ANALYSIS OF THE NEUTRAL GINSENOSIDES OF WILD AND PLANTATION ROOTS OF Panax ginseng GROWING IN MARITIME TERRITORY

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A procedure is proposed for determining ginsenosides with the aid of a Milikhrom liquid chromatograph. A comparative and quantitative analysis has been made of the neutral ginsenosides of the total glycoside fractions obtained from the roots of wild and plantation ginseng (Panax ginseng C. A. Meyer) growing in Maritime Territory. It has been shown that the amount of each ginsenoside and their total vary over a wide range in both wild and plantation roots.

Thanks to its unique physiological action, the well-known medicinal plant Panax ginseng C. A. Meyer has long been used in the medical practice of the peoples of the Far East. At the present time, ginseng is found fairly rarely in the wild form and it is therefore grown on the industrial scale in plantations in various regions of the world. It is considered that the main biological action of medicinal forms based on ginseng is due to the presence of ginsenosides – compounds characteristic of the geneus Panax. Various methods of analyziing these compounds have been described [1]. The HPLC method is being employed most widely, thanks to its ready availability and the possibility of the fairly rapid acquisition of information on the ginsenosides [2]. Using this method, Japanese and Chinese authors have made a comparative analysis of wild and plantation roots growing in China and Japan [3, 4].

In a preceding paper [5] we showed the possibility of using for these purposes a Milikhrom domestic microcolumn liquid chromatograph. The aim of the present work was to study the level of ginsenosides in various ginseng roots growing in Maritime Territory both in the wild form and in plantations.

We have previously described a gradient regime for separating ginsenosides R_{g1}, R_e, R_f, R_{b1}, R_c, R_{b2}, and R_d on a Milikhrom liquid chromatograph using a single volume (2.5 ml) of an acetonitrile–water gradient ((20:80)-(50:50)) created in a syringe pump, but we did not succeed in achieving a satisfactory separation of all the chromatographic peaks at a standard column efficiency of 4000-4500 t.p. We have now obtained good results when the analysis was performed in two stages (two volumes), the sample being added to the column only once. We conducted this two-stage evolution by using, first, an isocratic regime and then a gradient regime, or two successive gradient regimes. The proposed approach to the separation of ginsenosides on columns of the KAKh-2 type has enabled us to achieve good results. The KAKh-2 columns were used in combination with a Milikhrom instrument and were filled with the sorbents Silasorb C_{18}, 5 μm, and Separon C_{18}, 5 μm. We also used columns filled with other reversed-phase sorbents. The best results were obtained on the use of columns packed with the sorbent Spherisorb ODSi, 5 μm; the sorbents Diasorb-C16 T/130 fr. 5 μm and Silasorb C_{18}, 5 μm may also be used with success. It was impossible to achieve good results when using the sorbent Octadecyl-Si 100, 5 μm.

The proposed method for the quantitative determination of ginsenosides R_{g1} (1), R_e (2), R_f (3), R_{g2} (5), NG–R_2 (4), R_{b1} (6), R_c (7), R_{b2} (8), and R_d (9) has been used in an investigation of the total glycoside fraction (TGF) of ginseng (Panax ginseng C. A. Meyer) roots grown in various regions of Maritime Territory and also of wild roots gathered in the taiga (see scheme on p. 199). The results of these analyses are shwon in Table 1 and Fig. 1.

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TABLE 1. Levels of Ginsenosides in Various Roots of Ginseng Growing in Maritime Territory

Growth site of the roots and year of collection*	Ginsenoside content, mg/g of dry root										Rb/Rg
	R_{g1}	R_e	R_f	NG–R_1	R_{g2}	R_{b1}	R_c	R_{b2}	R_d	Σ	
1. Chuguevskii region, 1989	2,3	6,28	1 5	0,85	—	11,66	2,7	3,3	1,2	29,79	1,72
2. Anuchinskii region, 1990	2,59	1,96	0.72	0,29	0,31	1,66	1,47	0,91	4,65	14,56	1,48
3. Partizanskskii rgion, 1991	4,88	2,71	1,16	0,59	0,21	5,22	1,62	2,37	0,5	19,26	1,02
4. Oktyabr'skii region, 1990	1,02	2,19	0,43	0,25	0,27	1,79	1,7	2,02	1,19	10,86	1,61
5. Shkotovskii region, 1991	5,87	3,94	1,6	0,74	0,46	12,02	7,34	6,58	3,8	42,36	2,36
6. Shkotovskii region, 1991	7,37	5,3	2,43	0,23	0,6	6,02	3,57	3,5	1,57	30,59	0,92
7. Nadezhdinskii region, 1990	2,38	3,58	0,78	0,39	0,27	4,33	3,12	3,94	1,47	20,26	1,74
8. Ussuriiskii region, 1990	0,76	1,27	+	+	+	1,68	1,05	1,22	0,51	6,49	2,2
9. Anuchinskii region, 1989	2,60	1,9	0,75	0,5	—	5,2	3,2	3,3	1,3	18,7	2,26
10. Anuchinskii region, 1989	3.1	2,07	1,39	0,53	—	3,98	2,2	2,46	1,05	16,8	1,37
11. Anuchinskii region, 1989	2,2	4,7	1,3	0,5	—	4,1	2,8	3,6	1.2	20,4	1,34
12. Mikhailovskii region, 1990	3,42	0,92	0,71	+	+	4,95	1,63	2,0	0.26	13.89	1,75
13. Kirovskii region, 1990	2,45	2,72	0,89	0,25	0,37	1,91	1,57	1,2	1,03	12,44	0,86
14. Spasskii region, 1991	4,74	3,06	1,62	0,65	0,16	3,53	2,3	2,75	1,14	19,95	0,95
15. Anuchinskii region, 1990	2,39	1,24	0,69	+	+	2,07	0,96	1,04	0,36	8,85	1,05
16. Dal'negorskii region, 1990	4,25	2.46	1,77	0,89	—	11,02	4,16	0,52	1,18	26,25	1,8
17. Kavalerovskii region, 1991	1.87	3,06	0,85	0,42	0,38	2,94	2,22	2,67	0.72	15.13	1,30
18. Kavalerovskii region, 1991	1.25	2,86	0,53	0,39	0,42	1,02	1,18	1,3	0,96	9,91	0,82
19. Dal'begorskii region, 1991	1,4	2.4	0,6	0,4	0,35	1,35	1,3	1,25	0,9	9,95	0,93

*1-3) samples of wild ginseng; 4-19) plantation ginseng; +) presence of ginsenosides in trace amounts.

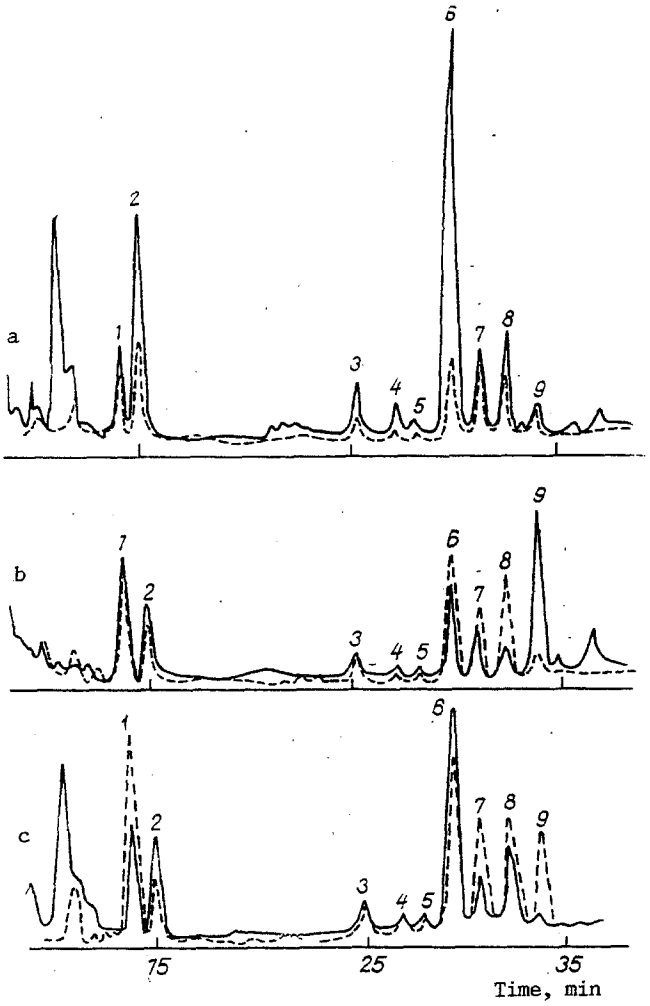
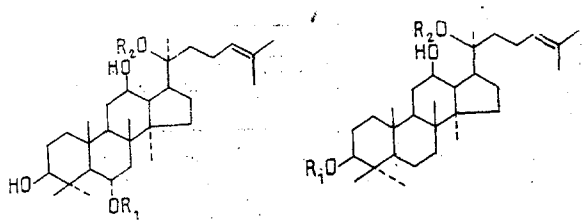


Fig. 1. HPL chromatograms of the total glycosidic fraction (TGF) obtained from the roots of wild ginseng Panax ginseng C. A. Meyer (full line: a) Chuguevskii region; b) Anuchinskii region; c) Partizanskii region) and of ginseng grown on plantations in various regions of Maritime territory (dashed line: a) Kirovskii region; b) Mikhailovskii region; c) Shkotovskii region). Column (2 × 62 mm) filled with the sorbent Spherisorb ODS, 5 μm.

As can be seen from Table 1, the total amount of ginsenosides in roots grown in various regions of Maritime Territory varied within wide limits: from 6.49 to 42.36 mg/g of dry root. The amounts of the individual ginsenosides also varied within a wide interval. Since all ginsenosides are derivatives of two aglycons (the R_b group (R_{b1}, R_c, R_{b2}, and R_d) are derivatives of protopanaxadiol, and the R_g group (R_{g1}, R_e, R_f, R_{g2}, and NG–R_2) are derivatives of protopanaxatriol), the ratio of these groups (R_b/R_g) shows that in most of the roots studied the R_b group was produced in larger amounts than the R_g group. It is probable that this is connected with the fact that the route to the biosynthesis of protopanaxadiol is realized faster. We did not detect any interrelationship whatever between the level of ginsenosides in the ginseng roots and their growth site. It is possible that the observed fluctuations in the levels of ginsenosides depend on the growth conditions in each concrete locality.

The amounts of ginsenosides in Panax ginseng roots growing in the wild form in various regions of Maritime Territory (samples 1-3) varied within a considerable range so far as concerns both their total and each individual ginsenoside separately. The changes in the amounts of ginsenosides within the R_b and R_g groups are apparently connected with different levels of activity of the corresponding glycosidases in each root individually. No substantial differences in the spectra of the ginsenosides and their amounts were observed between plantation and wild ginseng roots.



1) R_{g1}: R_1 = —Glc
R_2 = —Glc

2) R_e: R_1 = Rha(1→2)Glc—
R_2 = Glc—

3) R_f: R_1 = Glc(1→2)Glc—
R_2 = H

4) R_{g2}: R_1 = Rha(1→2)Glc—
R_2 = H

5) NG—R_2: R_1 = Xyl(1→2)Glc—
R_2 = H

6) R_{b1}: R_1 = Glc(1→2)Glc—
R_2 = Glc(1→6)Glc—

7) R_{b2}: R_1 = Glc(1→2)Glc—
R_2 = $Ara_{(p)}$(1→6)Glc—

8) R_c: R_1 = Glc(1→2)Glc—
R_2 = $Ara_{(f)}$(1→6)Glc—

9) R_d: R_1 = Glc(1→2)Glc—
R_2 = Glc—

All this shows that in the preparation of standard medicinal forms from ginseng roots it is necessary to monitor both the various batches of ginseng roots and also their medicinal forms for their levels of the individual ginsenosides.

EXPERIMENTAL

Milikhrom microcolumn liquid chromatograph (Nauchpribor Scientific Combine, Orel), 2 × 62 and 2 × 64 mm steel columns filled with the sorbents Silasorb C_{18}, 5 μm, and Separon C_{18}, 5 μm, KAKn-2 (Nauchpribor Scientific Combine, Orel). Sorbents: Spherisorb ODSi, 5 μm, Phase Sep (Holland); Diasorb-S 16 T/130, 5 μm, SP Biokhimak, Moscow; Silasorb C_{18} (LC), 5 μm, Lachema n.p. Brno; and Octadecyl-Si 100, 0.005 mm, Serva.

The ginseng roots were dried at 40°C in a drying chest. The moisture content of the roots was 6-8% and the ash content not more than 5%. The roots were supplied both by cooperatives and by amateur ginseng gatherers from various regions of Maritime Territory. The age of the cultivated roots was 5-6 years, and of the wild roots 16-17 years. The weight of the crude plantation roots was 12.0-41.0 g, and of the wild roots 3.5-13.6 g.

To obtain the TGF, the ground roots were extracted 3-4 times with 85% methanol, and then the evaporated methanolic extract was dissolved in water, and the solution was extracted first with pentane and then with water-saturated butanol. The butanol fraction was evaporated under reduced pressure to constant weight, and the TGF was determined (%). In the wild roots the TGF amounted to 6.2-13.0%, and in the plantation roots to 2.7-6.2%.

The total glygoside fraction so obtained was dissolved in a definite volume of 85% aqueous methanol in a proportion of 25-30 mg/ml. The following gradients were used to separate the ginsenosides: acetonitrile—water (20:80 → 25: 75) and (27:73 → 50:50). Rate of fedd of eluent, 100 μl/min. Detection at 204 nm.

LITERATURE CITED

1. B. Meier, A. Meier-Bratschi, K. Dallenbuch-Tölke, and O. Sticher, Advances in Chinese Medicinal Materials Research, World Scientific Publishing, Singapore (1985), p. 471.
2. F. Soldati and O. Sticher, Planta Med., 39, 348 (1979).
3. H. Yamaguchi, H. Matsuura, R. Kasai, O. Tanaka, M. Satake, H. Konda, H. Izumi, M. Nuno, S. Katsuki, S. Isoda, J. Shoji, and K. Goto, Chem. Pharm. Bull., 36, 4177 (1988).
4. T. T. Yip, C. N. S. Lau, P. P. H. But, and J. C. Kondu, "Quantitative analysis of ginsenosides in fresh Panax ginseng," Am. J. Chin. Med. 13, 77 (1985).
5. V. V. Makhan'kov, N. F. Samoshina, G. V. Malinovskaya, L. N. Atopkina, V. A. Denisenko, V. V. Isakov, A. I. Kalinovskii, and N. I. Uvarova, Khim. Prir. Soedin., 57 (1990).