Chemistry

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Abstract

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On the Structure of the Aglycone of Panaxoside A*

The investigation of the active principles of one of the most interesting remedies of Chinese medicine, ginseng (*Panax Ginseng*), led to the isolation of 6 individual glycosides—panaxosides A, B, C, D, E, and F**. In a preliminary study of the structure of panaxoside A, all attempts to obtain its genin in the native form were unsuccessful ([1]). At present it has been possible to obtain data that permit certain conclusions to be drawn concerning the structure of this genin. Under mild conditions of hydrolysis of panaxoside A (0.1 N aqueous-methanolic HCl, 65°), glucose is split off and only two different progenins are formed; formation of the genin was not observed in this case ([1]). Under more severe conditions (20% aqueous-methanolic HCl, 65°), a complex mixture of hydrolysis products is formed which, according to thin-layer chromatography, consists of not fewer than six substances, designated by us in order of decreasing polarity as genins A_1, A_2, A_3, A_4, A_5, A_6, which are in equilibrium with one another, since each of these substances, taken separately, under hydrolysis conditions gives the same mixture with predominance of the least polar genin A_6. The question of whether one of the A substances is the native genin remains open. Under still more severe hydrolysis (80°), genin A_6 and still less polar products of further decomposition are formed; when hydrolysis is carried out at 100°, these are the only reaction products. Hydrolysis of the individual panaxosides B ([1]) and C, and also of a mixture of panaxosides A, B, and C, gives a completely identical result. It follows from this that panaxosides A, B, and C have one and the same aglycone or closely related aglycones that, under hydrolysis conditions, give an equilibrium mixture of substances.

It is significant that we have never been able to establish the formation of panaxadiol, isolated by Japanese authors upon severe hydrolysis of the total glycosidic fraction of ginseng ([2]); moreover, none of the substances A_1-A_6 obtained in individual form could be converted into panaxadiol. Thus, panaxoside A, as well as panaxosides B and C, has a genin that differs from panaxadiol or from the corresponding native genin recently described ([3]). On the contrary, upon hydrolysis of panaxosides D, E, and F, panaxadiol is formed as the main reaction product. This indicates that all ginseng glycosides isolated so far are divided into two groups, one of which (D, E, and F) gives panaxadiol upon severe hydrolysis, while the other (A, B, and C) gives a genin different from it.

In order to form a definite idea of the structure of the genin of panaxoside A, the mixture of glycoside hydrolysis products was subjected to preparative separation by chromatography on alumina; as a result, fractions were obtained containing substances A_5 and A_6; substances A_2, A_3, and A_4; and pure substance A_1. Repeated chromatography of the first fraction on silica gel gave the individual A_5 and A_6, and of the second fraction—the individual A_2 and A_3. Substance A_4, present in the mixture in very small amounts, was not isolated; for the same reason A_3 could not be characterized sufficiently fully.

* Communication V in the series "Glycosides from ginseng roots."
** Data on the isolation of these glycosides are being published by us in a series of papers in *Izvestiya AN SSSR, Otd. khim. nauk.*

The data of elemental analysis of substances A_1, A_2, A_5, and A_6 show that the amount of oxygen in them progressively decreases on going from A_1 to A_6. If one takes into account the parallel decrease in polarity according to thin-layer chromatography data, this corresponds to a decreasing number of hydroxyl groups. Thus, the mixture of products of hydrolysis of panaxoside A is evidently a group of substances that differ from one another only in the number of hydroxyl groups and that most likely arise from the native genin of panaxoside A by successive hydration or dehydration during hydrolysis, the conditions of which (aqueous-methanolic HCl) allowed the formation of an equilibrium mixture of this type.

Analysis of the crystalline complete acetate of panaxoside A (absence of hydroxyls in the IR spectrum) indicates the presence in it of 11 acetoxyl groups. If the presence of three monosaccharide residues in the glycoside molecule* is taken into account, this corresponds to the presence in the genin of two hydroxyls, one of which is bound by a glycosidic bond and the other is free. Since the products obtained upon hydrolysis of panaxoside A are much richer in oxygen, it is evident that hydration of double bonds of the native genin takes place during hydrolysis. For judging the structure of the native genin of panaxoside A, it is essential that the IR spectrum (1) and NMR spectrum (4) of one of the hydrolysis products—substance A_6—are close to the spectra of panaxadiol (2), which indicates a close relationship between both compounds. At the same time, the UV spectrum of the initial panaxoside A (1) has a maximum at 213-214 mμ ($\varepsilon = 96\,000$), which indicates the presence in the native genin of a conjugated system of double bonds.

All these data, together with data on the structure of panaxadiol (2), make it possible to propose for the native genin of panaxoside A a structure of type I, in which the position and number of unsaturated bonds have not been determined. Apparently, during hydrolysis, along with cleavage of the glucose residues, hydration of multiple bonds occurs with simultaneous closure of the pyran ring (cf. [3]), which may be conventionally represented by the transformation scheme I, II, III, if the presence of multiple bonds at C_{13}, C_{20}, and C_{25} is assumed; if the double bonds are located in other positions, then the position of the hydroxyl group must be changed accordingly, which does not affect the general conclusion concerning the nature of the native genin.

Structural transformation scheme showing compounds (I), (II), and (III)

Figure 1: Structural transformation scheme showing compounds (I), (II), and (III)

The least polar of the hydrolysis products of panaxoside A—genin A_6—on oxidation with chromic anhydride in acetone gave a triketone, which indicates the presence in it of three hydroxyl groups. The less polar substances A_1—A_5 evidently contained a larger number of hydroxyls. It is interesting that genins A_2 and A_5, according to analysis data, contain, respectively, 1 and 2 methoxyl groups, which is the result of addition of methanol to the double bonds of the native genin; this also fits completely into the transformation scheme given above. It should be noted in conclusion—

It should be noted that preliminary data on the mass spectrum of genin A_6 are also in agreement with our ideas about the structure of the aglycone of panaxoside A*. The mass spectrum contains no peak of the molecular ion. The peak corresponding to the largest value (422$±1)*isduetotheeliminationofthreemoleculesofwaterfromasubstanceoftypeIII*, *towhichgeninA*_{6}$ evidently corresponds. Further fragmentation of the molecule, in particular the presence of peaks at 389, 253, 239, 263, corresponds to the generally accepted pathways of fragmentation of polycyclic compounds (successive elimination of oxygen functions, side chains, and methyl groups) and is close to the data reported for panaxadiol.

Thus, the data presented in this article indicate that panaxoside A, as well as panaxosides B and C, are glycosides of a polycyclic alcohol or of alcohols of similar structure containing a system of readily hydrogenated short bonds.

Experimental Part

Melting points were determined in a Kofler block and corrected. For chromatography, Al_2O_3 (activity group I) and SiO_2 KSK (200-270 mesh) were used. For eluting substances from columns the following solvent systems were used (all by volume) with a continuous gradient: A) chloroform—methanol (100 : 1 → 95 : 5), B) chloroform—ethyl acetate (100 : 0 → 0 : 100), C) chloroform—ethyl acetate (100 : 0 → 50 : 50). Fractions of 28 ml were collected. For analysis of the fractions, chromatography on a thin layer of aluminum oxide and on a thin layer of fixed silica gel was used. For development the following solvent systems were used: D) chloroform—methanol (95 : 5), E) chloroform—ethyl acetate (1 : 1). To detect genin spots on the plates, conc. H_2SO_4 and a saturated solution

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* In the preceding article (1) it was stated that panaxoside A is a bioside. Detailed analysis of the substance by methods accepted in carbohydrate chemistry showed that in fact panaxoside A contains not two, but three glucose residues.

of $SbCl_3$ in chloroform at 120° were used. Genin acetates were obtained by treating the corresponding compounds with acetic anhydride in pyridine for 48 h at room temperature.

Before analysis, determination of specific rotation, and recording of the IR spectrum and mass spectrum, the substances were dried for 12 h at 100°/0.1 mm Hg over P_2O_5. IR spectra were recorded on a UR-10 spectrophotometer**. The molecular weight was determined by the method of isothermal distillation.

Hydrolysis of panaxoside A. A solution of 14 g of panaxoside A in a mixture of 100 ml of methanol and 40 ml of conc. hydrochloric acid was heated for 5 h at 65°. After cooling, the reaction mixture was diluted with 200 ml of water and extracted with a mixture of ether and chloroform (150 ml × 10). The combined extracts were washed with water, 2 N $NaHCO_3$, again with water, dried over ignited Na_2SO_4, and evaporated. Yield of the total genins: 8.4 g.

Separation of the mixture of genins. 8.4 g of the mixture of genins was chromatographed on 500 g of Al_2O_3. The substances were eluted with 4 liters of system A. As a result, the mixture of genins was separated into three fractions: I (A_1; 0.6 g), II (A_2, A_3, A_4; 2.64 g), III (A_5, A_6; 3.53 g).

Fraction I is an individual genin A_1; after recrystallization from a methanol—methyl ethyl ketone—water mixture (1 : 12 : 1), it has m.p. 252-254°, $[\alpha]_D^{22} +30°$ (C 1.8, methanol—chloroform (5 : 1)). IR: 3450, 3220 cm^{-1} (OH).

Found, %: C 71.27; 71.05; H 10.96; 10.75
$C_{30}H_{52}O_6$. Calculated, %: C 70.87; H 10.70

* Preliminary data on the mass spectrum of genin A_6 were obtained in the mass-spectrometry laboratory of the Institute of Chemistry of Natural Compounds, Academy of Sciences of the USSR, by B. A. Zolotarev (head of the laboratory N. S. Wulfson), to whom the authors express their deep gratitude. Detailed data on the mass spectrometry of ginseng genins will be published by us later.

** All analyses, determinations of molecular weight, and analyses for functional groups were carried out by L. I. Glebko and Zh. I. Ul' kina, coworkers of the Far Eastern Institute of Biologically Active Substances, Siberian Branch of the Academy of Sciences of the USSR; IR spectra were recorded by M. Yu. Nefedova and R. G. Ovodova, to whom the authors express their gratitude.

Fraction II was chromatographed on 300 g of SiO_2. The substances were eluted with 3 l of system B. As a result, 2 g of genin A_2 and 0.1 g of genin A_3 were obtained. Genin A_4 is present in the mixture in a very small amount. Genin A_2, after two crystallizations from acetone, separates as thin needles with m.p. 253-254°, $[\alpha]_D^{22} + 30.5 \pm 2°$ (C 3.14, chloroform). IR: 3300 cm^{-1} (OH), 1675 cm^{-1}.

Found %: C 71.51; 71.62; H 11.34; 11.40; OCH_3 4.82; 5.40
$C_{30}H_{51}O_5(OCH_3)$. Calculated %: C 71.22; H 10.41; OCH_3 5.9

Acetate of genin A_2, m.p. 162.5-163.5° (from *n*-hexane), $[\alpha]_D^{22} + 15.5°$ (C 2.06, chloroform). IR: 3575 cm^{-1} (OH), 1745 cm^{-1} (CH_3CO).

Found %:	C 70.48; 70.72; H 9.73; 9.50
$C_{33}H_{56}O_7$. Calculated %:	C 70.17; H 9.99

Fraction III was chromatographed on 500 g of SiO_2. The substances were eluted with 3 l of system C. As a result, 0.87 g of genin A_5 and 1.71 g of genin A_6 were obtained.

Genin A_5, after two recrystallizations from ethyl acetate, separates as dense crystals with m.p. 159-163°, $[\alpha]_D^{22} + 34.0°$ (C 1.94, chloroform). IR: 3400, 3280 cm^{-1} (OH).

Found %:	C 73.66; 73.40; H 11.15; 10.97; OCH_3 10.15; 10.20; H_{act} 0.63
$C_{30}H_{49}(OH)_3(OCH_3)_2$. Calculated %:	C 73.51; H 11.18; OCH_3 11.8; H_{act} 0.58

Genin A_6, after two recrystallizations from ethyl acetate, separates as dense crystals with m.p. 225-227°, $[\alpha]_D^{22} + 18.2$ (C 1.98, chloroform). IR: 3380 cm^{-1} (OH).

Found %:	C 75.35; 75.24; H 11.19; 11.05; H_{act} 0.637
$C_{30}H_{49}O(OH)_3$. Calculated %:	C 75.63; H 10.93; H_{act} 0.633

Molecular weight found 474, calculated 476.76.

Acetate of genin A_6, m.p. 287-289, $[\alpha]_D^{22} + 33.9°$ (C 2.12, chloroform). IR: 3400 cm^{-1} (OH); 1740 cm^{-1} ($CH_3 \cdot CO$).

Found %:	C 73.05; 72.86; H 10.42; 10.42; H_{act} 0.196
$C_{34}H_{56}O_6$. Calculated %:	C 72.81; H 10.06; H_{act} (1 OH group) 0.18

Oxidation of genin A_6. To a solution, cooled to 0°, of 0.8345 g of genin A_6 in 334 ml of acetone, 4.4 ml of Kiliani mixture (26.6 g Cr_2O_3, 23 ml H_2SO_4, diluted with water to 100 ml) is added dropwise, and the mixture is stirred for 1 hour at room temperature, evaporated to 93 ml, diluted with water to 465 ml, and extracted with ether (6 × 500 ml). The extracts are washed with water, bicarbonate solution, and water, dried over Na_2SO_4, and evaporated. The residue was purified chromatographically on 30 g of SiO_2 (200-270 mesh) in the system benzene (0.3 l)—benzene : ethyl acetate (95 : 5) (0.3 l). Yield 0.6241 g, substance with m.p. 236.5-238.5°; $[\alpha]_D^{22} 0°$. IR (KBr): 1715 cm^{-1} (C=O in a six-membered ring), OH band absent.

Found %:	C 76.96; 76.74;	H 9.83; 9.72
$C_{30}H_{46}O_4$. Calculated %:	C 76.53;	H 9.85

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