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84. Shoji Shibata, Osamu Tanaka, Toshio Ando, Masako Sado, Susumu Tsushima, and Tomihiko Ohsawa : Chemical Studies on Oriental Plant Drugs. XIV.*1 Protopanaxadiol, a Genuine Sapogenin of Ginseng Saponins.*2

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Previously, the structure (I) (C/D *cis*, 17β-H) was proposed for panaxadiol,*1,1) which was isolated from the hydrolysate of the saponin of Ginseng roots. A further study on the saponin and the sapogenin of this drug has led us to a conclusion that panaxadiol (I) is a secondary product formed during the process of acid hydrolysis of the saponin, and the genuine sapogenin named protopanaxadiol is formulated as II.*2 It has been also found that the configuration of panaxadiol (I) should be amended to C/D *trans* fused and 17α-H structure.2)

The thin-layer chromatography of the crude saponin of the Ginseng roots on silica gel (solvent; upper layer of a mixture of *n*-BuOH-AcOH-H_2O=5:1:4) indicated the presence of several saponins which were designated according to the sequence of Rf values from the lower to the higher as ginsenosides-Rx (x=o,a,b,c,d,e,(f),g,and h).

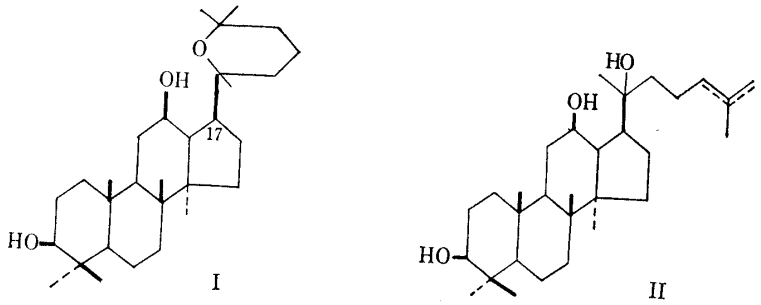

Chart 1.

By means of the thin-layer chromatography using the lower layer of a mixture of chloroform-methanol-water=65:35:10 as a developing solvent, it has been demonstrated that ginsenoside-Rb is a mixture of ginsenoside-Rb_1 and -Rb_2, and ginsenoside-Rg is consisted of -Rg_1, -Rg_2, and Rg_3 (Fig. 1). By the latter solvent system, ginsenoside-Rc showed the almost same Rf value as that of ginsenoside-Rb_2.

The present paper deals with a study on the genuine sapogenin of ginsenosides-Rb_1, -Rb_2, and -Rc. The methanolic extract of Ginseng roots was fractionated by modified Kotake's method3) (Fig. 2), to obtain a mixture of saponins (tentatively named GNS) as colorless powder, which contains ginsenosides-Rb_1, -Rb_2, -Rc, and a small amount of -Ra, Rd, and Re. By the preparative thin-layer chromatography (solvent; upper layer of a mixture of *n*-BuOH-AcOEt-H_2O=5:1:4) ginsenoside-Rc and a mixture of ginsenosides -Rb_1 and -Rb_2 were obtained. The separation of -Rb_1 and -Rb_2 was achieved by the

*1 Part XIII. S. Shibata, O. Tanaka, M. Nagai, T. Ishii : This Bulletin, **11**, 762 (1963). (Preliminary report : Tetrahedron Letters, **1962**, 1239). The title of this series of papers has now been amended as above.

*2 Preliminary communication of this paper : Tetrahedron Letters, **1963**, 795.

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1) S. Shibata, M. Fujita, S. Itokawa, O. Tanaka, T. Ishii : *Ibid.*, **1962**, 419; This Bulletin, **11**, 759 (1963).

2) O. Tanaka, M. Nagai, S. Shibata : Tetrahedron Letters, **1964**, 2291.

3) M. Kotake : Nippon Kagaku-Kaishi (J. Chem. Soc. Japan), **51**, 357 (1930).

second preparative thin-layer chromatography using a solvent system : lower layer of chloroform-methanol-water=65:35:10. On hydrolysis with boiling dil. mineral acid, ginsenosides-Rb_1, Rb_2, and -Rc, as well as the saponin mixture(GNS) yielded panaxadiol (I).

On treating with conc. hydrochloric acid at room temperature, the neutral saponin mixture (GNS) gave an unstable chloride (III), $C_{30}H_{53}O_3Cl$, m.p. 219~220°, which was proved to be identical with Kotake's chloride derived from α-panaxin (the crude prosapogenins of GNS).[3]

Heating this chloride (III) with diethylaniline or potassium *tert*-butoxide yielded a compound (IV), $C_{30}H_{52}O_3$, m.p. 236~238°, which showed in the IR spectrum (in $CHCl_3$) a free OH band at 3600 cm^{-1} and an intramolecularly hydrogen bonded OH band (concentration independent) at 3340 cm^{-1}. Acetylation of this compound (IV) with acetic anhydride and pyridine at room temperature afforded a diacetate (V), $C_{34}H_{56}O_5$, m.p. 125~127°, which still showed in the IR spectrum (in CS_2) a concentration independent OH band at 3537 cm^{-1}. The IR absorption band of carbonyl groups of acetoxyls of this acetate (V) appeared at 1743 and 1758 cm^{-1} (in CS_2). The latter absorption can be assigned to the carbonyl of the acetoxyl group, whose singly bonded oxygen (alcoholic) is hydrogen bonded with an OH group.[4] Consequently, the compound (IV) possesses three hydroxyls, one of which is sterically hindered and hydrogen bonded with another hydroxyl. In the NMR (nuclear magnetic resonance) spectrum of the compound (IV) in deuterochloroform, the signals in the region of τ 6.5~6.9 (2H, overlapped broad multiplet), which are shifted to the lower field by ca. 1.00 p.p.m. on acetylation, can be assigned to two protons such as $\underline{H}$-C-OH; the signal at τ 8.87 (3H, singlet) would be attributed to a methyl on a carbon atom bearing a tertiary hydroxyl, such as $C\underline{H}_3$-C-OH. On treatment with dil. mineral acid under the same condition as that of the hydrolysis of the saponin, the compound (IV) afforded panaxadiol (I). The catalytic hydrogenation of the compound (IV) gave the dihydro compound (VI), $C_{30}H_{54}O_3$, m.p. 246~248°. In regard to these findings, the compound (IV) can be formulated as II.

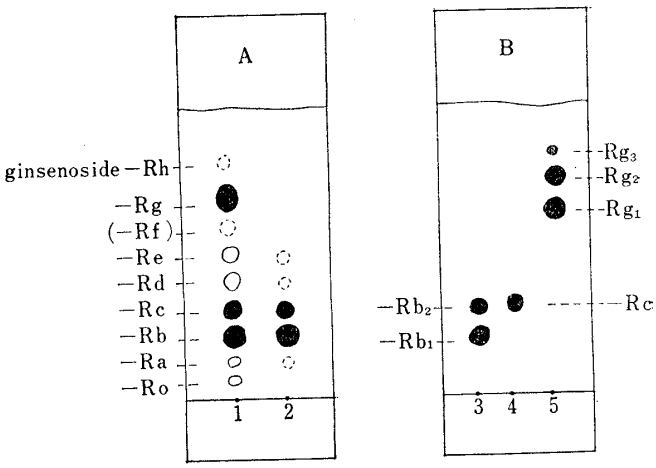

Fig. 1. The Thin-layer Chromatogram of Ginseng Saponins
A. Solvent: Upper layer of *n*-BuOH-AcOH-H_2O=5:1:4
1. Crude saponin mixture of Ginseng
2. The saponin mixture(GNS) (see Fig. 2)
B. Solvent: Lower layer of $CHCl_3$-MeOH-H_2O=65:35:10
3. Ginsenoside-Rb 4. Ginsenoside-Rc
5. Ginsenoside-Rg

In our previous communication,*2 the compound (IV) was named protopanaxadiol, for which the structure (IVb) has been proposed. The presence of the isopropenyl type double bond in its side chain was suggested by the IR absorption at 882 cm^{-1} and by an analogy of the dehydrochlorination reaction of the chlorides of butyrospermol, cyclolaudenol, and their related compounds.[5,6] However, examination of NMR spectrum of the compound (IV) indicates that this compound would be a mixture of the isomers at the position of the double bond. In the NMR spectrum in deuterochloroform (Fig.

4) F. Dalton, J. I. McDougall, G. D. Meakins : J. Chem. Soc., **1963**, 4068.
5) J. A. Henry, D. S. Irvine, F. S. Spring : *Ibid.*, **1955**, 1607; M. C. Dawson, T. G. Halsall, E. R. H. Jones, P. A. Robins : *Ibid.*, **1956**, 3172.
6) L. F. Fieser, M. Fieser : Steroids, p. 392. Reinhold Publ. Co., N. Y. (1959); G. Ourisson, P. Crabbé : Les triterpenes tetracycliques, p. 80 Hermann, Paris (1961).

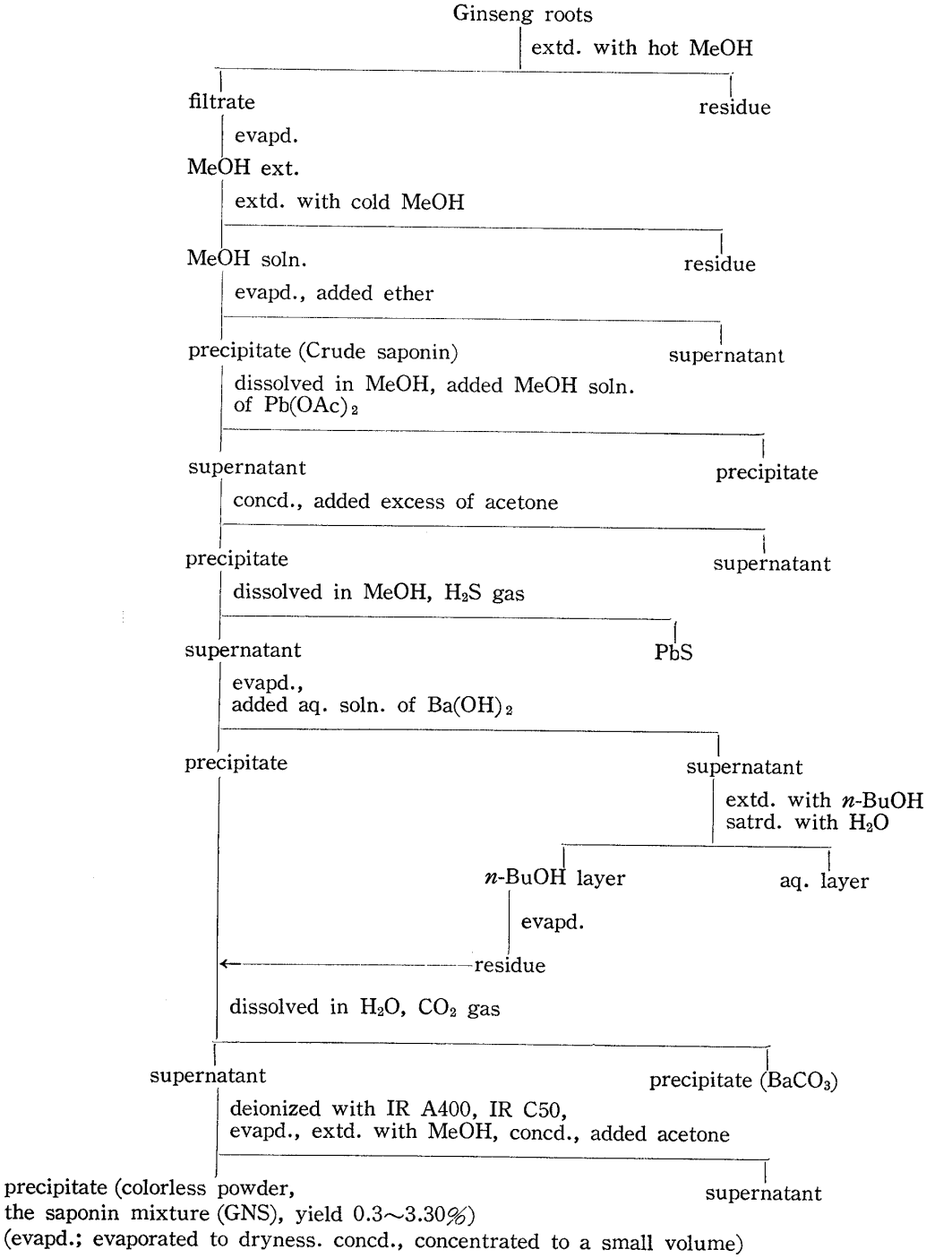

Fig. 2. Separation of the Ginseng Saponin

3), the signals at τ 8.30~8.35 (more than 3H), which are due to the methyls on a double bond, are not clearly resolved and the vinylic proton signals appear both at τ 4.80~5.00 and near τ 5.30. Betulafolienetriol (Ⅶ), which has an isopropylidene type double bond in its side chain,shows clearly resolved methyl signals at τ 8.30 and 8.36 (3H each, broad singlets due to long range coupling with a vinylic proton at C-24), and a vinylic proton signal near τ 4.80~5.00 (1H)(Fig. 3). These observations strongly suggest that the compound (Ⅳ) is a mixture of the compound (Ⅳa) (isopropylidene type double bond in the side chain) and the compound (Ⅳb) (isopropenyl type double bond in the side chain). The ratio of the formation of both isomers from the chloride (Ⅲ)

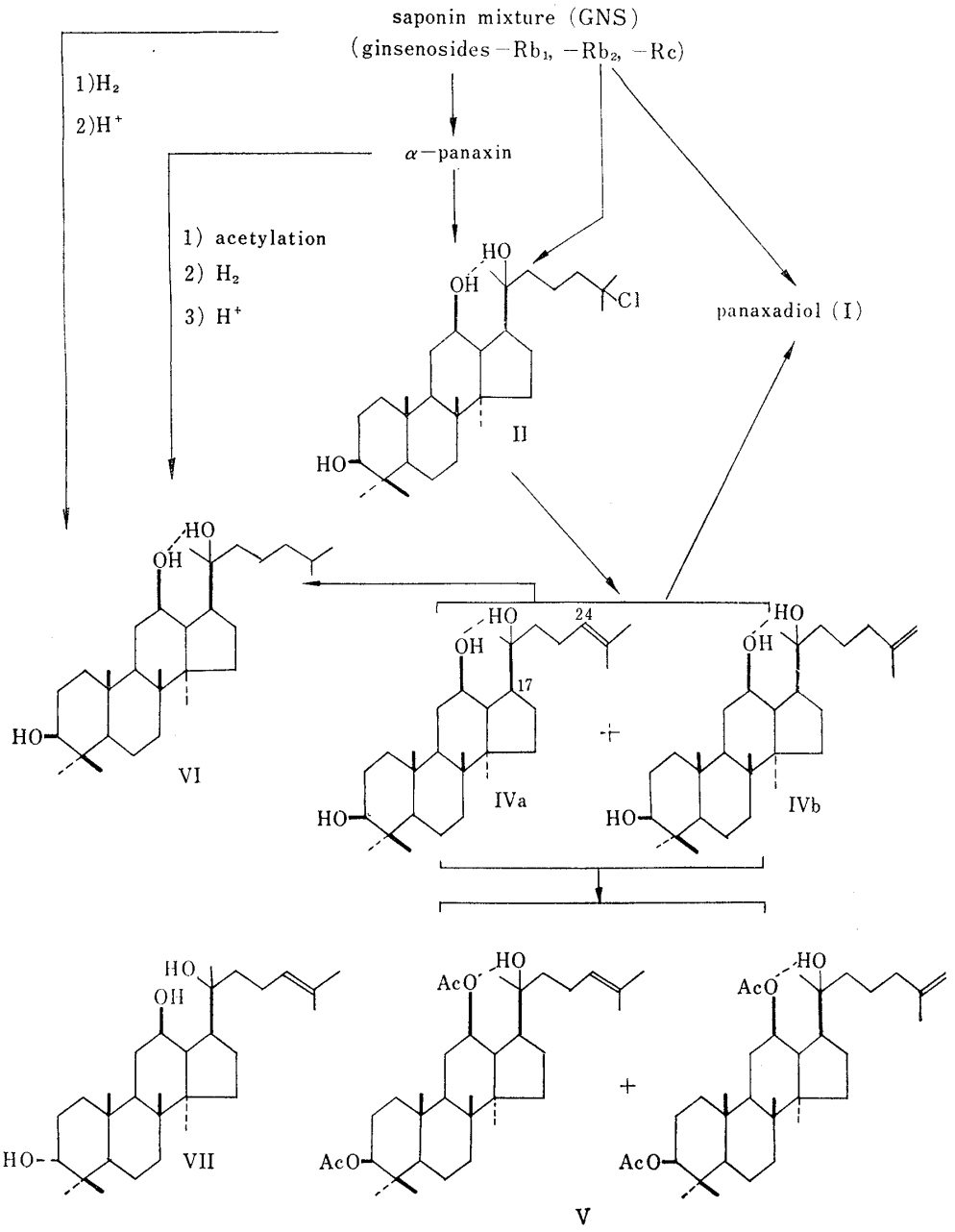

Chart 2.

would depend upon the condition of the dehydrochlorination reaction.[7] Although the separation of both isomers has not been achieved, the names of protopanaxadiol and isoprotopanaxadiol are now proposed for the compounds (Ⅳa and Ⅳb), respectively.

The NMR spectrum of the chloride (Ⅲ) in deuterochloroform exhibited peaks at τ 8.42 (6H, singlet, CH_3-C(-CH_3)-Cl) and τ 8.87 (3H, singlet, CH_3-C(-CH_3)-OH), and its IR spectrum in chloroform showed a concentration independent OH band (intramolecularly hydrogen bonded) and a free OH band at almost same positions as those observed in the spectrum of the compound (Ⅳ). These evidences led to the formula Ⅲ for this chloride.

As already mentioned before, the saponin mixture (GNS), as well as ginsenosides-

7) T. Oliver : J. Chem. Soc., **1961**, 2353; F. C. Chang, N. F. Wood : Steroids, **14**, 55 (1964); H. C. Brown, *et al.* : J. Am. Chem. Soc., **78**, 2190 (1956); *Ibid.*, **88**, 1425 (1966).

Rb_1, -Rb_2, and -Rc, afforded panaxadiol (I) on hydrolysis with boiling dil. mineral acid, whereas, the acid hydrolysis of the catalytically hydrogenated saponin mixture (GNS) gave no panaxadiol (I) but yielded dihydropanaxadiol (Ⅵ) in a good yield. Hydrogenation of Kotake's α-panaxin acetate and subsequent hydrolysis also gave dihydroprotopanaxadiol (Ⅵ). Panaxadiol (I) is stable either to the catalytic reduction or to the action of conc. hydrochloric acid at room temperature. The NMR spectra of acetylated ginsenosides-Rb_1, -Rb_2, and -Rc show the clearly resolved signals due to two methyls on a double bond in the region of τ 8.30~8.40 as observed in the spectrum of betulafolienetriol (Ⅷ), indicating the presence of an isopropylidene type double bond. Consequently, it has now been concluded that the genuine sapogenin of these saponins should be represented by protopanaxadiol (Ⅳa), and the trimethyltetrahydropyrane ring of panaxadiol (I) is formed during the process of the acid hydrolysis of these saponins by the acid catalyzed ring closure of the side chain of protopanaxadiol (Ⅳa).

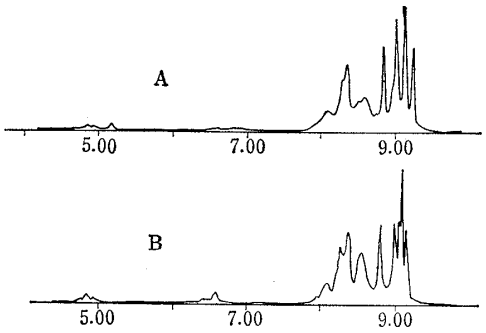

Fig. 3. Nuclear Magnetic Resonance Spectra (in $CDCl_3$, 60 mc.)
A. The compound (Ⅳ); B. Betulafolienetriol (Ⅷ)

The formation of panaxadiol (I) has been observed in the hydrolysis of the saponin mixture obtained from the following plants of *Panax* species[8] : *P. ginseng* C.A. MEYER (roots, leaves), *P. pseudoginseng* WALL (roots, rhizomes, leaves),[9] *P. japonicus* C. A. MEYER (roots, rhizomes), *P. quinquefolium* L. (American Ginseng, roots), and "Sanchi-ginseng" (人参三七).

The stereochemistry of protopanaxadiol and panaxadiol (the correlation of dihydroprotopanaxadiol with dammaranediol-I)[2] and the structure of the prosapogenin of ginsenosides-Rb_1, -Rb_2, and -Rc (3-(β-sophoroside) of protopanaxadiol) will be reported in the forthcoming papers.

Experimental*4

Preparative Thin-layer Chromatography of the Saponin Mixture (GNS, ref. Chart 2)——Silica gel G was applied on 20×20 cm. glass plates to a thickness of 250μ. After developing with the solvent mentioned in the theoretical part, the chromatogram was exposed to vapor of iodine. The resulted yellow bands were collected, allowed to stand in the dark until the yellow color disappeared, and extracted with methanol. To the concentrated methanolic solution was added excess of acetone to precipitate the saponin as colorless powder (negative to Beilstein's test). From 1 g. of the saponin mixture (GNS), there was obtained ginsenoside-Rc (105 mg.), m.p. 192~194° (decomp.) and a mixture-Rb_1 and -Rb_2 (440 mg.) (50 Plates were used for the separation of 1 g. of GNS, solvent *n*-BuOH-AcOH-H_2O=4 : 1 : 5 (upper layer)). Second thin-layer chromatography of the mixture (440 mg.) (25 Plates were used for the separation of 440 mg. of the mixture of -Rb_1 and -Rb_2, solvent $CHCl_3$-MeOH-H_2O=65 : 35 : 10 (lower layer)) gave ginsenosides-Rb_1 (150 mg.), m.p. 197~200°, (decomp.), and -Rb_2 (120 mg.), m.p. 198~201°(decomp.).

For the measurement of NMR spectra in $CDCl_3$, each saponin was acetylated with acetic anhydride and pyridine in the usual way, yielding the corresponding acetate as colorless powder (from aqueous ethanol). The detail of the properties of each saponin and their acetates will be described in the forthcoming paper.

Hydrolysis of the Saponin Mixture (GNS) with Conc. Hydrochloric Acid——The mixture (GNS) (3 g.) was treated with conc. HCl (15 ml.) at room temperature. During the course of the reaction, sticky oil deposited, which solidified on standing overnight. The reaction mixture was poured into ice water, and the precipitate was collected, washed with water, dried, and extracted with ether. The etherial solution was concentrated to a small volume to obtain crystalline precipitate which was recrystallized from benzene or ethyl acetate to give the chloride (Ⅲ) as colorless crystals, m.p. 219~220° (250 mg.) *Anal.* Calcd. for $C_{30}H_{53}O_3Cl$: C, 72.50;

*4 All melting points were determined on a Kopfler micro-hot stage and uncorrected. Thin-layer chromatography was performed on silica gel G acc. to Stahl and spots were detected by heating at 100° after spraying 10% H_2SO_4 in case of qualitative analysis.

8) S. Shibata, T. Ando, O. Tanaka, Y. Meguro, K. Soma, Y. Iida : Yakugaku Zasshi, **85**, 753 (1965).

9) Collected in Sikkim, India by Prof. H. Hara of this University.

H, 10.69; Cl, 7.14. Found : C, 72.49; H, 10.74; Cl, 6.90, 7.04. The identity of this chloride (Ⅲ) with the chloride obtained from α-panaxin (prosapogenin mixture of GNS) by Kotake[3] was confirmed by mixed fusion and comparison of the IR spectra.

Dehydrochlorination of the Chloride (III). The Formation of the Compound (IV) (A Mixture of Protopanaxadiol (IVa) and Isoprotopanaxadiol (IVb))——a) With diethylaniline : A solution of the chloride (Ⅲ) (160 mg.) in diethylaniline (3 ml.) and xylene (15 ml.) was heated under reflux for 10 hr. The solution was diluted with ether after cooling and the etherial solution was washed with dil. HCl, and water, dried, and concentrated to dryness. The residue was crystallized from ethyl acetate or benzene to give the compound (Ⅳ) as colorless crystals (50 mg.), m.p. 236～238°, $[\alpha]_D^{25}$ + 20.5 (c=1.03, $CHCl_3$), *Anal.* Calcd. for $C_{30}H_{52}O_3$: C, 78.20; H, 11.38. Found : C, 78.36; H, 11.36.

b) With potassium *tert*-butoxide : A mixture of the chloride (Ⅲ) (9 g.), potassium *tert*-butoxide (10 g.), *tert*-butanol (500 ml.), and dimethyl-sulfoxide (800 ml.) was heated at 75° for 3.5 hr. After dilution with water, the reaction mixture was extracted with ether, and the organic layer was washed with water, dried, and concentrated to dryness to afford the colorless residue which was crystallized from ethyl acetate to give the compound (Ⅳ) (5.8 g.), m.p. 236～238°.

The dehydrochlorination of the chloride (Ⅲ) was also observed during the column chromatography on silica gel.

Acetylation of the Compound (IV)——The compound (Ⅳ) was acetylated with acetic anhydride and pyridine at room temperature standing overnight. After working up in the usual method, the product was crystallized from 90% aqueous methanol to give the diacetate (Ⅴ), colorless crystals, m.p. 125～127°, $[\alpha]_D^{24}$ −5.6° (c=1.03, $CHCl_3$). *Anal.* Calcd. for $C_{34}H_{56}O_5$: C, 74.95; H, 10.36. Found : C, 74.95; H, 10.42

Panaxadiol (I) from the Compound (IV)——The compound (Ⅳ) (250 mg.) was heated with a mixture of conc HCl (7 ml.), ethanol (15 ml.), and water (15 ml.) on a boiling water bath for 1.5 hr. The reaction mixture was diluted with water, and extracted with ether. After working up in the usual method, the oily product was purified by chromatograyhy on silica gel to afford panaxadiol (I), colorless crystals (50 mg.), m.p. 250° (from ethyl acetate), which was compared with authentic sample by thin-layer chromatography (solvent : $CHCl_3$ -ether=2 : 1), mixed fusion, and the IR spectra.

Dihydroprotopanaxadiol (VI)——The compound (Ⅳ) (200 mg.) was catalytically hydrogenated with the Adams catalyst (50 mg.) in a mixture of ethanol and ethyl acetate. The product was crystallized from methanol to give colorless crystals, dihydroprotopanaxadiol (Ⅵ), m.p. 246～248°, $[\alpha]_D^{27}$ + 22.9° (c=1.00, $CHCl_3$), *Anal.* Calcd. for $C_{30}H_{54}O_3$: C, 77.86; H, 11.76. Found : C, 78.15; H, 11.68.

Hydrolysis of the Hydrogenated Saponin Mixture (GNS)——The saponin mixture (GNS) (2 g.) was catalytically hydrogenated with the Adams catalyst in a mixture of ethanol, methanol, and acetic acid. (41 ml. of hydrogen was absorbed within 50 min.). The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo* to dryness. The residue was heated under reflux with a mixture of conc. HCl (10 ml.), ethanol (20 ml.), and water (20 ml.) for 4 hr. to yield colorless precipitate, which was taken up in ether. After working up in the usual method the product was crystallized from methanol to give dihydroprotopanaxadiol (Ⅵ), colorless needles (350 mg.), m.p. 246～248°. The absence of panaxadiol (I) in the reaction product was demonstrated by the thin-layer chromatography.

Hydrolysis of the Acetate of Hydrogenated α-Panaxin——α-Panaxin (prosapogenin mixture), prepared from the saponin mixture (GNS) (900 mg.) according to Kotake's procedure,[3] was acetylated with acetic anhydride and pyridine in the usual way, and the resulted acetate was catalytically hydrogenated with the Adams catalyst (200 mg.) in ethyl acetate containing 5 % acetic acid. The catalyst was removed and the product was hydrolyzed by refluxing with a mixture of conc. HCl (10 ml.), ethanol (20 ml.), and water (20 ml.). After working up in the usual way, the hydrolysate was purified by chromatography on silica gel to afford dihydroprotopanaxadiol, colorless crystals from methanol, m.p. 246～248°. The thin-layer chromatography of each fraction of the above chromatography indicated the absence of panaxadiol (I) in the hydrolysate.

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Summary

It was proved that the genuine sapogenin of ginsenosides-Rb_1, -Rb_2, and -Rc, saponins of Ginseng roots, was represented by protopanaxadiol (Ⅳa), and panaxadiol (I) was an artifact formed during the process of the acid hydrolysis of these saponins.

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