GLYCOSIDES FROM GINSENG ROOTS
IV. ISOLATION OF NEW GLYCOSIDES FROM GINSENG

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The isolation of the first two individual glycosides, panaxosides A and B from the root of ginseng (Panax ginseng C. A. Mey) – one of the most interesting materials of Tibetan medicine – has been reported previously [1-3]. On further investigation of the glycoside fraction it has been shown that the use of Sephadex permits the separation of the glycosides of ginseng into two fractions: a group of less polar glycosides including panaxosides A and B, and a group of glycosides of higher polarity*. In the present paper data are presented on the isolation of new individual glycosides from these groups.

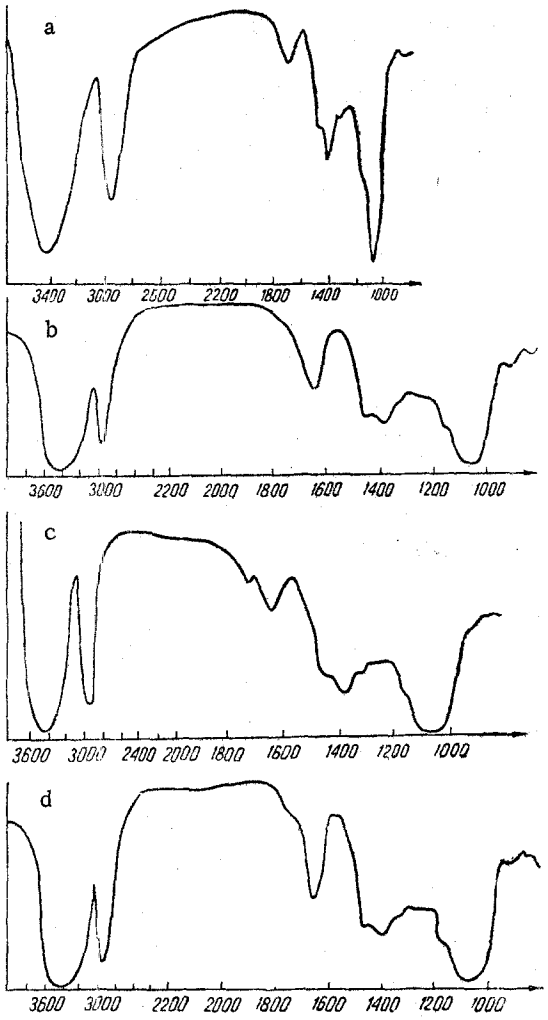

Fig. 1. IR spectra of panaxosides C (a); D (b); E (c); and F (d).

On partition chromatography on silica gel or alumina of the less polar group of glycosides, which issues from Sephadex in the form of a second peak, in addition to panaxosides A and B a third new glycoside called panaxoside C was isolated, this consisting of a crystalline substance homogeneous to paper chromatography and thin-layer chromatography. When it was hydrolyzed with a mixture of sulfuric acid and methanol, two monosaccharides were found to be split off – glucose and rhamnose. A quantitative determination of the mixture carried out by a recognized method [4] showed that they were present in a ratio of 3:1. This permits the assumption that panaxoside C is a tetraoside. The results obtained in a molecular weight determination confirmed this conclusion.

The acetylation of panaxoside C did not give an individual acetate, in spite of repeated attempts. Even under the most varied conditions it always led to a mixture of reaction products; further acetylation of the individual fractions isolated from these mixtures gave the initial mixture once more. The IR spectrum of panaxoside C (Fig. 1a) is very similar to the spectra of panaxosides A and B.

The hydrolysis of panaxoside C forms a complex mixture of the decomposition products of its genin. Investigation of this mixture by means of thin-layer chromatography showed complete identity with the results obtained in the hydrolysis of panaxosides A and B. This, together with the results of IR spectroscopy, shows that panaxoside C is a derivative of the same genin as panaxosides A and B.

The more polar part of the glycoside fraction obtained from the root of ginseng (in the separation on Sephadex it issues in the form of the first peak), when investigated by partition chromatography in a thin layer of silica gel, showed the presence of three glycosides. By means of preparative partition chromatography on silica gel or alumina, they were separated into the individual constituents, and we have called them panaxosides D, E, and F (in order of increasing polarity). Panaxosides D and F were obtained in the individual chromatographically pure state directly after a single chromatography, while panaxoside E, contaminated by other glycosides, was purified by rechromatography.

Acid hydrolysis of panaxisodes D and F gave a single monosaccharide (glucose), while acid hydrolysis of panaxoside E gave glucose and arabinose in a ratio of 4:1. These results permit the assumption that panaxoside E is a pentaoside. The results of a molecular weight determination confirm this conclusion. Judging from its chromatographic behavior and its molecular weight, panaxoside D is a tetra- or a pentaoside and panaxoside F is most probably a hexaoside.

* The data on the separation of the glycosides of ginseng will be published separately.

The IR spectra of the three new panaxosides (Fig. 1, b, c, d) are practically identical and show the absence of carboxyl groups from their molecules. The hydrolysis of all three panaxosides forms a complex mixture of decomposition products of the genin which, from the results of thin-layer chromatography in a fixed layer of silica gel, proved to be completely identical for all three individual glycosides (Fig. 2). This shows that panaxosides D, E, and F are derivatives of one and the same genin.

Among the decomposition products of the genin obtained in the acid hydrolysis of panaxosides D, E, and F, panaxadiol [5] (panaxagenin B [6]) was identified by thin-layer chromatography. We may mention that the chromatographic pictures of the hydrolysis products of panaxosides A, B, and C and of the hydrolysis products of panaxosides D, E, and F differ fundamentally. In particular, panaxadiol is absent from the hydrolyzates of the glycosides of the first group, while it is a constant component of the mixture of decomposition products of the genin from the second group. These results justify us in dividing all the individual glycosides of ginseng known up to the present time into two different groups not only from their behavior during separation on Sephadex but also from the genins which they contain. An investigation of the genins of the two groups which is being carried out at the present time confirms their difference. The elucidation of the details of the structure of the genins will permit an explanation of the formation of panaxadiol in the hydrolysis of one of the groups of glycosides of ginseng and its absence from the hydrolyzates of the other group.

EXPERIMENTAL

Neutral alumina of activity grade II was used for chromatography and silica gel of type "KSK" (200 mesh) was used for thin-layer chromatography on plates with a fixed layer of adsorbent. Column chromatography was carried out on silica gel of the same type (150-200 mesh). Paper chromatography was carried out on paper of the Leningrad "Goznak" mill with a density of 110 g/m^2.

The following solvent systems were used in the work (by volume):

A – Toluene-butanol (various ratios);
B – Butanol-ethanol-water (10:2, to saturation);
C – Butanol-acetic acid-water (4:1:5);
D – Butanol-10% ammonia-ethanol (9:9:2);
E – Butanol-pyridine-benzene-water (5:3:1:3);
F – Benzene-ethyl acetate (3:7).

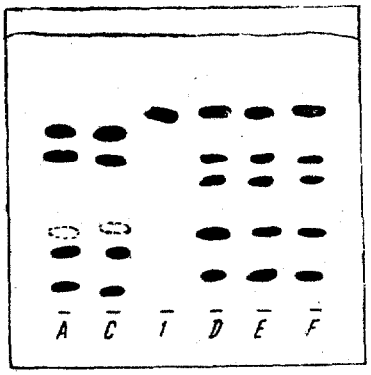

Fig. 2. Chromatography of the decomposition products of the genins of the panaxosides obtained by hydrolysis of the glycosides in a thin fixed layer of silica gel. Developing agent – ethyl acetate. The spots were revealed with conc. H_2SO_4 or $SbCl_3$ in chloroform at 120°. A, C, D, E, F – hydrolyzates of the corresponding panaxosides; 1 – panaxadiol.

Molecular weights were determined by the method of isothermal distillation in pyridine [7]. The IR spectra were taken in KBr tablets on a UR-10 spectrophotometer*. All the solutions were evaporated in vacuum at 40-50°. The spots of the glycosides of ginseng were shown up by a solution of $SbCl_3$ in chloroform with heating, and the spots of the products of the decomposition of the genins by the same solution or by H_2SO_4.

Isolation of panaxoside C. a) 6.7 g of a mixture of panaxosides A, B, and C (the second maximum in the separation of the mixture of glycosides of ginseng on Sephadex) was transferred to a column (6.7 × 56 cm) of 730 g of silica gel containing 550 ml of water. Development was begun with 1.5 liter of system A (2:1) and then with 7 liters of the same system (1:1), 30-ml fractions being collected. Analysis of the fractions was carried out by chromatography in a thin layer of alumina in system B. This gave 1.61 g of panaxoside A contaminated with a small amount of panaxoside B and 1.57 g of panaxoside C.

b) 36 g of a mixture of panaxosides (the second maximum in the separation on Sephadex) was transferred to a column (9 × 60 cm) containing 4 kg of alumina saturated with 1 liter of water, and development was carried out with system A (2:1 and then 1:1), 250-ml fractions being collected. The separation was followed by thin-layer chromatography on alumina in system B. This gave 6.6 g of panaxoside A and 5.4 g of panaxoside C.

Panaxoside C is a powder with a creamy tinge. After crystallization from butanol containing a small amount of methyl ethyl ketone, it had mp 185-187°, $[\alpha]_D^{20} - 4.3 \pm 2°$ (c 2.76; methanol).

Found %: C 58.53; 58.47; H 8.94; 8.87; M 1031; 1064.

Hydrolysis of panaxoside C. Ten milligrams of panaxoside C was heated for 3 hr at 100° in 1 ml of aqueous methanol (1:1) containing 0.02 ml of conc. H_2SO_4. The reaction mixture was neutralized with Dowex-1 anion-exchanger (HCO_3

* The determination of the molecular weights of the panaxosides and their analyses were carried out by colleagues of the laboratory of the chemistry of natural compounds of the Far Eastern Branch of the Siberian Division of the U. S. S. R. Academy of Sciences L. I. Glebko and Zh. I. Ul'kina, and the Ir spectra were taken by M. Yu. Nefedova and R. G. Ovodova.

form) and this was filtered off and washed with 25 ml of methanol. The filtrate and washings were evaporated to 1 ml. Paper chromatography in system E showed the presence of glucose and rhamnose. Quantitative determination by a recognized method [5] showed their ratio to be 3:1.

Isolation of panaxosides D, E, and F. a) Three grams of a mixture of panaxosides D, E, and F (first maximum in the separation of the glycoside fraction on Sephadex) was transferred to a column (4.1 × 76 cm) of 325 g of silica gel containing 240 ml of water. Development was begun with system A (2:1, 1.5 liter) and then successively with the same system in various ratios: 2 liters (1:1); 1.5 liter (1:2); 2 liters (1:3); and 2 liters (1:4). The column was washed with 4 liters of aqueous butanol. Analysis of the fractions was carried out by thin-layer chromatography in a fixed layer of silica gel in systems B and D. This gave 0.4 g of panaxoside D, 0.2 g of panaxoside E containing traces of other panaxosides, and 1.7 g of panaxoside F.

b) 22 g of a mixture of panaxosides D, E, and F (first maximum from Sephadex) was transferred to a column (9 × × 36 cm) of 1.5 kg of alumina containing 375 ml of water and was eluted as described above. This gave 1.55 g of panaxoside D, 3.5 g of panaxoside E contaminated with other panaxosides, and 11.1 g of panaxoside F.

c) 5.1 g of panaxoside E contaminated with panaxosides D and F was transferred to a column (6 × 60 cm) containing 530 g of silica gel saturated with 390 ml of water. The column was developed with the systems of solvents used in experiment a. This gave 3.5 g of pure panaxoside E.

Panaxoside D was recrystallized from ethyl alcohol with the addition of acetone, panaxoside E from propyl alcohol with the addition of a small amount of methyl ethyl ketone, and panaxoside F from butanol containing a small amount of methyl ethyl ketone. The glycosides so obtained consisted of yellowish powders with the following physicochemical characteristics:

Panaxoside	Mp, °C	$[\alpha]_D^{20}$ in methanol	*M*	Found C	Found H	Ratio of monosaccharides
D	157—160	+29° (*c* 4.82)	1178	58.27 58.10	8.86 8.76	Glucose
E	185—187	+21.5° (*c* 4.18)	1222 1230	58.23 58.36	8.88 8.73	Glucose and arabinose (4 : 1)
F	185—187	+20.6° (*c* 5.34)	1388 1424	56.07 56.18	8.48 8.49	Glucose

Hydrolysis of panaxosides D, E, and F. a) 10 mg of the glycoside was heated for 3 hr at 100° with 1 ml of 50% aqueous methanol containing 0.02 g of concentrated sulfuric acid. The reaction mixture was neutralized with Dowex-1 anion-exchanger (HCO_3^- form), the resin was filtered off and washed on the filter with 25 ml of aqueous methanol, and the filtrate and the washing liquid were evaporated to a volume of 1 ml. The resulting solution was used for paper chromatography.

Chromatography in systems B and E showed that the hydrolysis of panaxosides D and F gave only glucose while the hydrolyzate of panaxoside E contained glucose and arabinose. A quantitative determination of the monosaccharides by a recognized method [5] showed the ratio of glucose to arabinose to be 4:1.

b) 10 mg of the glycoside was heated for 6 hr at 60° with 1 ml of a mixture of conc. HCl and methanol (1:4 by volume). The reaction mixture was diluted two-fold with water and the products of the decomposition of the genin were extracted with an equal volume of chloroform. The chloroform extract was washed with water and evaporated to a volume of 1 ml. The solution was chromatographed on plates with a thin fixed layer of silica gel and development was carried out with ethyl acetate (see Fig. 2).

SUMMARY

1. New glycosides – panaxosides C, D, E, and F – have been isolated from the root of ginseng by partition chromatography.

2. The glycosides of ginseng contain genins of two groups: the first consists of the genins of panaxosides A, B, and C, and the second of those of panaxosides D, E, and F. When panaxosides D, E, and F are hydrolyzed, panaxadiol is formed as the main product of the decomposition of the genins.

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