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STRUCTURE OF THE PRODUCTS OF ACID HYDROLYSIS OF PANAXOSIDES D, E, AND F

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We have previously [1] reported the isolation of six individual glycosides, panaxosides A, B, C, D, E, and F, forming two groups (A, B, C and D, E, F) according to the structure of the native genin. The main products of the acid hydrolysis of each of them are panaxatriol [1, 2] and panaxadiol [3], respectively, both these substances being artefacts. The structures of the native genins which lead to the formation of panaxatriol and panaxadiol under the conditions of acid hydrolysis that were proposed by Shibata [2] are extremely probable but the presence of a tertiary hydroxyl at C-20 cannot be considered as proved since under ordinary conditions pure panaxosides A gives crystalline peracetate the IR spectrum of which lacks the absorption band of a free hydroxyl.

Since it has not yet been possible to obtain a native genin, an equilibrium mixture of the substances formed in the hydrolysis of panaxosides A, B, and C (20% HCl, CH_3OH, 65° C) was studied. We have suggested [1] that the formation, together with panaxatriol, of these substances which contain hydroxy and methoxy functions at C-20 and C-25 (and are correspondingly more polar) is due to the hydration (methoxylation) of multiple bonds in the side chain. However, the position of the hydroxy (methoxy) groups was not demonstrated with certainty.

We give the results of a separation of an analogous mixture obtained by the acid hydrolysis of panaxosides D, E, and F. Hydrolysis was carried out as described for panaxoside A [1] and the mixture of substances formed (F_1-F_5) was separated by chromatography first on Al_2O_3 and then on SiO_2.

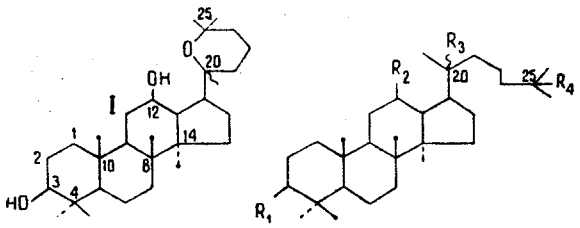
Table 1 gives information on substances F_1, F_2, and F_4 obtained in the separation of 7 g of the mixture. Substance F_3 was present in the mixture in very small amounts and could not be completely characterized. Three grams of substance F_5, panaxadiol, the main component of the mixture, was isolated.

Substances F_1, F_2, and F_4 and their acetates were studied further by NMR spectroscopy. Investigations of the methyl region of the NMR spectra gave information on the location of the hydroxy and methoxy groups in the side chain. The chemical shifts of the signals of the methyl groups are given in Table 2. The signals of the groups of rings A, B, C, and D have similar chemical shifts for all these substances, which confirms the identity of the structure of their skeletons.

The shift of the signals of the three methyl groups of the side chain in a weak field (1.3 ppm $-$ 1 CH_3 and 1.6 ppm $-$ 2 CH_3) as compared with the signals of the methyl groups of the side chain containing no oxygen functions [4] in the spectrum of F_4 show that the methyl groups are located adjacent to the oxygen functions, and the absence of splitting of the signals shows that the oxygen functions are tertiary. Since two methoxy groups are present in F_4 and its skeleton is identical with that of panaxadiol, the signal at 1.13 ppm must be assigned to the 21-methyl group (OCH_3 at C-20) and the signal at 1.16 ppm the intensity of which is twice that of the signal at 1.13 ppm, to the methyl groups at C-26 and C-27 (OCH_3 at C-25). Consequently, F_4 has structure (III).

In the spectrum of the most polar substance, F_1, containing four hydroxy groups, the signal at 1.13 ppm corresponds to the 21-methyl group (OH at C-20) [5], and that at 1.22 ppm to the 26- and 27-methyl groups (OH at C-25). Thus, F_1 has structure (IV). The spectrum of the acetate of F_1 confirms these conclusions.

From a comparison of the spectrum of F_2 with the spectra of the preceding substances, taking the analytical data (3 OH and 1 OCH_3, cf. Tables 1 and 2) into account, it follows that the OH is located at C-20 and the OCH_3 at C-25 (1.16 ppm). The absence of a signal at 1.22 ppm excludes the presence of a hydroxy group at C-25, and therefore F_2 has structure (IV).



II $R_1 = R_2 = R_3 = OH$, $R_4 = H$;
III $R_1 = R_2 = OH$, $R_3 = R_4 = OCH_3$;
IV $R_1 = R_2 = R_3 = OH$, $R_4 = OCH_3$;
V $R_1 = R_2 = OAc$, $R_3 = OH$, $R_4 = OCH_3$;
VI $R_1 = R_2 = R_3 = R_4 = OH$;
VII $R_1 = R_2 = OAc$, $R_3 = R_4 = OH$.

Table 1

Substance	Amount, g	mp, °C	IR $\Delta\nu$ cm^{-1} $CHCl_3$	$[\alpha]_D^{20}$, deg	Found, % C	Found, % H	Found, % OCH_3	Empirical formula	Calculated, % C	Calculated, % H	Calculated, % OCH_3
F_1	0.62	257—261	3615 3380	+15 (*c* 5.6; CH_3OH)	73.90 74.00	11.30 11.33	0.00	$C_{70}H_{54}O_4 \cdot 1/2\ H_2O$	73.53	11.36	0.00
F_2	0.45	253—256	3615 3380	+10 (*c* 2.4; CH_3OH)	75.79 75.89	11.30 11.26	5.86	$C_{31}H_{56}O_4$	75.55	11.47	6.30
F_4	0.2	196—199	3615 3390	+16,2 (*c* 6.12; $CHCl_3$)	75.49 75.67	11.67 11.82	11.64 11.49	$C_{32}H_{58}O_4$	75.80	11.35	11.46
Acetate of F_1	—	191—198	3380 1740	+35,5 (*c* 3; $CHCl_3$)	72.73 72.97	10.48 10.46	$OOCCH_3$ 19.55 19.13	$C_{34}H_{56}O_8$	72.55	10.38	$OOCCH_3$ 20.90
Acetate of F_2	—	157—162	3530 1740	+18 (*c* 1.1; $CHCl_3$)	73.23 73.49	10.29 10.58	15.87 16.11	$C_{35}H_{60}O_5$	72.80	10.48	20.00

Table 2

Compound*	Chemical shift of the methyl groups, ppm 4α	4β	10β	8β	14α	20	25	25	OCH_3	OAc
Panaxadiol (I)	0.80	1.00	0.90	1.00	0.90	1.20	1.24	1.29	—	—
Dihydroprotopanaxadiol** (II)	0.80	1.00	0.90	1.00	0.90	1.13	0.90	0.90	—	—
F_4 (III)	0.80	1.00	0.90	1.00	0.90	1.13	1.16	1.16	3.18; 3.18	—
F_2 (IV)***	0.80	1.00	0.90	1.00	0.90	1.16	1.16	1.16	3.19	—
Ac F_2 (V)	0.84	0.84	0.84	1.00	0.94	1.10	1.15	1.15	3.18	2.04; 2.04
F_1 (VI)	0.80	1.00	0.90	1.00	0.90	1.13	1.22	1.22	—	—
Ac F_1 (VII)	0.84	0.84	0.87	1.01	0.96	2.09	1.22	1.22	—	2.04; 2.05

*The protons located at C-3 and C-12 give, respectively, a triplet with τ = 8 Hz and a quadruplet with τ = = ~5–10 Hz in the 3–4 ppm region in the spectra of the genins and in the 4–5 ppm region in the spectra of the acetates of the genins. The spin-spin coupling constant shows the equatorial location of the hydroxy and acetoxy groups [6].

**Substance provided by S. Shibata at our request.

***The third hydroxyl gives a singlet at 1.25 ppm.

Experimental

Neutral alumina (activity grade II) was used for chromatography and silica gel of type KSK (270 mesh) for thin-layer chromatography on plates with a fixed layer of adsorbent. The silica gel for preparative chromatography was of the same type (150–270 mesh).

The following systems of solvents (by volume) were used: 1) chloroform – ethyl acetate (various ratios), and 2) chloroform – methanol (various ratios). The IR spectra were taken on a U-10 spectrophotometer in KBr tablets and in chloroform solution. All the melting points were determined on a Boetius heated stage and are uncorrected. The samples of materials for analysis and for IR and NMR spectroscopy were dried at 100° C over phosphorus pentoxide for 6 hr.

The acetylation of F_1 and F_2 was carried out under the usual conditions of treatment with acetic anhydride in pyridine for 48 hr at room temperature. The specific rotation was determined on a Hilger M 412 polarimeter. The NMR spectra were taken on a JNM-C-60 spectrometer in $CDCl_3$ using tetramethylsilane as internal standard.

Hydrolysis of panaxosides D, E, and F. A mixture of 35 g of the total panaxosides, 200 ml of methanol, and 80 ml of concentrated hydrochloric acid was heated at 65° C for 4 hr. The reaction mixture was diluted with 50 ml of water and extracted with a mixture of ether and chloroform; the extract was washed with water, sodium hydrogen carbonate solution, and water again, and was evaporated to dryness. The residue (7 g) was subjected to chromatographic separation on a column of alumina (600 g). Elution was carried out in system 2 (100:0 → 97:3). This gave fraction I, containing 3 g of panaxadiol, and fraction II containing 2 g of substances $F_1 - F_4$ combined.

Isolation of substances $F_1 - F_4$. Two grams of the mixture of substances was transferred to a column of SiO_2 (200 g) and was eluted with system 1 (100:0 → 70:30). Analysis of the fractions was carried out by thin-layer chromatography in a fixed layer of silica gel in system 1 (1:1).

The constants and analytical data of the substances are given in Table 1. Substance F_1 was crystallized from ethyl acetate, F_2 from acetone, F_4 from aqueous methanol, and the acetates of F_1 and F_2 from methanol.

Summary

The substances obtained in the acid hydrolysis of panaxosides D, E, and F have been isolated and characterized. Probable structures for these substances have been given on the basis of the results of chemical analysis and NMR spectroscopy.

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