used for medical purposes. The biological activity of ginseng roots is connected with the presence in them of ginsenosides – glycosides of the dammarane series. However, it has been found comparatively recently that some of the ginsengosides of ginseng roots are also present in the leaves, buds, and the flesh of the fruit in amounts of 6, 4, 1, and 6.4%, respectively, in several variations [1, 2]. As a rule, the total amount of ginsengosides in the epigeal parts of ginseng exceeds their amount in roots (1.45) [3]. A direct dependence of the biosynthesis of the ginsenosides in the leaves and buds of ginseng on the growth has also been found [4-6].

The increase in the plantations of cultivated ginseng in Maritime Territory is responsible for interest in the study of its epigeal part with the aim of establishing its qualitative and quantitative content of ginsenosides. The high yield of ginsenosides from the epigeal part of ginseng shows not only a potential possibility of isolating individual components but also the utilization of the whole epigeal part.

On investigating the glycoside composition of commercial ginseng roots, we isolated and identified 12 ginsenosides by the methods of ^{13}C and ^{1}H NMR, mass spectrometry, and high-performance liquid chromatography (HPLC) [7]. The procedure developed for the quantitative analysis of ginsenosides by the HPLC method [8] has permitted a comparative study to be made of extracts of the epigeal part of ginseng (leaves, flower buds, flesh of the fruit) of different populations, different growth sites, and different years of collection.

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TABLE 1. Glycosides of the Epigeal Parts of Panax ginseng

Epigeal part	Amount of ginsenosides, mg/kg										
	R_{g1}	R_e	R_f	R_{b1}	R_c	R_{b2}	R_d	F2	NG–R_2	R_{g2}	Σ
1. Buds, Japanese population, 1989*	+		+	+	0.59	4,04	0,16		0,39		5,18
2. Japanese population, 1991*	4,07	9,85		0,75	+	1,53	3,98			0,29	20,47
3. Buds, Japanese population, 1991**	3,02	5,82		1,08	2,44	1,31	3,86			0,35	17,88
4. Buds, Martitime Territory population, 1989**	+		+	+	0,78	3,56	0,20		0,35		4,89
5. Buds, Maritime Territory population, 1990**	2,28	7,87	3,89	1,15	1,45	1,74	4,41		0,71		23,5
6. Buds, Maritime Territory population, 1991*	+	7,55		0,95	1,88	1,34	3,60			0,28	15.6
7. Buds, Maritime territory population, 1991**	+	19,85		3,85	7,26	4,92	12,83			+	48,71
8. Buds of yellow-fruited P. ginseng, 1989*	+		+	+	1,09	4.2	0.29		0,39		5,97
9. Buds of yellow-fruited P. ginseng, 1991*	1,40	15.03		1,65	+	2.86	7,76			0,66	29,36
10. Buds, Korean population, 1989*	+		+	+	0,61	4,44	+		0,28		5,33
11. Buds, Korean population, 1991*	3,35	8,74		1,68	2,96	2,25	5,89			0,36	25,25
12. Buds, Chinese population, 1989*	+		+	+	0.96	3,66	+		0.56		5,18
13. Buds, Chinese population, 1991*	3,86	17,95		1,38	+	2,22	6,89			0,86	33,16
14. Buds, Chinese population, 1991**	3,96	10,9		2,0	3,95	3,06	6.12			0,64	30,63
15. Buds from a commerical root, 1989*	+		+	+	1,11	5,24	+			0,72	7,07
16. Buds from a commercial root, 1991*	8,37	26,39		3,57	+	4,28	18,71			0,82	62,14
17. Flesh of the fruit of the Martitime Territory population, 1989**	0,27	27,56		7,05	8.55	10,5	11,66	1,44	0,58		67,61
18. Flesh of the fruit of the Maritime Territory population, 1991**	28,72	21,64	0,69		3,67	2,95	6,88	0,5	1,13		66,18
19. Leaves (1 year) from a commercial root, 1989**	7,22	15,12	2,41		3.77	5,83	9.15	1,49			45,04
20. Leaves (5-6 yr.) of the Maritime Territory population, 1990**	3,08	8,1	3,33	0.8	3,34	1.94	6,58	1,84	0,62		30,13
21. Leaves of the Maritime population (5 yr), 1991*	11,11	12,75		0,86	5,91	3,65	8,15	0,79			43,22
22. Leaves of the Maritime Territory population (405 yr), 1991****	7.22	23,26		0,77	11,19	6,55	19,5	1,35			69,84

*Material was collected on the plantation of the Far Eastern Zone Experimental Station, Artem, Maritime Territory.

**Collected in the plantations of the Research Station of DV ZOS VILR [Far Eastern Zonal Experimental Station of the All-Union Institute of Medicinal Plants], village of Starovarvarovks, Auchino region, Maritime Territory.

***Collected in the village of Taiga, environs of Dal'nerechenska, Maritime Territory, August.

****Denotes the presence of traces of ginsenoside the amount of which it is impossible to determine quantitatively because of poor separation of the peak on a chromatogram.

To increase the number of standards of ginsenosides with known structures, we have obtained glycoside R_{g_2} – 20(S)-protopanaxatriol 6-O-[O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside] by the alkaline hydrolysis of ginsenoside R_e – 20(S)-protopanaxatriol 20-O-β-D-glucopyranoside 6-O-[O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside] – excluding epimerization at C_{20} [9]. The structure of R_{g_2} was confirmed by physicochemical methods. Ginsenoside F2 has previously been synthesized in our laboratory [10]. The results of quantitative analysis are given in Table 1.

The study of extracts of ginseng buds from various populations showed that their content of ginsenosides depended on the year in which the raw material was collected, i.e., it is obvious that the biosynthesis of ginsenosides is affected by weather conditions. Thus, the buds of ginseng of the Japanese (No. 1), and of the Maritime Territory (No. 4) Korean (No. 10), and Chinese (No. 12) populations and of yellow-fruited ginseng (No. 8) gathered in 1989 contained a minimal amount of ginsenosides, while the protopanaxatriol derivatives R_{g_1} and R_e were almost completely absent from these samples. The buds of the Japanese (Nos. 2 and 3) and Chinese Nos. 13 and 14) populations gathered in the same year but from different sites had close total amounts of ginsenosides, while the buds from different growth sites in the Maritime Territory (Nos. 6 and 7) showed a substantial difference in the amounts of ginsenosides.

In the flesh of ginseng fruit from the Maritime Territory population (Nos. 17 and 18), depending on the year of collection, the concentration of individual ginsenosides varied, but the total amount of ginsenosides remained approximately the same. A high content of ginsenoside R_e (27.56 mg/g) was found in the flesh of the fruit (No. 17), as Japanese scientists have also reported [1, 2]. But it must be mentioned that in different years of collection the quantitative composition of the individual components in the flesh of the fruit varied; thus, in the flesh of the fruit (No. 18) in 1991 a larger amounts of ginsenosides R_{g_1}, NG-R2, and R_f (with the aglycon protopanaxatriol) was detected, while the concentration of ginsenosides derived from protopanaxadiol, R_{b_1}, R_c, R_{b_2}, R_{d_2} and F2 were smaller than in the flesh of the fruit of the 1989 harvest (No. 17).

On comparing extracts of the leaves of ginseng of one-year and four- to six-year growths from the Maritime Territory population (Nos. 19, 20, and 21) we detected no appreciable qualitative differences in the composition of ginsenosides. It was found that the concentration of ginsenosides in the leaves depended to a considerable extent on the growth site of the ginseng (Nos. 21 and 22). The leaves of ginseng of the Maritime Territory population gathered in 1991 at the zonal experimental station (No. 21) had a smaller amount of total ginsenosides than the leaves collected in the north of the Maritime Territory (No. 22) at one and the same period and in one and the same year. Such analogies are also found in the literature. Thus, a study of the leaves of P. japonicus showed that its glycoside composition depended greatly on the locality in Japan in which it was collected [6].

EXPERIMENTAL

For the analysis of extracts by the HPLC method we used a Milikhrom microcolumn liquid chromatograph. Columns (2 × 64 mm) were filled with Spherisorb ODSi 5 μm. As the mobile phase we used a mixture of the solvents acetonitrile and water. Analysis was performed in a gradient elution regime which was created by the assembly of definite volumes of the eluent acetonitrile–water (20:80) → (60:40) into syringe-type pumps.

We used betulafolienetetraol diglucoside as an internal standard. The detection of the eluates was carried out in the signle-wave regime at 204 nm, the rate of feed of the solutions being 100 μl/min. 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 ginsenosides, which were prepared by dissolving the individual compounds in methanol. The concentrations of ginsenosides in the standard solutions were 1, 5, and 10 mg/ml.

Samples of extracts of the epigeal part of Panax ginseng were prepared in the following way. The air-dry leaves, buds, and freeze dried flesh of the fruit were extracted with methanol at room temperature. The evaporated ethanolic extract was dissolved in water, and the solution was then treated successively with pentane and with water-saturated butanol. For analysis we used the butanolic fraction, which was evaporated to dryness under reduced pressure and dissolved in a definite volume of 85% methanol in a proportion of 25-30 mg/ml. The volume of an analytical sample introduced into the column was 2-4 μl.

^{13}C NMR spectra were recorded on a Bruker HX-90E instrument. The chemical shifts are expressed on the δ scale relative to TMS. For column chromatography we used type KSK SiO_2. The individuality of the substances was checked by the TLC method on silica gel in the systems chloroform–butanol–methanol–water (10:5:5:1.8), and chloroform–butanol–methanol–water (20:20:15:20), upper layer. The substances were revealed on the chromatograms with a 10% solution of sulfuric acid in methanol.

Alkaline Hydrolysis of Ginsenoside R_e. A 380 mg sample of ginsenoside R_e was added to 3.7 g of NaOH in 76 ml of butanol. The mixture was heated in the water bath at 80°C for 4 h. The course of the reaction was monitored by TLC. Then the reaction mixture was placed in a separatory funnel and was washed with 100 ml of water. The butanol layer was washed another three times with small amounts of water. Then it was evaporated under reduced pressure, and 252 mg of the dry residue was chromatographed on a column of SiO_2 in the chloroform–methanol–water (500:100:10) system.

Four fractions were obtained. It was established by the TLC, HPLC, and ^{13}C NMR methods that fraction 2 (35 mg) consisted of R_{g1}, 3 (22 mg) of R_{g2}, and 4 (104 mg) of the initial ginsenoside R_e. It was shown by the ^{13}C NMR method that fraction 1 (28 mg) was a mixture of the two ginsenosides R_{h1} and F_1. Fraction 3 (R_{g2}) was crystallized from ethanol, giving colorless crystals with mp 186-189°C. ^{13}C NMR spectrum (ppm): 39.39 (C^1), 27.70 (C^2), 78.66 (C^3), 39.62 (C^4), 60.83 (C^5), 78.04 (C^6), 45.91 (C^7), 41.18 (C^8), 49.79 (C^9), 39.89 (C^{10}), 32.10 (C^{11}), 70.94 (C^{12}), 48.26 (C^{13}), 51.64 (C^{14}), 32.10 (C^{15}), 28.82 (C^{16}), 54.63 (C^{17}), 17.57 (C^{18})*, 17.42 (C^{19})*, 74.43 (C^{20}), 27.0 (C^{21}), 35.78 (C^{22}), 23.0 (C^{23}), 126.19 (C^{24}), 130.47 (C^{25}), 25.67 (C^{26}), 17.18 (C^{27})*, 31.31 (C^{28}), 17.18 (C^{29})*, 16.98 (C^{30})*. 6-O-Glucose: 101.63 (C^1), 79.16 (C^2), 78.31 (C^3), 72.17 C^4), 78.66 (C^5), 63.18 (C^6). Rhamnose: 101.63 (C^1), 72.23 (C^2), 72.23 (C^3), 74.11 (C^4), 69.32 (C^5), 18.59 (C^6).

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*The assignment of these signals may be interchanged.