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Abstract

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STRUCTURE OF THE CARBOHYDRATE CHAINS OF PANAXOSIDES D, E, and F

The isolation from ginseng roots of 6 individual triterpene glycosides—panaxosides A, B, C, D, E, and F—was described by us earlier ([1]); in accordance with the structure of the genins, the isolated glycosides are divided into two groups (A, B, C and D, E, F), each of which has one and the same, or very similar, genins ([1]), whose structure has not yet been completely elucidated because of their very high lability ([1, 2]). In the present work the first information is reported on the structure of the carbohydrate chains of the second group of ginseng glycosides—panaxosides D, E, and F. In all calculations of the analytical data we proceeded from the structure of the native genin recently proposed by Shibata and co-workers ([3]). As was indicated earlier ([1]), panaxosides D and F are penta- and hexaglucosides, respectively, while panaxoside E contains one arabinose residue and 4 glucose residues.

Chromatographic examination of the hydrolysates of fully methylated glycosides, obtained after double treatment of the panaxosides according to Kuhn ([4, 5]), and then according to Purdie ([6]), made it possible to establish the complete absence, among the hydrolysis products, of mono- and dimethylated monosaccharides, which indicates the linearity of the carbohydrate chains of all three glycosides. The identification of the methylated monosaccharides was carried out chromatographically on paper in various systems; 2,3,4,6-tetra-*O*-methyl-D-glucose was isolated preparatively, and 3,4,6-tri-*O*-methyl-D-glucose was proved by oxidation with Bonner' s reagent ([7]) and by formation of a complex with boric acid; the corresponding data are given in Table 1.

From these data it follows that in panaxosides D and F there are 2 terminal glucose residues (the presence of 2 moles of 2,3,4,6-tetra-*O*-methyl-D-glucose), and in panaxoside E the terminal residues are those of glucose and arabinose (the presence of 1 mole of 2,3,4,6-tetra-*O*-methyl-D-glucose and 1 mole of 2,3,5-tri-*O*-methyl-D-arabinose). These same data indicate the presence in the carbohydrate chain of panaxosides D and E of one $(1\$\rightarrow 6)$ and two $(1\rightarrow 3)$ linkages, and in the carbohydrate chain of panaxoside F, in addition, of one $(1\rightarrow\$2)$ linkage between glucose residues.

Since branching is absent in the carbohydrate chains of all three glycosides, the

facts presented indicate the presence in each of panaxosides D, E, and F of two linear carbohydrate chains.

The results obtained upon methylation are confirmed by periodate oxidation and partial hydrolysis of the panaxosides. All three glycosi-

Table 1

Methylated glycoside	Found, % C	Found, % H	Found, % OCH_3	Methylated monosaccharides (mol.)
Panaxoside D	62.7762.60	9.309.19	30.5330.47	2,3,4,6-tetra-*O*-methyl-D-glucose (2)2,3,4-tri-*O*-methyl-D-glucose (1)2,4,6-tri-*O*-methyl-D-glucose (2)
Panaxoside E	61.5461.60	9.069.02	31.2231.25	2,3,4,6-tetra-*O*-methyl-D-glucose (1)2,3,5-tri-*O*-methyl-D-arabinose (1)2,3,4-tri-*O*-methyl-D-glucose (1)2,4,6-tri-*O*-methyl-D-glucose (2)

Methylated glycoside	Found, % C	Found, % H	Found, % OCH_3	Methylated monosaccharides (mol.)
Panaxoside F	61.8161.77	8.998.99	32.2232.39	2,3,4,6-tetra-*O*-methyl-D-glucose (2)2,3,4-tri-*O*-methyl-D-glucose (1)2,4,6-tri-*O*-methyl-D-glucose (2)3,4,6-tri-*O*-methyl-D-glucose (1)

also are oxidized by periodate. In the hydrolysis products of the glycosides oxidized with periodate, glucose is detected chromatographically.

Thus, all three glycosides have a common fragment in their carbohydrate chains:

$$\sim O\text{–}D\text{-Glp} - (1 \to 3) - D\text{-Glp}\text{–}O \sim$$

Upon partial hydrolysis of panaxosides D, E, and F (a mixture B, 1.6°, 3 h), a progenin was isolated that proved to be identical for all three glycosides. According to the data of elemental analysis and molecular-weight determination, it is a trioside. In the products of hydrolysis of the completely methylated progenin, 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,4,6-tri-*O*-methyl-D-glucose were detected chromatographically. Consequently, in the carbohydrate chain of the progenin only the $(1 \to 3)$ bonds between glucose residues are retained, and only two structures are possible for its carbohydrate chain:

$$\text{D}-\text{Gl}-1 \to 3-\text{D}-\text{Gl}-1 \to 3-\text{D}-\text{Gl}-1 \to \text{ genin}; \quad \text{(I)}$$
$$\text{D}-\text{Gl}-1 \to 3-\text{D}-\text{Gl} \to \text{ genin} \leftarrow 1-\text{D}-\text{Gl}. \quad \text{(II)}$$

On oxidation with periodate (8), about 2 moles of $NaJO_4$ are consumed per 1 mole of progenin, which proves structure I for the progenin.

As a result of partial hydrolysis of panaxoside D, glucose was obtained; upon hydrolysis under the same conditions of panaxoside E–glucose and arabinose,

while glucose and gentiobiose-6-(β-glucosido)-D-glucose were isolated from the hydrolysate of panaxoside F.

The data obtained make it possible to propose the following variants of the structures of the carbohydrate chains of panaxosides D, E, and F, satisfying all the experimental facts:

Panaxoside D

$$\begin{array}{c} \mathrm{D{-}Gl^{*}-1\to 3-D{-}Gl-1\to 3-D{-}Gl-1} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\searrow \mathrm{g^{**}} \\ \mathrm{D{-}Gl-1\to 6-D{-}Gl-1} \\ \mathrm{D{-}Gl-1\to 6-D{-}Gl-1\to 3-D{-}Gl-1\to 3-D{-}Gl-1} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\searrow \mathrm{g} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\mathrm{D{-}Gl-1}\nearrow \end{array}$$

Panaxoside E

$$\begin{array}{c} \mathrm{D{-}Gl-1\to 3-D{-}Gl-1\to 3-D{-}Gl-1} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\searrow \mathrm{g} \\ \mathrm{D{-}Ar^{***}-1\to 6-D{-}Gl-1} \\ \mathrm{D{-}Gl-1\to 6-D{-}Gl-1\to 3-D{-}Gl-1\to 3-D{-}Gl-1} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\searrow \mathrm{g} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\mathrm{D{-}Ar-1}\nearrow \\ \mathrm{D{-}Ar-1\to 6-D{-}Gl-1\to 3-D{-}Gl-1\to 3-D{-}Gl-1} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\searrow \mathrm{g} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\mathrm{D{-}Gl-1}\nearrow \end{array}$$

Panaxoside F

$$\begin{array}{c} \mathrm{D{-}Gl-1\to 3-D{-}Gl-1\to 3-D{-}Gl-1} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\searrow \mathrm{g} \\ \mathrm{D{-}Gl-1\to 6-D{-}Gl-1\to 2-D{-}Gl-1} \\ \mathrm{D{-}Gl-1\to 2-D{-}Gl-1\to 3-D{-}Gl-1\to 3-D{-}Gl-1} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\searrow \mathrm{g} \\ \mathrm{D{-}Gl-1\to 6-D{-}Gl-1} \\ \mathrm{D{-}Gl-1\to 6-D{-}Gl-1\to 2-D{-}Gl-1\to 3-D{-}Gl-1\to 3-D{-}Gl-1} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\searrow \mathrm{g} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\mathrm{D{-}Gl-1}\nearrow \end{array}$$

* Glucose residues in the carbohydrate chains of the panaxosides are everywhere in the pyranose form.
** Arabinose residues are in the furanose form.
*** g–genin.

Experimental Part

For preparative chromatography, SiO_2 (60-200 mesh) was used, elution being carried out with the following systems (all by volume): A) chloroform–ethanol ($100 \rightarrow 75$; $0 \rightarrow 25$); chromatography in a thin fixed layer of SiO_2, in the systems: B) toluene–ethanol (10 : 1); C) butanol–ethanol–water (10 : 2 to saturation); D) chloroform–ethanol (1 : 1). Chromatography on paper of "Goznak" factory, grade M, was carried out in the systems: E) methyl ethyl ketone saturated with 1% NH_4OH; F) n-C_6H_9OH–C_6H_5N–H_2O (3 : 1 : 1). For detection of spots of methylated panaxosides on plates, H_2SO_4 was used; for protopanaxogenin, $SbCl_3$; for monosaccharides and their methyl ethers (on paper), aniline phthalate reagent. For neutralization of solutions, Dowex-1 anion exchanger (HCO_3^-) was used; for demineralization, Amberlite IRC-120 (H^+).

Hydrolysis was carried out with the following mixtures: A) CH_3OH–72% $HClO_4$ (10 : 1); B) CH_3OH–H_2O–H_2SO_4 (50 : 50 : 2). Solutions were evaporated on a rotary evaporator at 50°/20 mm. Analytical samples were dried for 6 h at 100°/1 mm over P_2O_5. IR spectra were recorded on a UR-10 spectrophotometer. Molecular weight was determined by the method of isothermal distillation.*

1. **Methylation.** a) 0.7 g of panaxoside D is stirred with 6 ml of DMFA, 4 ml of CH_3I, 3 g of BaO, 0.5 g of $Ba(OH)_2 \cdot 8H_2O$, and 0.02 g of BaO_2 for 12 h at 40-50°. It is methylated again in the presence of 2 g of Ag_2O and 8 ml of CH_3I at 40° for 12 h, and after chromatography on SiO_2, 0.25 g of methylated panaxoside D is obtained. The course of methylation is monitored by thin-layer chromatography in system B. The IR spectrum of methylated panaxoside D (practically complete absence of absorption in the region 3400-3600 cm^{-1}) is identical with the IR spectra of methylated panaxosides E and F.

b) Similarly, from 1.29 g of panaxoside E, 1.0 g of methylated panaxoside E is obtained.

c) From 4.8 g of panaxoside F, as a result of double methylation by Kuhn' s method, 3.81 g of methylated panaxoside F is obtained.

2. **Hydrolysis (methanolysis).** a) 10 mg of methylated panaxoside D is heated in an ampoule with 1 ml of hydrolyzing mixture A at 100° for 3 h. The contents of the ampoule are diluted with 1 ml of H_2O. The precipitate is filtered off and washed with methanol. The filtrate is evaporated to a volume of 1 ml and heated in an ampoule at 100° for 1 h. The hydrolysate is neutralized, evaporated to dryness, the residue is dissolved in 1 ml of methanol, and the methylated sugars are chromatographed on paper in

system E. The hydrolysis and identification of methylated sugars from methylated panaxoside E are carried out analogously.

b) From 3.8 g of methylated panaxoside F under the conditions described above, 1.87 g of a mixture of methylated sugars is obtained; these are chromatographed on a column with SiO_2 (130 g; 35 × 4) in system A, affording 0.51 g of 2,3,4,6-tetra-O-methyl-D-glucose, 0.13 g of 3,4,6-tri-O-methyl-D-glucose, and 0.83 g of a mixture of 2,3,4-, 2,4,6-, and 3,4,6-tri-O-methyl-D-glucose.

2,3,4,6-Tetra-O-methyl-D-glucose, recrystallized from petroleum ether with a small addition of ethyl ether, has mp 90-93°, $[\alpha]_D^{20} + 78$ (C 3.72; water).

3. **Partial hydrolysis.** a) 100 mg of panaxoside D is dissolved in 10 ml of hydrolyzing mixture B and heated under reflux for 4.5 h at 60°. The precipitate is filtered off, washed with 1 ml of water–methanol mixture (1 : 1), dried, and 15 mg of protopanaxogenin is obtained; its IR spectrum (absorption in the region 3400-3600 cm^{-1}) is identical with the IR spectra –

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* All analyses and molecular-weight determinations were carried out in the microanalysis laboratory of the Institute of Biologically Active Substances by L. I. Glebko and Zh. I. Ulkina; IR spectra were taken by M. Yu. Nefedova.

of the progenins from panaxosides E and F. The progenin of panaxoside D on a thin-layer chromatogram (fixed silica gel) in systems B and D gives the same R_f value as the progenins of panaxosides E and F.

In the hydrolysate of panaxoside D, glucose is identified by paper chromatography in system Zh.

b) From 0.5 g of panaxoside E, under the conditions of experiment a), 0.20 g of progenin is obtained, which is recrystallized from dry acetone.

Found, %: C 62.35; 62.23; H 8.91; 8.94

In the hydrolysate, glucose and arabinose are identified chromatographically.

c) From 2 g of panaxoside F, under the conditions of experiment a), 0.4 g of progenin is obtained, which is recrystallized from dry acetone.

Found, %: C 62.02; 61.16; H 9.03; 8.95
$C_{48}H_{80}O_{16}$. Calculated, %: C 60.86; H 8.72

In the hydrolysate, glucose and a disaccharide (0.8 g) are identified. The latter is recrystallized from absolute alcohol, m.p. 121-123°, $[\alpha]_D^{20} + 9.08$ (C 5.28; water).

Found, %: C 41.91; 41.98; H 6.56; 6.64
$C_{12}H_{22}O_{11}$. Calculated, %: C 52.14; H 6.47

From the behavior of the chromatogram on paper in system Zh, the disaccharide was identified as gentiobiose, taken as a standard. Its structure was confirmed by methylation followed by hydrolysis, which led to 2,3,4-tri-*O*-methyl-D-glucose and 2,3,4,6-tetra-*O*-methylglucose.

4. Periodate oxidation of progenin. On oxidation by the method described ([8]), 17.62 mg (0.0000186 mole) of progenin consumes 7.98 mg of $NaJO_4$ (0.000043 mole).

5. Methylation of progenin. 0.15 g of progenin is dissolved in 1.5 ml of DMFA, heated and stirred for 6 hr at 40-50° with 0.7 g of BaO, 0.2 g of $Ba(OH)_2 \cdot 8H_2O$, and 0.01 g of BaO_2. The procedure is then the same as for methylation of panaxoside D. 80 mg of methylated progenin is obtained; in its hydrolysate, by paper chromatography in system E, 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,4,6-tri-*O*-methyl-D-glucose are identified.

6. Periodate oxidation of panaxosides. a) A mixture of 0.2 g of panaxoside D, 0.2 g of $NaJO_4$, and 10 ml of water is left for 7 days in the dark at 20°, dialyzed in cellophane against water until a negative reaction for JO_4^-, evaporated, and 0.12 g of oxidation products is obtained.

b) The oxidation product (10 mg) is hydrolyzed at 100° for 3 hr with Kiliani mixture or hydrolyzing mixture B. It is neutralized, evaporated, and glucose is identified by paper chromatography in system Zh.

c) 30 mg of the oxidation product of panaxoside D is dissolved in 6 ml of aqueous 40% CH_3OH; 200 mg of $NaBH_4$ is added to the solution, and the mixture is stirred for 48 hr, demineralized, evaporated, and the residue is hydrolyzed as in experiment a). On the paper chromatogram in system Zh, glucose is identified.

Analogous results were obtained also on oxidation under the conditions of experiment a) of 0.5 g of panaxoside E and 1.0 g of panaxoside F.

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Note: Figure translations are in progress. See original paper for figures.