

# GLYCOSIDES FROM GINSENG ROOTS. VI. STRUCTURE OF THE CARBOHYDRATE CHAIN OF PANAXOSIDE A

G. B. Elyakov, L. I. Strigina, and N. K. Kochetkov

Khimiya Prirodnykh Soedinenii, Vol. 1, No. 3, pp. 149-152, 1965

We have previously isolated from the root of ginseng (*Panax ginseng* C. A. Mey) for the first time two individual glycosides which have been named panaxosides A and B [1, 2]. In the present paper we give data on the structure of the carbohydrate chain of panaxoside A, which is present in the plant in large quantity.

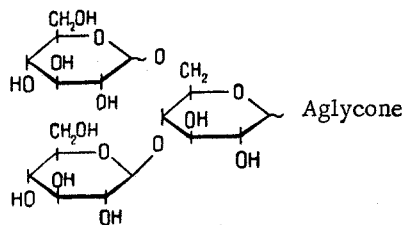
The results of a more detailed study of the elementary composition and molecular weight of panaxoside A, and also new data on the genin contained in it [3] indicate that panaxoside A is not a bioside, as we previously thought [1], but a trioside, containing three glucose residues and a genin with the composition  $C_{30-31}H_{48-52}O_{2-3}$ .

We have used the method of methylation, which has been widely employed in recent years to determine the structure of triterpene glycosides and oligosides [4], in order to establish the structure of the carbohydrate part of panaxoside A. Two-fold methylation by Kuhn's method [5] proved to be sufficient to achieve the complete methylation of panaxoside A. A more prolonged methylation leads to a loss of material and its contamination with products of the decomposition of the methylated panaxoside A. The methylated panaxoside A was purified by chromatography on alumina, this operation being checked by chromatography in a thin layer of alumina. The completely methylated panaxoside A was subjected to methanolysis with a solution of 72% perchloric acid in methanol (1:10 by volume) and the methyl glycosides were hydrolyzed with aqueous perchloric acid. The mixture of methylated sugars so produced consisted, according to paper chromatography results, of 2,3,4,6-tetra-O-methylglucose and 2,3-di-O-methylglucose in the ratio of 2:1.

The mixture of methylated monosaccharides was separated by partition in the chloroform-water system, and then each of the monosaccharides was purified by chromatography on silica gel with elution by mixtures of chloroform and alcohol with an increasing concentration of alcohol. The crystalline 2,3,4,6-tetra-O-methyl-D-glucose so isolated did not differ in its chromatographic behavior and specific rotation from an authentic sample.

The chromatographically purified dimethylglucose had a rotation coinciding with that given in the literature for 2,3-di-O-methyl-D-glucose. The structure of the methylated monosaccharide was shown unambiguously in the following way. It was not shown up on the chromatograms by reagents containing periodic acid and, therefore, had no free  $\alpha$ -glycol groups. Two dimethyl glucoses corresponding to this requirement are possible in practice: 2,3-di-O-methylglucose and 2,4-di-O-methylglucose. After reduction of the dimethylglucose obtained from the methylation of panaxoside A by the action of sodium borohydride in aqueous methanol and periodic oxidation of the dimethylsorbitol so formed, paper chromatography showed the presence in the reaction mixture of a substance identical in chromatographic behavior with 2,3-di-O-methylthreose and differing markedly from 2,4-di-O-methylxylose. In this way, it was established that the initial partially methylated monosaccharide was 2,3-di-O-methylglucose, and not 2,4-di-O-methylglucose (cf. [8]).

The results of the investigation of the fully methylated panaxoside A show that the carbohydrate part of panaxoside A has the structure given below:



The question of the configuration of the glycosidic bonds cannot yet be decided, since there are no data characterizing the native genin of panaxoside A.

## Experimental

The following systems of solvents were used for chromatography (ratios by volume): a) toluene-ethanol (10 : 1), b) ethyl acetate-chloroform (3 : 2), c) methyl ethyl ketone saturated with 1% aqueous ammonia. The substances were shown up on the plates with a solution of antimony trichloride in chloroform or with concentrated sulfuric acid and heating to 120°, and the methylated monosaccharides with a solution of hydrogen aniline phthalate in aqueous butanol with subsequent heating of the chromatograms (105-110°). All solutions were evaporated in vacuum at a temperature of 40-50°.

Methylation of panaxoside A. A solution of 0.67 g of panaxoside A in 6.7 ml of dimethylformamide was treated with 2.7 g of BaO ground to a fine powder, 67 mg of Ba(OH)<sub>2</sub>, and 10 ml of methyl iodide. The reaction mixture was heated with stirring to 55-60°, and after the beginning of the reaction (development of a brown coloration) it was stirred for a further 3 hr at the same temperature and for 18 hr at room temperature; then 1.35 g of BaO and 1.35 ml of methyl iodide were added and it was heated with stirring for 3 hr. After this, the mixture was cooled, poured into 30 ml of saturated sodium thiosulfate solution, and filtered, and the filtrate was extracted 5 times with 30 ml portions of chloroform. The chloroform extracts were washed with water and evaporated to dryness. This gave 0.7 g of a mixture of fully methylated panaxoside A and the products of its partial methylation (mixture I). 0.7 g of mixture I was remethylated under similar conditions. This gave 0.79 g of a mixture of fully methylated panaxoside A with a small amount of partial methylation products (mixture II).

Purification of methylated panaxoside A. 0.7 g of methylated panaxoside A (mixture II) was chromatographed on a column containing 50 g of alumina, elution being carried out with 200 ml of chloroform and then with mixtures of chloroform and ethyl acetate (9:1 → 3:7, by volume; 100-ml portions, the concentration of ethyl acetate being increased by 10% each time). The fractions (20 ml each) were analyzed in a thin layer of alumina in systems a and b; fractions 41-43 containing the fully methylated panaxoside A were combined and evaporated to dryness. Yield 0.21 g. Methylated panaxoside A consists of a yellowish amorphous powder readily soluble in ether and insoluble in water.

Found %: C 65.99, 66.08; H 9.45, 9.57.

Methanolysis of methylated panaxoside A. 310 mg of the fully methylated panaxoside A was heated with 10 ml of a mixture of 72% perchloric acid and methanol (1:10 by volume) in a sealed tube for 5 hr at 95°. The solution was diluted with water in a ratio of 1:3 and the precipitate of methylated genin which deposited was filtered off and washed with water. The filtrate and the wash water were evaporated to a small volume, 5-10 ml of water was added, and evaporation was repeated. The residue was treated with water to give a total volume of 10 ml and with a small amount of activated carbon, and the mixture was boiled for 4 hr at 95° under reflux in order to hydrolyze the methyl glycoside. The solution was cooled, filtered, neutralized with the anion-exchanger Dowex-1 (HCO<sub>3</sub><sup>-</sup> form), and evaporated. This gave 0.12 g of a mixture of methylated sugars in the form of a colorless viscous syrup.

#### Isolation of the methylated monosaccharides.

1. Separation of the mixture of monosaccharides. 0.12 g of mixture of methylated monosaccharides obtained from the methanolysis of methylated panaxoside A was dissolved in 10 ml of a mixture of chloroform and water (1:1 by volume). The chloroform layer was separated off, and the aqueous layer was extracted with chloroform five more times. Evaporation of the combined chloroform extract gave 70 mg of tetramethylglucose, and from the aqueous layer 50 mg of dimethylglucose was obtained.

2. Purification and identification of 2,3,4,6-tetra-O-methyl-D-glucose. 70 mg of tetramethylglucose from the chloroform extracts was chromatographed on a column containing 10 g of silica gel. The substance was applied in the form of a solution in a mixture of benzene and chloroform (1:1 by volume) and was eluted with mixtures of benzene and chloroform (5:5 → 0:10 by volume; 10-ml portions, the concentration of chloroform being increased by 10% each time), and then with mixtures of chloroform and ethanol (99:1 → 90:10 by volume; 10-ml portions, the concentration of ethanol being increased by 1% each time). The fractions (10 ml each) were analyzed in a thin layer of alumina by chromatography in system b. After evaporation, fraction II, containing pure tetramethylglucose, gave 40 mg of a syrup which crystallized on standing. Paper chromatography in system c showed that the methylated monosaccharide did not differ in its chromatographic behavior from an authentic sample of 2,3,4,6-tetra-O-methylglucose and did differ from 2,3,5,6-tetra-O-methylglucose and from the tri-O-methylglucoses.  $[\alpha]_D^{20} = 84 \pm 1^\circ$  (c 3.45; water).

Literature data [6]: 2,3,4,6-tetra-O-methyl-D-glucose,  $[\alpha]_D^{20} + 84^\circ$  (water).

3. Purification and identification of 2,3-di-O-methyl-D-glucose. 50 mg of dimethylglucose from the aqueous layer was chromatographed on a column containing 20 g of silica gel. The substance was eluted with mixtures of chloroform and ethanol (100:0 → 91:9 by volume; 9 portions of 10 ml each; 60 ml of a 90:10 mixture; 80:20 → 0:100, 9 portions of 10 ml each). The fractions (10 ml each) were analyzed by paper chromatography in system c. Evaporation of fractions 25-26, containing the pure dimethylglucose, gave 40 mg of a syrup. As the paper chromatography in system c showed, the monosaccharide did not differ in its chromatographic behavior from an authentic sample of 2,3-di-O-methylglucose.  $[\alpha]_D^{20} + 47 \pm 3^\circ$  (c 2.12; acetone).

Literature data [7]: 2,3-di-O-methyl-D-glucose,  $[\alpha]_D^{20} + 48.3^\circ$  (acetone).

The dimethylglucose (30 mg) from the methylated panaxoside A was dissolved in 1 ml of 50% aqueous methanol, 10 mg of sodium borohydride was added, and the mixture was stirred for 4 hr at room temperature. After the addition of a new portion of sodium borohydride (10 mg), stirring was continued for a further 12 hr. Then the solution was passed through a column containing Amberlite IR-120 (H<sup>+</sup> form), the column was washed with aqueous methanol, and the eluate

was evaporated to dryness. The boric acid was eliminated by repeatedly evaporating the residue with dry methanol. The resulting syrup was dissolved in 1.5 ml of a 2% solution of periodic acid in acetate buffer (pH 4.5) and was left for 24 hr at room temperature. Then the reaction mixture was passed successively through a column containing Dowex-1 ( $\text{HCO}_3^-$  form) and Amberlite IR-120 ( $\text{H}^+$  form). The eluate was evaporated to a volume of 1 ml and the residue was chromatographed on paper in system c. The substance obtained had the same  $R_f$  value and spot color as 2,3-di-O-methylthreose and differed sharply from 2,4-di-O-methylxylose.

The preparative chromatography and the thin-layer chromatography were carried out with alumina of Brockmann's activity, grade II and with silica gel of "KSK" grade (150 mesh), and the paper chromatography was carried out on chromatographic paper of the Leningrad "Goznak" mill with a density of 110 g/cm<sup>2</sup>.

#### Summary

The structure of the carbohydrate part of panaxoside A has been established by methylation with subsequent hydrolysis and identification of the methylated monosaccharides.

#### REFERENCES

1. G. B. Elyakov, L. I. Strigina, A. Ya. Khorlin, and N. K. Kochetkov, *Izv. AN SSSR, OKhN*, 2064, 1962.
2. G. B. Elyakov and L. I. Strigina, *Izv. SO AN SSSR*, 5, 126, 1962.
3. G. B. Elyakov, L. I. Strigina, and N. K. Kochetkov, *DAN SSSR*, 158, 892, 1964.
4. N. K. Kochetkov and A. Ya. Khorlin, *DAN SSSR*, 150, 1289, 1963.
5. R. Kuhn et al., *Angew. Chem.*, 72, 805, 1960.
6. J. C. Irvine and J. W. H. Oidham, *J. Chem. Soc.*, 119, 1744, 1921.
7. J. C. Irvine and J. P. Scott, *J. Chem. Soc.*, 103, 575, 1913.
8. N. K. Kochetkov, A. Ya. Khorlin, and V. E. Vas'kovskii, *Izv. AN SSSR, OKhN*, 1398, 1963.

24 November 1964

Far Eastern Branch of the Siberian Division  
AS USSR and Institute of the Chemistry of  
Natural Compounds AS USSR