STUDY OF THE CARBOHYDRATE CHAINS OF PANAXOSIDES B' AND C, GLYCOSIDES FROM THE ROOTS OF PANAX GINSENG

N. I. Uvarova, N. A. Ferens, G. I. Shaposhnikova, and G. B. Elyakov

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We have described the structures of the carbohydrate components of the glycosides of Panax ginseng C. A. Meyer (ginseng), panaxosides D, E, and F [1], which are the first known representatives of neutral triterpene glycosides of the dammarane series which have two carbohydrate chains. Recently Tschesche et al. have reported the isolation of some steroid saponins of the furostane series containing carbohydrate chains attached to the hydroxyl at C-3 and C-26 of the genin [2].

In this paper we give the results of a study of the structure of the carbohydrate chains of panaxosides B' and C. We have statedpreviously [3] that panaxoside C is a tetraoside whose carbohydrate moiety includes glucose and rhamnose in a ratio of 3 : 1. However, a study of hydrolysate of panaxoside C by gas-liquid chromatography (GLC) showed that these monosaccharides are present in a ratio of 2 : 1 (Fig. 1). The results obtained are in agreement with the elementary analysis of panaxoside C for an empirical formula of $C_{48}H_{80}O_{17} \cdot 2H_2O$. As the genin we took the structure proposed previously [4], according to which the hydroxyl groups are present at the C-3, C-6, and C-12 atoms.

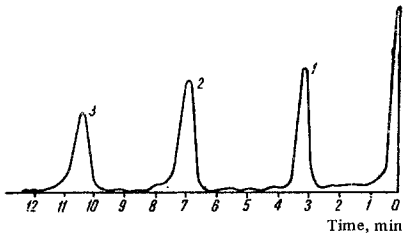

Fig. 1. GL chromatogram of the TMS ethers of the reduced monosaccharides: 1) rhamnitol, 2) glucitol, and 3) i-inositol (standard).

The methylation of panaxoside C by Kuhn's method [5] and by Purdie's method [6] with subsequent acid cleavage has shown that the hydrolysate contains two terminal methylated monosaccharides: 2, 3, 4, 6-tetra-O-methyl-D-glucose and 2, 3, 4-tri-O-methyl-L-rhamnose, and also 3, 4, 6-tri-O-methyl-D-glucose, which was identified by comparison with an authentic sample [7] and also by its capacity for being oxidized both by Bonner's reagent [8] and periodate with the formation of 2, 3, 5-tri-O-methylarabinose [7]. The absence of dimethylated glucose and the presence of two terminal monosaccharides shows that the carbohydrate chains are grafted to panaxoside C in two positions, and the presence of 3, 4, 6-tri-O-methyl-D-glucose shows the 1 → 2 bond between the monosaccharide fragments. What has been said above is confirmed by the GLC of the carbohydrate moiety of methylated panaxoside C, for whose composition a 1 : 1 : 1 ratio was obtained (Fig. 2), agreeing with the results of the GLC of an authentic equimolecular mixture.

Consequently, the structure of the carbohydrate moiety of panaxoside C can be represented by the following two variants:

$$\left.\begin{matrix} D-Glc \to 2D-Glc \to \\ L-Rha \to \end{matrix}\right\} \text{ and } \left.\begin{matrix} L-Rha \to 2D-Glc \to \\ D-Glc \to \end{matrix}\right\}.$$

Panaxoside B' has constants which differ from those of the panaxoside B described previously [9]. The hydrolysate of the completely methylated panaxoside B' obtained by successive methylation using the methods of

Hakomori [10] and Purdie [6] contained 2,3,4,6-tetra-O-methyl-D-glucose and 3,4,6-tri-O-methyl-D-glucose in a ratio of 2 : 1 (Fig. 3). Taken in combination with the results of elementary analysis for the empirical formula $C_{48}H_{80}O_{18}$, this means that panaxoside B' is a trioside with two carbohydrate chains whose glucose fragments are connected by a 1 → 2 bond. For the carbohydrate chains the following structure may be proposed:

$$\left.\begin{matrix} D-Glc \rightarrow 2D-Glc \rightarrow \\ D-Glc \rightarrow \end{matrix}\right\}.$$

EXPERIMENTAL

Chromatography was carried out with KSK silica gel, alumina (activity grade III), M paper of the Leningrad No. 2 paper mill, and the following solvent systems: 1) chloroform–methanol–water (2 : 1, to saturation), 2) butan-1-ol–ethanol–water (10 : 2, to saturation), 3) benzene–butan-1-ol–pyridine–water (1 : 5 : 3 : 3), 4) toluene–ethanol (9 : 1), 5) methyl ethyl ketone saturated with 1% ammonia solution, 6) benzene–ethyl acetate (3 : 2), 7) chloroform–methanol, 8) chloroform–ethyl acetate, and 9) chloroform–ethanol.

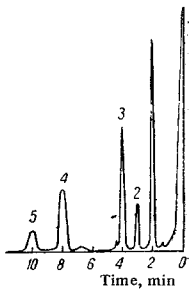

Fig. 2. GL chromatogram of the methyl glycoside of the methylated monosaccharides of panaxoside C: 1) the 2,3,4-tri-O-methyl-L-rhamnoside, 2 and 3) the 2,3,4,6-tetra-O-methyl-D-glucosides (α- and β-isomers), 4 and 5) the 3,4,6-tri-O-methyl-D-glucosides (α- and β-isomers).

The monosaccharides were revealed with aniline phthalate and the glycosides and their derivatives with a saturated solution of $SbCl_3$ in chloroform and with conc H_2SO_4. Gas-liquid chromatography was carried out in a Tsvet-2 instrument (produced by the Experimental Design Bureau for Automation, Dzerzhinsk) fitted with a differential system having two flame ionization detectors (consumption of hydrogen and nitrogen 33 ml/min and of air 260 ml/min) and in a Pye Argon chromatograph with a β-ionization detector (rate of flow of argon 40 ml/min). Gas-liquid chromatography was carried out in stainless-steel columns (100 × 0.3 cm) containing 5% neopentyl glycol succinate on Chromosorb W (60–80 mesh) (column 1) and 10% SE-30 on Chromosorb W (60–80 mesh) treated with hexamethyldisilazane (column 2), and also in a glass column (120 × 0.3 cm) containing 10% poly(ethylene glycol succinate) on Chromosorb W (60–80 mesh) treated with dichlorodimethylsilane (column 3). All the solutions were evaporated in vacuo at temperature not exceeding 55° C. The elementary analyses of all the compounds corresponded to the calculated figures.

Panaxoside B'. A mixture of panaxosides B' and C, 0.9 g, was transferred to a column of silica gel (3 × 30 cm) and eluted in system 7 with a gradient of increasing concentration of methanol from 0 to 20%. The fractions collected were monitored by thin-layer chromatography on silica gel in systems 1 and 2. This gave 0.4 g of panaxoside B' with mp 175–177° C (from a mixture of methyl ethyl ketone and butan-1-ol); $[\alpha]_D^{20}$ + 11.3° (*c* 0.106, ethanol).

Acid hydrolysis of panaxosides B' and C. A mixture of 20 mg of one of the glycosides and 1 ml of 3.5% H_2SO_4 was heated at 100° C for 3 hr. The hydrolysate was neutralized with Dowex 1 × 4 anion-exchange resin (HCO_3^- form) and evaporated in vacuo. Using PC and GLC, glucose was identified in the mixture of products from the hydrolysis of panaxoside B', and glucose and rhamnose from panaxoside C.

Methylation of panaxoside B'. With stirring, 0.06 g of sodium hydride was heated with 2 ml of dimethyl sulfoxide at 60–70° C in a current of nitrogen for 1 hr. Then 0.15 g of the glycoside in 2 ml of dimethyl sulfoxide was added to the cooled reagent. The mixture was kept at 20–22° C for 8 hr and then, with ice water cooling, 4 ml of CH_3I was added and the reaction was continued under the same conditions for 3 hr. The mixture was diluted with water and

extracted with chloroform, and the organic layer was washed with water and evaporated to dryness. The residue was dissolved in 3 ml of CH_3I and treated with 0.7 g of Ag_2O, and the mixture was stirred in a current of nitrogen at 20–22° C for 20 hr. After the addition of half-amounts of CH_3I and Ag_2O, stirring was continued for another 20 hr. The solid matter was filtered off and the filtrate was evaporated in vacuo. This gave 0.09 g of chromatographically homogeneous product (system 4).

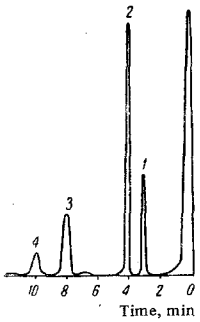

Fig. 3. GL chromatogram of the methyl glycosides of the methylated monosaccharides of panaxoside B': 1 and 2) the 2,3,4,6-tetra-O-methyl-D-glucoside (α- and β-isomers), 3 and 4) the 3,4,6-tri-O-methyl-D-glucoside (α- and β-isomers).

Hydrolysis of methylated panaxoside B'. A mixture of 0.02 g of the panaxoside and 1 ml of 3% HCl in methanol was heated at 100° C for 6 hr. Then 1 ml of water was added and the mixture was heated for another 5 hr. After neutralization with Dowex 1 × 4 anion-exchange resin (HCO_3^- form) the hydrolysate was shown by chromatography on paper (system 5) and in a thin layer of silica gel (system 6) with authentic samples to contain 2,3,4,6-tetra-O-methyl-D-glucose and 3,4,6-tri-O-methyl-D-glucose.

Methylation of panaxoside C. A solution of 4 g of the glycoside in 15 ml of dimethylformamide was treated with 18 g of finely ground BaO, 4 g of $Ba(OH)_2 \cdot 8H_2O$, 0.04 g of BaO_2, and 15 ml of CH_3I, and the mixture was heated with vigorous stirring at 55° C for 10 hr. Then it was cooled and poured into 40 ml of saturated $Na_2S_2O_3$ solution. The precipitate was separated off, the filtrate was extracted with chloroform (5 × 30 ml), and the organic layer was washed with water and evaporated to dryness. After remethylation, 3.2 g of a yellow vitreous product appearing on plates coated with alumina (system 6) in the form of two spots was obtained. The product, 3.2 g, was dissolved in 25 ml of CH_3I and, with stirring, 15 g of Ag_2O was added in six portions at short intervals. Methylation with the addition of fresh portions of reagents at 35–40° C lasted 40 hr. The precipitate was filtered off and the filtrate was evaporated in vacuo. The chromatographic behavior of the mixture was identical with that of the product of the preceding methylation. The product obtained was transferred to a column of silica gel (3 × 60 cm) and was eluted with system 8 with a gradient of increasing concentration of ethyl acetate from 0 to 100%. Two substances were isolated: 1.82 g with $[\alpha]_D^{20} - 8.0°$ (*c* 3.0, methanol) and 0.2 g with $[\alpha]_D^{20} + 3.7°$ (*c* 1.36, methanol), whose hydrolysates were found to contain the same set of methylated monosaccharides: 2,3,4,6-tetra-O-methyl-D-glucose, 3,4,6-tri-O-methyl-D-glucose; and 2,3,4-tri-O-methyl-L-rhamnose.

Separation of the mixture of methylated monosaccharides. Methylated panaxoside C, 1.82 g, was hydrolyzed under the conditions described for methylated panaxoside B'. The mixture of methylated monosaccharides (0.72 g) was transferred to a column of silica gel (2 × 60 cm) and eluted in system 9 with a gradient of increasing concentration of ethanol from 0 to 100%. The fractions were analyzed in a thin layer of silica gel in system 6. Fraction I contained 0.04 g of 2,3,4-tri-O-methyl-L-rhamnose giving an aniline derivative with mp 115–117° C (from a mixture of ether and petroleum ether). Literature data: mp 123–125° C |11|. A mixture with an authentic sample gave no depression of the melting point. Fraction II consisted of 0.3 g of a mixture of 2,3,4-tri-O-methyl-L-rhamnose and 2,3,4,6-tetra-O-methyl-D-glucose. Fraction III consisted of 0.07 g of 2,3,4,6-tetra-O-methyl-D-glucose with mp 76.79°C (from petroleum ether), $[\alpha]_D^{20} + 78.0°$ (c 2.46, methanol). Literature data: mp 98°C, $[\alpha]_D^{20} + 81.3°$ |12|. A mixture with an authentic sample showed no depression of the melting point. Fraction IV consisted of 0.11 g of a mixture of 2,3,4,6-tetra-O-methyl-D-glucose and 3,4,6-tri-O-methyl-D-glucose; and fraction V consisted of 0.15 g of 3,4,6-tri-O-methyl-D-glucose.

Qualitative and quantitative analysis of mixtures of derivatives of the monosaccharides by the GLC method. A) To 15 mg of the panaxoside was added 3 mg of i-inositol, which had been suggested as an internal standard |13|, and

the mixture was hydrolyzed with 3.5% aqueous H_2SO_4. The mixture of monosaccharides and i-inositol was reduced with sodium borohydride |14| and was silylated and hydrolyzed by Sweeley's method |15|. The mixture of trimethylsilyl (TMS) ethers of the polyols was chromatographed in a Tsvet-2 instrument (column 1) with a temperature program ranging from 155 to 200° C at a rate of heating of 2° C/min and with the evaporator at a temperature of 200–300° C. The value of K (correction factor for the flame-ionization detector) was calculated for the TMS ether of glucitol as 1.1, and for the TMS ether of rhamnitol as 1.4.

B) After the hydrolysis of 15 mg of methylated panaxoside with 1 ml of 3% HCl in methanol (10 hr at 100° C) a mixture of methyl glycosides of methylated sugars was obtained. Qualitative analysis was carried out on a Pye Argon chromatograph (column 3) under isothermal conditions (column temperature 164° C). The molar ratio of the components of the mixture was determined on a Tsvet-2 instrument (column 2) with a temperature programmed from 110 to 195° C at a rate of heating of 10° C/min. Standard mixtures were prepared by taking aliquots of methanolic solutions of the synthesized monosaccharide methyl ethers, 2,3,4,6-tetra-O-methyl-D-glucose (78.3 mg/25 ml), 3,4,6-tri-O-methyl-D-glucose (74.0 mg/25 ml), and 2,3,4-tri-O-methyl-L-rhamnose (68.7 mg/25 ml), and converting them into the methyl glycosides by the method described above. The chromatograms for the standard mixture and for the mixture of methyl glycosides of methylated monosaccharides of panaxoside C were similar. Chromatography of the methyl glycosides of methylated panaxoside B' gave a pattern identical with the chromatogram of an authentic mixture of 2,3,4,6-tetra-O-methyl-D-glucoside and 3,4,6-tri-O-methyl-D-glucoside (molar ratio 2 : 1).

CONCLUSIONS

It has been found that panaxosides B' and C are triosides containing two carbohydrate chains, in which the monosaccharide residues are connected by a 1 → 2 bond.

The carbohydrate moiety of panaxoside B' contains glucose and that of panaxoside C contains glucose and rhamnose (2 : 1).

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Institute of Biologically Active Substances, Far Eastern Division, Siberian Branch, AS USSR