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THE PROBABLE STRUCTURE OF THE TRUE AGLYCONES OF GINSENG GLYCOSIDES

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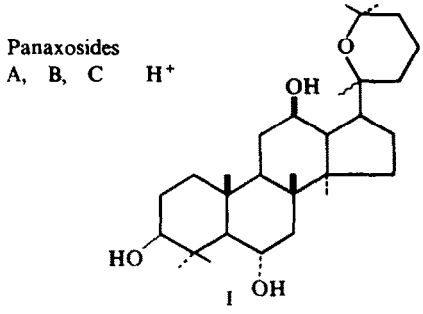
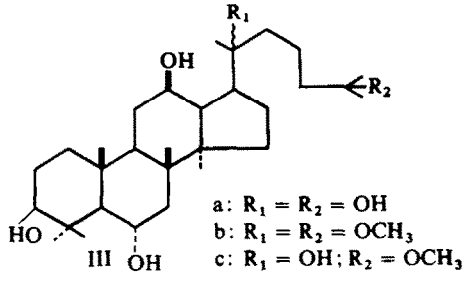
Abstract—Hydrogenation and oxidation of genuine panaxosides A and F with osmium tetroxide, under conditions eliminating possibility of transformation during acid hydrolysis,[1] has established that the double bond, in the side chain of native aglycones (for which the most probable structure XI was suggested) is located in the 24 (25) position.

THE triterpenoid glycosides[1] of Ginseng root (panaxosides A, B, C, D, E, F) fall into two groups, (A, B, C) and (D, E, F) in accordance with the structure of their true aglycones. Acid hydrolysis of the glycosides, belonging to either of these two groups yields complex equilibrium mixtures of open-chain tetracyclic triterpenoids (III–IV) together with the major products—panaxatriol (I) and panaxadiol (II).

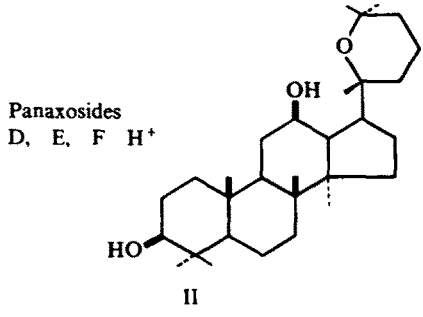


I

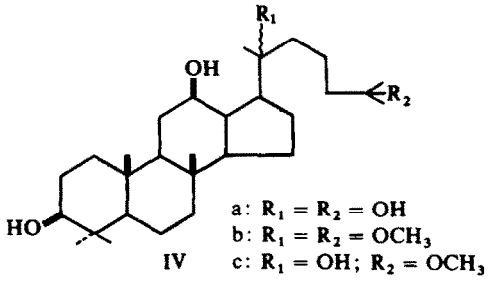


III

a: $R_1 = R_2 = OH$
b: $R_1 = R_2 = OCH_3$
c: $R_1 = OH; R_2 = OCH_3$



II



IV

a: $R_1 = R_2 = OH$
b: $R_1 = R_2 = OCH_3$
c: $R_1 = OH; R_2 = OCH_3$

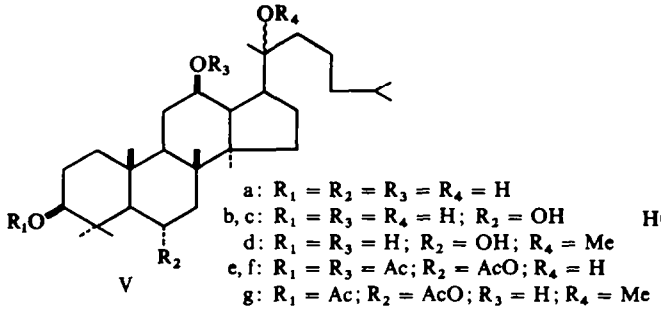
The structures III–IV were confirmed by chemical methods, NMR and mass spectra (2–4).*

A comparison of the data on the starting glycosides[2,5] and compounds I–IV strongly suggests, that the latter could not be the *genuine* panaxosides aglycones.

* The data on the isolation and structural analysis of these substances were published in *Chemistry of Natural Compounds* and *Dokl. Acad. Nauk SSSR.*

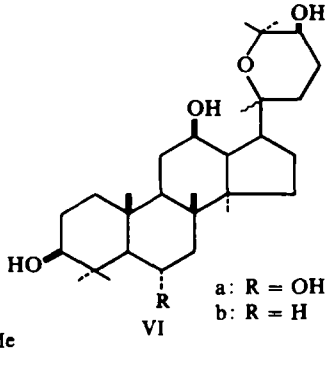
The starting glycosides have double bonds which can be hydrogenated;[6] they have no tertiary oxygen functions at positions 20 and 25 (cf. III, IV), as revealed by the IR spectrum of crystalline panaxoside A acetate and they have no MeO groups.[1] Consequently, we concluded that the formation of compounds III–IV is caused by the addition of water (methanol) across both the double bonds of the *genuine* aglycones,[2] during hydrolysis. In order to determine the location of these double bonds and to obtain additional information on the *true* aglycones of both glycoside groups, panaxosides A and F were subjected to hydrogenation and oxidation with OsO_4.

Acid hydrolysis of dihydropanaxoside F afforded dihydroprotopanaxadiol Va as the only product and identified by direct comparison.* Hydrogenation of panaxoside A also afforded only one compound—dihydropanaxoside A, and acid hydrolysis of the latter resulted in a mixture of the three major compounds, Vb, Vc and Vd, isolated from the hydrolysate by chromatography on Al_2O_3 and SiO_2. Analytical data as well as the IR and NMR spectra revealed that the products Vb–Vd compared with the products of panaxoside A hydrolysis (IIIa–IIIc) lack an oxygen function (OH or OMe) at C-25. Compounds Vb and Vc, like IIIa, have OH groups at C-12 and C-20 as revealed by the presence of the corresponding absorption bands at 3615 (free OH) and 3385 cm^{-1} (intramolecular hydrogen-bonded OH). The absorption band (3540 cm^{-1}) present in the IR spectra of triacetates of Vb (Ve) and Vc (Vf) is due to the hydrogen-bond formed by the OH group at C-20 with the oxygen of the acetyl group at C-12. Beside the free OH band (3630 cm^{-1}), an absorption band at 3390 cm^{-1} in the IR spectrum of Vd is attributed to the stretching mode of the C-12 (OH) hydrogen-bonded with the oxygen of the C-20 methoxyl. Chemical shifts of signals in the NMR spectra of signals in the NMR spectra of compounds Vb, Vc, Vd and of their acetates are given in Table 1. The magnitude of the chemical shifts of signals of skeletal Me groups and the chemical shifts and the coupling constants of signals from protons at C-3, C-6 and C-12 proved the identity of the rings A, B, C and D structure in Vb, Vc, Vd and in the products of hydrolysis of panaxoside A (IIIa-c).[3] A difference was observed in the signals of side chain Me groups. The chemical shift of the two Me groups at C-25 (δ = 0·9 ppm J = 6 c/s) suggested the absence of neighbouring oxygen functions, but the magnitude of the chemical shift of the third side chain Me group (1·12–1·16 ppm) indicated the presence of a neighbouring oxygen function: OH in Vb, c or OMe in Vd.



V

a: $R_1 = R_2 = R_3 = R_4 = H$
b, c: $R_1 = R_3 = R_4 = H$; $R_2 = OH$
d: $R_1 = R_3 = H$; $R_2 = OH$; $R_4 = Me$
e, f: $R_1 = R_3 = Ac$; $R_2 = AcO$; $R_4 = H$
g: $R_1 = Ac$; $R_2 = AcO$; $R_3 = H$; $R_4 = Me$



VI

a: R = OH
b: R = H

* A sample of dihydroprotopanaxadiol was kindly supplied by Professor Shibata.

TABLE 1. CHEMICAL SHIFTS* OF PROTON SIGNALS IN THE NMR SPECTRA†

No.	Substance	4α Me	4β Me	10β Me	8β Me	14α Me	20 Me	25 Me	25 Me	3α H	12α H	6β H	24 H	12β OH	21 OH	OMe	COOMe
1	Vb	0·95	0·95	1·15	0·99	0·95	1·16	0·90	0·90	3·15	3·55	4·08					
2	Ve	0·93	1·00	1·00	1·15	0·93	1·12	0·90	0·90	4·50	4·75	5·35			2·85		2·05
3	Vf	1·00	1·00	1·00	1·15	0·95	1·12	0·90	0·90	4·40	4·70	5·30			3·15		2·03
4	Vd	0·95	0·95	1·15	1·03	0·95	1·12	0·90	0·90	3·20	3·55	4·08		5·85		3·20	
5	Vg	0·95	1·05	1·05	1·14	0·95	1·14	0·90	0·90	4·50	3·55	5·40		5·85		3·20	2·03
6	I	0·89	0·95	1·15	1·03	0·89	1·18	1·22	1·28	3·15	3·55	4·08		6·25			
7	VIa	0·89	0·95	1·15	1·03	0·89	1·18	1·22	1·28	3·15	3·50	4·08	3·85				
8	VIIa	0·89	0·95	1·06	1·03	0·89	1·28	1·25	1·25	3·15	3·50	4·08	3·90	5·65			
9	II	0·80	1·00	0·90	1·00	0·90	1·20	1·24	1·29	3·20	3·50			6·25			
10	VIb	0·78	0·97	0·88	0·97	0·88	1·10	1·18	1·22	3·20	3·50		3·90	5·50			
11	VIIb	0·76	0·97	0·85	0·97	0·88	1·10	1·25	1·25	3·20	3·50		3·70				

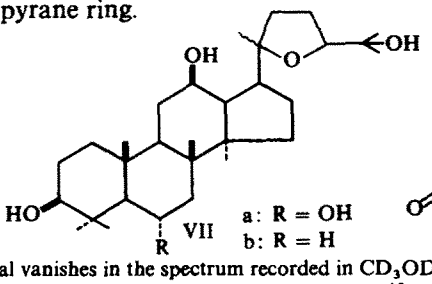
* Chemical shifts are given in ppm from TMS (= *0*) for $CDCl_3$ solns.

† All spectra were recorded at room temp—50 mg of substances were dissolved in 0·5 ml $CDCl_3$.

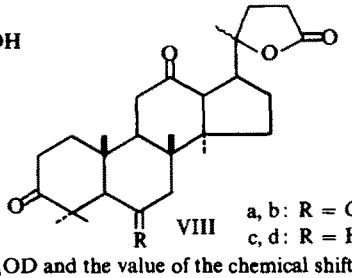
The signal of the OMe group occurs at 3·2 ppm. In the spectrum of Vd, the OH group at C-12 produces a signal at 5·85 ppm.* The downfield shift of the signal must be due to an intramolecular hydrogen-bond of this OH with the O atom of the OMe group. The signal also occurs in the spectrum of acetate of Vd (Vg), i.e. only the OH groups at C-3 and C-6 are involved in the acetylation. The NMR spectra of acetates of Vb (Ve) and Vc (Vf) differ in the chemical shifts of the C-20 hydroxyls (2·85 and 3·15 ppm, respectively); this difference is probably due to the different nature of the hydrogen-bond with the O atom of the acetate group caused by different stereochemistry at C-20. Thus, Vb and Vc have the structures of dammaran-3β,6α,12β, 20ξ-tetraols, and Vd—the structure of 20-O-methyl-dammaran-3β,6α,12β,20ξ-tetraol. The isolation of two tetraols epimeric at C-20 confirmed indirectly the absence of an OH-group at C-20 of the starting panaxoside A. Hence, the OMe and OH groups ought to appear at C-20 after hydration (methoxylation) of the second double bond resistant to hydrogenation. This makes it impossible to position this double bond at Δ 20(21) or at Δ 20(22), because analogous bonds in sammaran triterpenoids are readily hydrogenated [7]. The tetrasubstituted double bond Δ 17(20) survives hydrogenation but is readily oxidized with oxmium tetroxide.[8]

Treatment of panaxoside A with osmium tetroxide resulted in dihydroxypanaxoside A, which on acid hydrolysis gave rise to two substances, VIa and VIIa. Similar oxidation of panaxoside F followed by hydrolysis also afforded two products, VIb and VIIb. The mixtures were separated by chromatography on silica gel. TLC on silica gel (system F) revealed that VIa, b and VIIa, b cannot be oxidized with sodium metaperiodate and hence contain no α-glycol system. The analytical data as well as the NMR and IR spectra also suggested that VIa, b and VIIa, b contain no α-glycol system and are the products of further transformation of genins which initially contained the α-glycol system. The IR spectra of VIa and VIb exhibit absorption bands characteristic of the free OH (3620 cm^{-1}) and the absorption bands (3350, 3380 cm^{-1}) characteristic of the intramolecular hydrogen bonds of the panaxatriol type.[2] The IR spectra of VIIa, also has absorption bands at 3440 and 3330 cm^{-1} (cf. 3350, 3420 cm^{1} in VIIb) in addition to the band at 3620 cm^{-1}, tentatively attributed to two types of hydrogen-bonding.

The chemical shifts of the Me group signals in the NMR spectra of VIa and VIb (Table 1) were the same as those of the Me group signals in the spectra of panaxatriol (I) and panaxadiol (II). The only difference is the presence of a triplet with an SSC-constant $J_{aa} + J_{ae} = 10$ cps + 6 cps = 16 cps in the chemical shifts region of the protons at the OH-substituted C-atom, additional to signals of the C-3, C-6 and C-12 protons; this difference points to an equatorial position of the additional OH at the tetrahydropyrane ring.



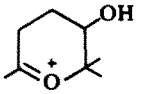
VII a: R = OH
b: R = H



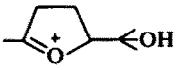
VIII a, b: R = O
c, d: R = H_2

* The signal vanishes in the spectrum recorded in CD_3OD and the value of the chemical shift does not depend on the concentrations of the soln at room temp.[13]

The location of this OH at C-24 follows from the structures of the two other substances (VIIa and VIIb) and from a general consideration of the oxidation with osmium tetroxide. The NMR spectra of VIIa and VIIb exhibit a different Me group signals pattern. Two Me groups have become equivalent, and the chemical shift value (1·25 ppm) points to the neighbourhood of a tertiary OH. The presence of the side chain isopropyl group in VIIa and VIIb is confirmed by the presence of a multiplet of the C-24 proton at 3·7–3·9 ppm. These data are in accord with the mass spectral evidence obtained from VIa, b and VIIa, b—their characteristic major peaks at *m/e* 143 correspond to the side chain fragment (IX or X) (cf. Ref. 9).



IX
m/e 143



X
m/e 143

The structure proposed is supported by the formation of lactones VIIIb, d and VIIIa, c on oxidation of VIIa, b and VIa, b, respectively, with chromic acid. In the latter case, the reaction involves ring contraction (cf. Ref. 10). The reaction mixtures after oxidation of VIa (VIb) contained a minor amount of lactones VIIIe (VIIIf) which showed no depression of m.ps on admixture with lactones obtained from VIIa (VIIb). The NMR spectra of these lactones were also identical. On the basis of the above evidence, VIa was considered to be 20,25-oxadammaran-3β,6α,12β,24ξ-tetraol, and VIIa, 20,24-oxadammaran-3β,6α,12β,25ξ-tetraol. Compounds VIb and VIIb are 20,25-oxadammaran-3β,12β,24ξ-triol and 20,24-oxadammaran-3β,12β,25ξ-triol respectively. The corresponding lactones can have one of the structures VIIIa, b and VIIIc, d. The structure of VIa, b and VIIa, b finally proved the 24 (25) position of the hydrogenated double bond in the side chain of panaxosides A and F and made it impossible to locate the hydrogenation-resistant double bond at position 17 (20). Bearing in mind the data on hydrogenation and hydroxylation, we may conclude that XI is the most probable structure of the *genuine* aglycones of Ginseng glycosides. It appears in fact, that an analogous 13 (17) double bond found in isotirucallenol[11] is resistant to hydrogenation and other characteristic reactions. The formation of C-20 oxygen-containing derivatives on acid hydrolysis may well proceed via rearrangement involving an intermediate formation of C-20 carbonium ion; the initial step may be protonation at C-13 (see Diagram I). This rearrangement is the reverse of that proceeding with dammaran-3,20-diol.[7]

The NMR spectrum of panaxoside A contains the only one signal of Me group in the range 1·10–1·15 ppm, corresponding to protons of Me group of the ring A (12), in accord with the absence of an OH at C-20. The suggested structure of the *genuine* genin agrees with all the experimental data. However, the alternative structure XII cannot be finally rejected until the *genuine* aglycone is obtained directly.

EXPERIMENTAL*

M.ps were measured using a Boetius table. Silica gel "KSK" (200–270 mesh) was used for chromatography. The following solvent systems with gradient elution were used as eluants in column (A) toluene–n-butanol; (B) $CHCl_3$–EtOAc; (C) benzene–EtOAc; (D) benzene–$CHCl_3$; (E) EtOAc–MeOH. TLC on

* The C and H analyses of samples are all probably high no doubt due to solvation.[14]

fixed silica gel was used for the analysis of fractions (25 ml). The following developing solvent systems were used: (F) $CHCl_3$–MeOH (2:1), saturated with water; (G) $CHCl_3$–EtOAc (9:1); (H) $CHCl_3$–EtOAc (2:3); (J) benzene–EtOAc (2:1). Conc H_2SO_4 was used for spot detection. The reaction mixtures were usually diluted with water and repeatedly extracted with ether–$CHCl_3$; the combined extracts were washed with water, 2N $NaHCO_3$, again with water, dried over Na_2SO_4 and evaporated to dryness. The samples intended for analysis, determination of specific rotation, IR and NMR spectroscopy were dried during 12 hr at 100°/0·1 mm over P_2O_5. The IR spectra were recorded using an UR-10 spectrophotometer. The NMR spectra were recorded using a JNM-C-60 spectrometer.

Hydrogenation of panaxoside A. Panaxoside A (0·63 g) was hydrogenated in EtOH (100 ml) in the presence of Adams catalyst and the product chromatographed on a column (35 × 2·7 cm) with silica gel packing containing 75% of water; elution with 1·5 l of system A (1:3 → 1:1). The separation afforded dihydropanaxoside A (0·6 g), m.p. 200–202° (from n-BuOH–MeCOEt), $[\alpha]_D^{20}$ +24·5° (MeOH). (Found: C, 61·19, 60·87; H, 8·91, 9·29. Calc for $C_{48}H_{82}O_{18}$: C, 60·87; H, 8·70%).

Hydrolysis of dihydropanaxoside A. A soln of dihydropanaxoside A (2·8 g) in MeOH (40·1 ml) and conc HCl (8·2 ml) was heated for 5 hr at 65°. After the usual treatment, 1·79 g mixture of genins was obtained.

Separation of the genins mixture. The genins mixture (1·79 g) was chromatographed on a column (80 × 4·5 cm) with silica gel. The substances were eluted with 3 l. of system B (1:1 → 1:9). The mixture was separated into 5 fractions: 1. (tar; 0·19 g); 2. (Vd; 0·41 g); 3. (Vd; 0·16 g); 4. (Vc, traces of Vb and Vd; 0·31 g); 5. (Vb, traces of Vc; 0·29 g).

Fraction 3 was repeatedly chromatographed on a column (25 × 2·5 cm) with silica gel. The substances were eluted with system B, stepwise gradient: 1:1; 1:2; 1:3; 1:4; 1:5; 1:6; 1:7; (each portion 20 ml) and gave Vd (0·09 g) m.p. 197–200° (from n-hexane), $[\alpha]_D^{22}$ +18·3° (MeOH). IR ($CHCl_3$): 3400 cm^{-1} (OH). (Found: C, 75·39; 75·48; H, 11·51; 11·60. Me 5·75. $C_{30}H_{53}O_3$ (OMe) requires: C, 75·50; H, 11·40%).

Diacetate Vd (Vg; 0·18 g) was chromatographed on a column (38 × 3 cm) with silica gel. The substances were eluted with 0·4 l. of system C (9:1 → 7:3). Repeated chromatography afforded acetate Vg (0·15 g), m.p. 155–157° (from n-hexane), $[\alpha]_D^{22}$ +30·8° (MeOH). IR ($CHCl_3$): 3400 cm^{-1} (OH); 1735 cm^{-1} (CH_3CO). (Found: C, 73·35; 73·16; H, 10·62; 10·64. $C_{35}H_{60}O_6$ requires: C, 72·87; H, 10·30%).

Fraction 4 was chromatographed on a column (60 × 3 cm) with silica gel. The substances were eluted with 0·6 l. of system B (100:1 → 50:50); the separation afforded genin Vc (0·12 g), m.p. 277–278° (from EtOAc), $[\alpha]_D^{22}$ +30·7° (pyridine); IR ($CHCl_3$): 3615 cm^{-1} (OH); 3385 cm^{-1} (OH). (Found: C, 75·80; 75·99; H, 11·39; 11·54. $C_{30}H_{54}O_4$ requires: C, 75·26; H, 11·30%).

Triacetate Vc (Vf; 0·2 g) was chromatographed on a column (50 × 3·5 cm) with silica gel. The substances were eluted with 0·6 l. of system C (9:1 → 7:3); acetate Vf (0·2 g) was obtained, m.p. 148–150° (from n-hexane), $[\alpha]_D^{20}$ +33° (MeOH); IR ($CHCl_3$): 3550 cm^{-1} (OH); 1735 cm^{-1} (Ac). (Found: C, 71·23; 71·35; H, 10·06; 9·94. $C_{36}H_{60}O_7$ requires: C, 71·48; H, 9·99%).

Fraction 5 was chromatographed on a column (60 × 3 cm) with silica gel, elution with 0·4 l. of system B (100:1 → 1:1), followed by 0·3 l. of 1:1 to afford Vb (0·13 g) contaminated with traces of Vc. Rechromatography afforded pure Vb (0·1 g), m.p. 228–231° (from aqueous acetone), $[\alpha]_D^{22}$ +34·4° (MeOH); IR ($CHCl_3$): 3615 cm^{-1} (OH); 3385 cm^{-1} (OH). (Found: C, 75·19; 74·96; H, 11·03; 11·39. $C_{30}H_{54}O_4$ requires: C, 75·26; H, 11·30%) Genin acetate Vb (Ve) was not obtained in a crystalline form.

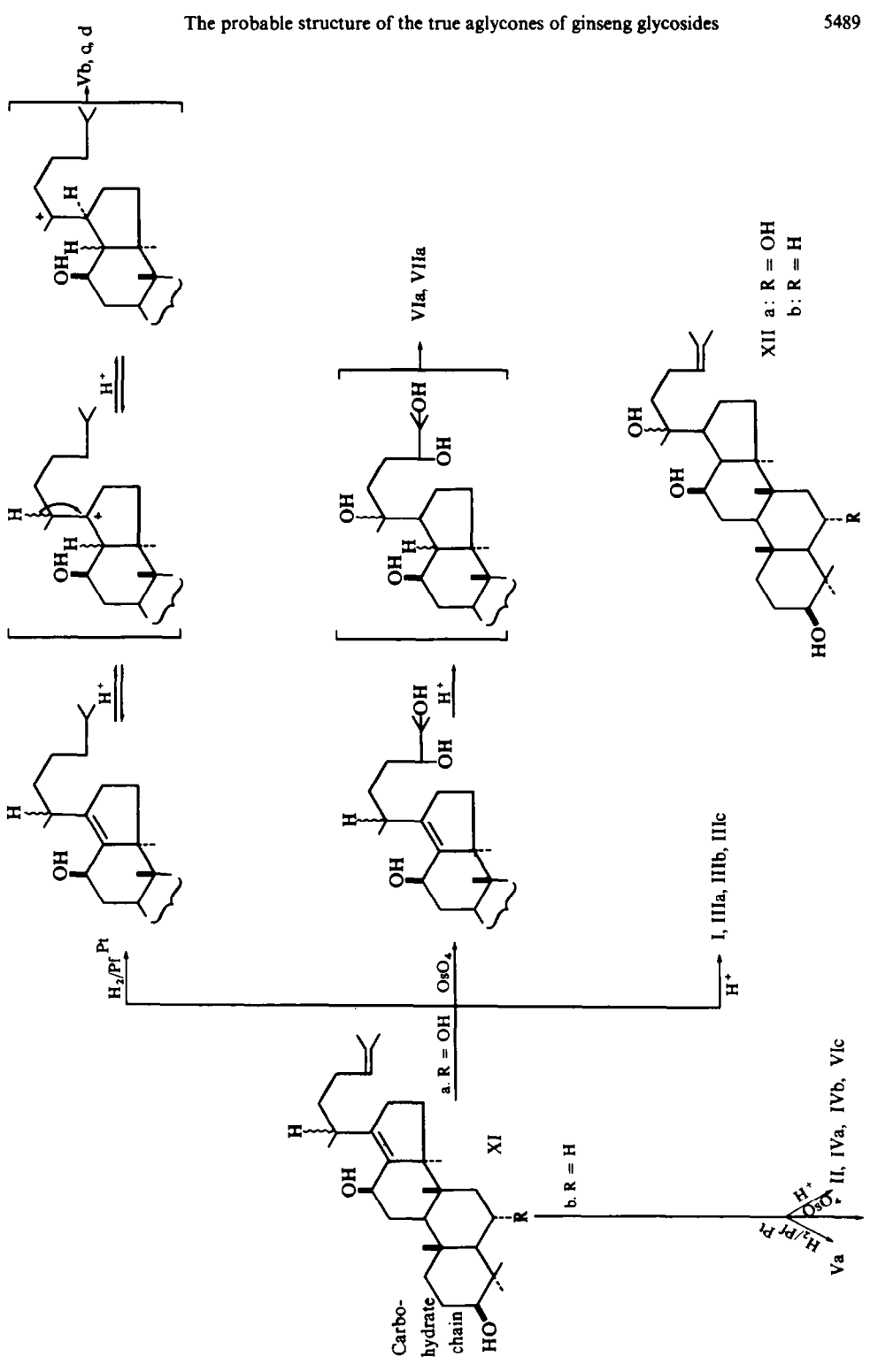
Oxidation of panaxoside A with OsO_4. Panaxoside A (6·11 g) was dissolved in dry pyridine (78 ml), the soln added dropwise to a soln of OsO_4 (3·28 g) in 82 ml pyridine and the mixture left for 18 days at room temp. The pyridine was evaporated under reduced press, the residue treated with mannitol (29·2 g), KOH (46·8 g), EtOH (234 ml), H_2O (117 ml) and the mixture heated under reflux for 8 hr. After deionization with KU-2 (H^+)* and AV-17 (OH^-),† the reaction mixture was evaporated to dryness. After removal of mannitol by its repeated crystallization from aqueous MeOH, 4·68 g of crude oxidation product were obtained.

Isolation and purification of the oxidation products by chromatography

Oxidation product (4·68 g) was chromatographed on silica gel (1250 g) saturated with water (837 g). Elution was performed with system A: 5 l. (2:1); 3 l. (1:1); 3 l. (1:2); 2·8 l. (1:3); 3 l. (1:4); 5·4 l. (1:8) and afforded oxidized panaxoside A (4·29 g), m.p. 208–212° (from n-BuOH–MeCOEt), $[\alpha]_D^{22}$ +30·8° (MeOH). (Found: C, 59·19; 59·45; H, 9·31; 9·57. $C_{48}H_{82}O_{20}$ requires: C, 58·87; H, 8·45%).

* RU-2-strong sulphated cationite.

† AV-17—strong polystyrene anionite.


Vb, c, d
Via, VIIa
XII a: R = OH
b: R = H
I, IIIa, IIIb, IIIc
a. R = OH
b. R = H
XI
Carbo-
hydrate
chain
II, IVa, IVb, VIc
VIb, VIIb
Va

Hydrolysis of the oxidation product. A soln of oxidized panaxoside A (4·1 g) in a mixture of MeOH (58·6 ml) and conc HCl (11·9 ml) was heated during 5 hr at 65°. After usual treatment, the genins mixture (2·22 g) was obtained.

Separation of the genins mixture. The genins mixture (2·22 g) was chromatographed on a column (67 × 5·6 cm) with silica gel, elution with 2 l. of system B (100:0 → 50:50), followed by 3 l. of (1:1 → 0:1), 1·5 l. (0:1) and next with system E—1 l. (95:5), 1 l. (90:10), 1 l. (80:20), 1 l. (70:30), 1 l. (0:100). As a result, three fractions were obtained: fraction 1 (VIIa—0·39 g); fraction 2 (VIa, traces of VIIa—0·24 g); fraction 3 (VIa—1·42 g).

Fraction 1 after recrystallization from EtOAc with a small amount of MeOH gave VIIa, m.p. 258–260°, $[\alpha]_D^{22}$ +32·1° (MeOH). IR ($CHCl_3$): 3620, 3440, 3330 cm^{-1}. (Found: C, 73·31; 73·29; H, 10·77; 10·79. $C_{30}H_{52}O_5$ requires: C, 73·13; H, 10·57%).

Fraction 3 was subjected to additional chromatography on a column (60 × 3·5 cm) with silica gel; elution with 2 l. of system B (1:1 → 1:4), then with system E—0·5 l. (1:4) afforded genin VIa (0·88 g), m.p. 233–234° (from EtOAc with a small amount of MeOH), $[\alpha]_D^{20}$ +32·1° (MeOH); IR ($CHCl_3$): 3620, 3350, 3380 cm^{-1}. (Found: C, 73·33; 73·11; H, 10·74; 10·76. $C_{30}H_{52}O_5$ requires: C, 73·13; H, 10·58%).

Oxidation of VIa. Kiliany mixture (1·56 ml) (CrO_3—26·6 g, H_2SO_4— 23 ml adjusted with H_2O to 100 ml) was added dropwise to a vigorously stirred soln of VIa (0·22 g) in acetone (176 ml) cooled to 0°. The stirring was continued for 1 hr, the reaction mixture was evaporated to ⅓ volume (initial) and the usual treatment resulted in 0·2 g of the reaction products.

Separation of oxidation products. The reaction products (0·2 g) were chromatographed on a column (38 × 2·5 cm). Elution was performed with 0·6 l. of system C (1:0 → 1:1), followed by 0·5 l. of (1:1 → 0:1) and afforded lactone VIIIa (0·13 g), m.p. 288–291° (from benzene), $[\alpha]_D^{22}$ +12·9° ($CHCl_3$); IR ($CHCl_3$): 1770 (γ-lactone), 1715 cm^{-1} (C=O). (Found: C, 72·69; 72·88; H, 8·62; 8·72. $C_{27}H_{38}O_5$ requires: C, 73·27; H, 8·65%). 0·03 g of lactone VIIIe emerged in the first few fractions, IR ($CHCl_3$): 1770 cm^{-1} (γ-lactone), 1715 cm^{-1} (C=O), m.p. 291–292° (VIIIe).

Oxidation of VIIa. 0·14 g of VIIa were oxidized as above. 0·12 g of the reaction product was chromatographed on a column (24 × 2 cm) with silica gel; elution with 0·5 l. of system B (1:0 → 1:1) afforded VIIIb (0·1 g), m.p. 291–292·5° (from MeOH), $[\alpha]_D^{22}$ +35·1° ($CHCl_3$); IR ($CHCl_3$): 1780 cm^{-1} (γ-lactone), 1725 cm^{-1} (C=O). (Found: C, 72·68; 72·87; H, 8·68; 8·80. $C_{27}H_{38}O_5$ requires: C, 73·25; H, 8·65%). Admixture of a sample with lactone VIIIe gave no depression of m.p. (m.m.p. 289–291°).

Oxidation of panaxoside F with OsO_4. Panaxoside F (4·5 g) was dissolved in dry pyridine (57 ml) and poured into a soln of OsO_4 (1·6 g) in dry pyridine (40 ml) and left for 14 days. The pyridine was evaporated under reduced press and the residue was treated with mannitol (14·2 g), KOH (22·8 g), EtOH (114·2 ml) and H_2O (57·1 ml); the mixture was heated under reflux for 8 hr. After deionization with KU-2 (H^+) and AV-17 (OH^-) and precipitation of mannitol, 3·85 g of the oxidation product were obtained.

Hydrolysis of the oxidation product. A soln of oxidized panaxoside F (3·85 g) in MeOH (28 ml) and conc HCl (11 ml) was heated for 4 hr at 65° yielding a mixture of genins (0·47 g).

Separation of the genins mixture. The genins mixture (0·77 g) was chromatographed on a column (40 × 3 cm) with silica gel. Elution with 1·5 l. of system B (100:0 → 50:50) resulted in two fractions: fraction 1 (VIIb, traces of VIb—0·126 g), fraction 2 (VIb—0·324 g).

Fraction 1 was rechromatographed on a column (23 × 2·2 cm) with silica gel. Elution was performed with 1·5 l. of system D (15:85 → 0:100) and gave VIIb (0·09 g), m.p. 218–220° (from light petroleum), $[\alpha]_D^{20}$ +21° ($CHCl_3$); IR ($CHCl_3$): 3620, 3350, 3420 cm^{-1}. (Found: C, 75·12; H, 11·07. $C_{30}H_{52}O_4$ requires: C, 75·58; H, 10·99%).

Fraction 2 was recrystallized from acetone to give VIb, m.p. 239–241·5°, $[\alpha]_D^{20}$ +21° (EtOH); IR ($CHCl_3$): 3620, 3350, 3380 cm^{-1}. (Found: C, 74·91; 74·97; H, 11·02; 11·07. $C_{30}H_{52}O_4$ requires: C, 75·58; H, 10·99%).

Oxidation of VIb. VIb (200 mg) dissolved in AcOH (3 ml) was treated with 393·7 mg CrO_3 in AcOH (6 ml) and left for 12 hr. After decomposing excess oxidant with isopropanol, 200 mg of crude product was obtained. The reaction product (0·2 g) was chromatographed on a column (24·7 × 2·2 cm) with silica gel. Elution with 1·580 l. of system C (1:0 → 2:1) resulted in lactone VIIIc (0·1 g), m.p. 199–203° (from light petroleum), $[\alpha]_D^{20}$ + 38·9° ($CHCl_3$); IR ($CHCl_3$): 1760 cm^{-1} (γ'-lactone), 1710 cm^{-1} (C=O). (Found: C, 75·51; 75·71; H, 9·89; 9·74. $C_{27}H_{40}O_4$ requires: C, 75·66; H, 9·40%). Lactone VIIIf (0·022 g) was eluted in the first few fractions; IR ($CHCl_3$): 1770 cm^{-1} (γ-lactone) 1715 cm^{-1} (C=O) m.p. 210–216°.

Oxidation of VIIb. VIIb (0·0695 g) was oxidized as above (CrO_3 in AcOH) and gave the reaction product (0·05 g). Chromatography was performed on a column (20 × 2 cm) with silica gel. Elution with 0·5 l. of system C (1:0 → 2:1) afforded lactone VIIId (0·048 g), m.p. 212–215° (from light petroleum), $[\alpha]_D^{20}$ +45·4°

($CHCl_3$); IR ($CHCl_3$): 1770 cm^{-1} (γ-lactone), 1715 cm^{-1} (C=O). Admixture of a sample with lactone VIIIf gave no depression (m.m.p. 210–215°). (Found: C, 75·29; 75·26; H, 9·62; 9·63. $C_{27}H_{40}O_4$ requires: C, 75·66; H, 9·40%).

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