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Chemical Studies on the Oriental Plant Drugs. XXVIII.[1] Saponins and Sapogenins of Ginseng: Stereochemistry of the Sapogenin of Ginsenosides -Rb_1, -Rb_2 and -Rc[2]

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The sapogenin of Ginseng saponins, ginsenosides-Rb_1, -Rb_2 and -Rc, was once reported to be protopanaxadiol (III). But it was found recently that dammarane type triperpenes with hydroxyl or methoxyl group at $C_{(12)}$ and $C_{(20)}$ give an equilibrated mixture of epimeric compounds at $C_{(20)}$.

On mild hydrolysis of the saponins 20-*epi*-protopanaxadiol has been obtained as the genuine sapogenin for which the name of 20(*S*)-protopanaxadiol (V) is proposed. The prosapogenin (XI), which was obtained by the partial hydrolysis of the above mentioned three saponins is found to be 3-O-(2β-D-glucopyranosyl-β-D-glucopyranosyl)-20 (*R*)-protopanaxadiol.

The remaining glycosidic linkage in ginsenosides-Rb_1, -Rb_2 and -Rc should be located at $C_{(20)}$-OH.

As already reported, the thin-layer chromatography (TLC) of the Ginseng extract has demonstrated the presence of numerous saponins designated as ginsenosides-Ro, a, b_1, b_2, c, d, e, f, g_1, g_2, g_3, and h.[4] Of these saponins, ginsenosides-Rb_1, -Rb_2, and -Rc afforded panaxadiol (I) on the hydrolysis with boiling dil. mineral acid.[5,6] Hydrolysis of these saponins with conc. HCl and the subsequent treatment of the resulted chloride (II) with potassium *tert.* butoxide yielded protopanaxadiol (III) (dammar-24-en-3β,12β,20(*R*)-triol), which gave panaxadiol (I) on acid treatment.[1,4] On acid hydrolysis the hydrogenated ginseng saponins afforded dihydroprotopanaxadiol (IV).[1,4] On the basis of these results and some other spectrometric evidences, protopanaxadiol (III)[4] was assigned to the genuine sapogenin.

However, as has been described in the preceding paper,[1] the $C_{(20)}$ hydroxyl group of dammarane type triterpenes having 12β hydroxyl group (or methoxyl group) is epimerized by the treatment with acid to form an equilibrated mixture of epimers. Therefore the chirality at the $C_{(20)}$ of the genuine sapogenin must be settled as being represented by either protopanaxadiol (III) or its $C_{(20)}$ epimer (V). The present paper describes the experimental details which lead to the conclusion that 20(*S*)-protopanaxadiol (V) is favorable to represent genuine sapogenin of ginsenosides-Rb_1, -Rb_2, and -Rc, and the partial structure of these

1) Part XXVII: O. Tanaka, M. Nagai, T. Ohsawa, N. Tanaka, K. Kawai and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **20**, 1204 (1972).
2) Preliminary communication of this study: M. Nagai, T. Ando, O. Tanaka and S. Shibata, *Tetrahedron Letters*, **1967**, 3579, and in part: O. Tanaka, M. Nagai, T. Ohsawa, N. Tanaka and S. Shibata, *ibid.*, **1967**, 391.
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4) S. Shibata, O. Tanaka, T. Ando, M. Sado, S. Tsushima and T. Ohsawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 595 (1966).
5) M. Fujita, S. Itokawa and S. Shibata, *Yakugaku Zasshi*, **82**, 1638 (1962).
6) M. Nagai, O. Tanaka and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **19**, 2349 (1971). Preliminary report: M. Nagai O. Tanaka and S. Shitata, *Tetrahedron Letters*, **1966**, 4797.
7) S. Shibata, T. Ando and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **14**, 1157 (1966).

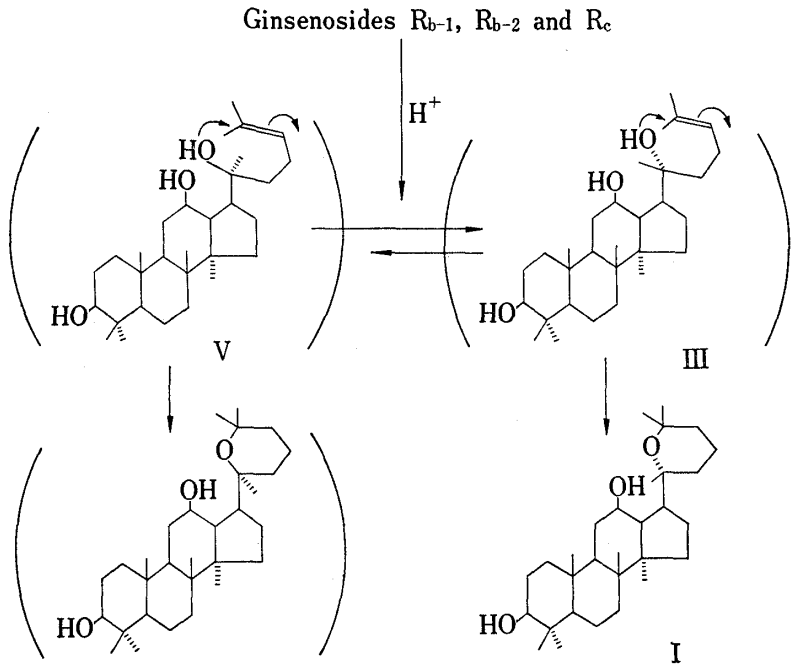

Chart 1

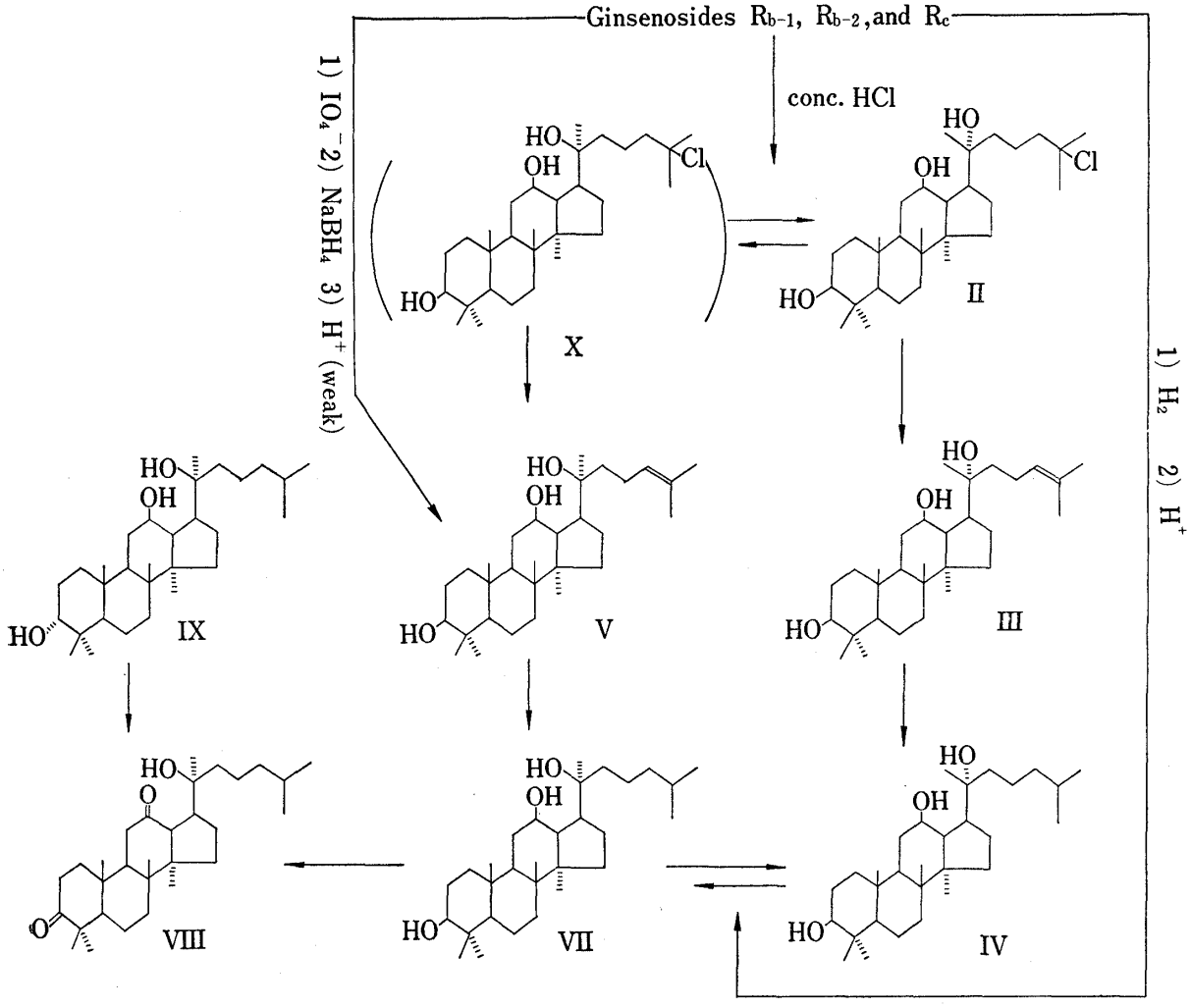

Chart 2

saponins is formulated as VI. The chloride (II) which yielded III by base treatment has been obtained from the ether less soluble portion of the hydrolyzate of the prosapogenin[7] (*vide infra*) or the original saponins with conc. HCl.[4,8] The ether soluble portion of this hydrolyzate was treated with base, and the products were purified by column chromatography on silica gel to give a new compound (V), $C_{30}H_{52}O_3$, which gave a dihydro compound (VII) on catalytic hydrogenation. This compound (V) showed a close *Rf* value to that of III on thin-layer chromatogram, but the retention time of the gas chromatography of V was clearly different from that of III. Oxidation of VII afforded a diketone (VIII), which was proved to be identical with the 3,12-diketone prepared from betulafolianetriol (IX),[9] the epimer of IV at $C_{(3)}$ and $C_{(20)}$. By this result and the nuclear magnetic resonance (NMR) spectral evidences, the compound (V) can be formulated as dammar-3β,12β,20(*S*)-triol, an epimer of III at $C_{(20)}$. The compounds III and V are now designated as 20(*R*)- and 20(*S*)-protopanaxadiol, respectively. The isolation of V indicates the presence of the epimer (X) of the chloride (II) in the above hydrolyzate of the saponins, but X could not be isolated due to its higher solubility and its transformation into a dehydrochlorinated compound during the process of chromatography on silica gel or alumina.

A mixture of ginsenosides-Rb_1, -Rb_2, and -Rc tentatively named GNS was subjected to the hydrolysis under a mild condition, *i.e.* Smith's degradation[10] to give V. The gas chromatography of this reaction mixture strictly showed the absence of 20(*R*)-protopanaxadiol (III). Each of these saponins separated from GNS[4] was also hydrolyzed respectively in a small scale by the same procedure as above. In every case, the aglycone was shown to be

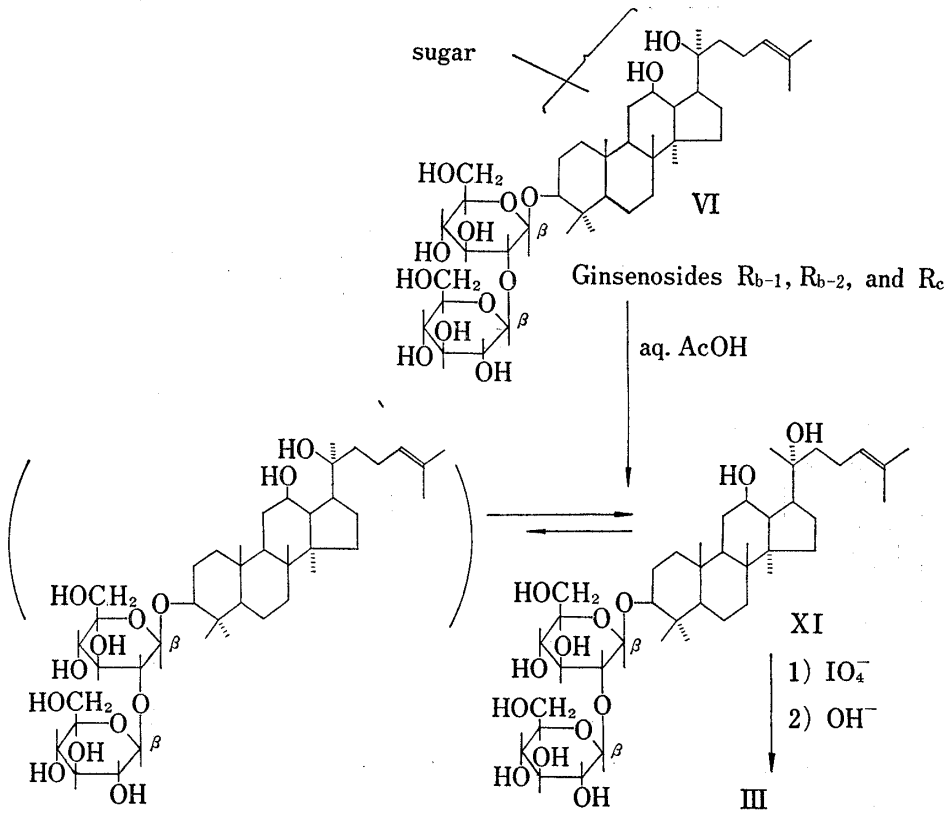

Chart 3

8) M. Kotake, *Nippon Kagakuzasshi*, **51**, 357 (1930).
9) O. Tanaka, M. Nagai and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **14**, 1150 (1966). Preliminary report: O. Tanaka, M. Nagai and S. Shibata, *Tetrahedron Letters*, **1964**, 2291.
10) F. Smith, G.W. Hay and B.A. Lewis, "Methods in Carbohydrate Chemistry," Vol. 5, Academic Press, New York and London, 1965, p. 361.

V by TLC and gas chromatography, and the formation of III was not detected. Now it has been concluded that the genuine sapogenin of ginsenosides-Rb_1, -Rb_2, and -Rc is 20(*S*)-protopanaxadiol (V). Panaxadiol ($C_{(20)}$ R) (I) and 20(*R*)-dihydroprotopanaxadiol (IV) are less soluble and readily crystallized to give a predominant yield than the corresponding $C_{(20)}$ S compounds in the equilibrated mixture of hydrolyzates.

On heating with aqueous acetic acid, ginsenosides-Rb_1, -Rb_2, or -Rc afforded their common prosapogenin (XI), which has been formulated as 3-O-(2β-D-glucopyranosyl-β-D-glucopyranosyl)-protopanaxadiol.[7] Oxidation of XI with periodate followed by alkaline treatments[11] yielded 20(*R*)-protopanaxadiol (III) indicating that the sapogenin of the prosapogenin (XI) should be represented by III. Since the epimerization of the $C_{(20)}$ hydroxyl group has been found to take place even by heating with aqueous acetic acid,[1] it is evident that XI was formed from the saponins as a result of the partial hydrolysis of the sugar moiety accompanying the epimerization of hydroxyl at $C_{(20)}$. The predominant isolation of XI would also be due to its less solubility in the solvent than its $C_{(20)}$ epimer.

The acetates of ginsenosides-Rb_1, -Rb_2, and -Rc showed no OH absorption in their infrared (IR) spectra to suggest the presence of O-glycosyl linkage at $C_{(20)}$, because the tertiary hydroxyl group at $C_{(20)}$ of this type has been found to resist against acetylation, and the OH band should be observed even after acetylation if the $C_{(20)}$-hydroxyl group is free. The IR spectrum of octaacetate of prosapogenin exhibited an intramolecularly hydrogen bonded OH band at 3542 cm^{-1} (in CCl_4)[7] to show the occurrence of free hydroxyl at $C_{(20)}$. The presence of O-glycosyl group at $C_{(20)}$ was also supported by the mass spectra of the acetates of ginsenosides-Rb_1, -Rb_2, and -Rc[12] as well as by the result of the hydrolysis of the saponin with the soil bacteria.[13] On the basis of the present finding, the common partial structure of ginsenosides -Rb_1, -Rb_2, and -Rc can now be formulated as shown in Chart 3.

Experimental[14]

20(*S*)-Protopanaxadiol (V)——A mixture of saponins, ginsenosides –Rb_1, –Rb_2 and –Rc, (VI) (8 g)[7] was dissolved in conc. HCl (40 ml), and the reaction was allowed to stand at room temperature overnight. The reaction mixture was poured into ice water and the resulting precipitate was collected by filtration, washed with water, dried *in vacuo*, and dissolved in a large amount of ether. The ethereal solution was concentrated to a small volume to deposit the crude chloride (II). The supernatant was evaporated to dryness and the residue was subjected to dehydrochlorination as follows.

The residue was dissolved in a mixture of DMSO (150 ml) and sodium (0.62 g) in *tert*-BuOH (150 ml). The solution was heated at 100—120° for 5 hr and then poured into water. The precipitate was taken up in ether, and the ether layer was washed with water repeatedly, dried, and concentrated to dryness. The residue was purified by chromatography on silica gel followed by recrystallization from benzene to give 20(*S*)-protopanaxadiol (V) (180 mg), colorless needles, mp 197—200°, $[\alpha]_D$ +26.7° ($CHCl_3$). *Anal.* Calcd. for $C_{30}H_{52}O_3$: C, 78.20; H, 11.38. Found: C, 78.09; H, 11.33. NMR δ: 0.78 (3H), 0.88 (6H), 0.98 (6H), 1.16 (3H) (all singlets, $-\overset{|}{\underset{|}{C}}-CH_3$), 1.61, 1.67 (singlets, 3H each, $-\overset{|}{C}=\overset{|}{C}-CH_3$), 3.2, 3.7 (broad, 1H each, $-\overset{|}{C}H-OH$), and 5.13 ppm (triplet-like, 1H, $-\overset{|}{C}=CH-$).

20(*S*)-Dihydroprotopanaxadiol (VII)——V (80 mg) was hydrogenated with the Adams catalyst (50 mg) in a mixture of EtOAc (15 ml) and EtOH (25 ml). The product was recrystallized from benzene to give VII (55 mg) as colorless needles, mp 213—215°, $[\alpha]_D$ +2.1($CHCl_3$). *Anal.* Calcd. for $C_{30}H_{54}O_3$: C, 77.87; H, 11.76. Found: C, 77.90; H, 11.68.

Oxidation of VII to the Diketone (VIII)——To a solution of VII (300 mg) in acetone (30 ml) was added gradually the Jones reagent until the constant orange color was observed. After working up in the usual way, the reaction product was recrystallized from a mixture of benzene and *n*-hexane yielding dammarane-

11) J.J. Dugan and P. de Mayo, *Can. J. Chem.*, **43**, 2033 (1965).
12) T. Komori, O. Tanaka and Y. Nagai, unpublished result.
13) I. Yoshioka, K. Imai, I. Kitagawa, S. Shibata, O. Tanaka and T. Ando, 88th Annual Meeting of Pharm. Soc. of Japan, Tokyo, April, 1968.
14) All melting points were determined on the Kofler block and remain uncorrected. NMR spectra were taken at 100 Mc in $CDCl_3$ solution with TMS as the internal standard.

20(*S*)-ol-3,12-dione (VIII) (70 mg), mp 160.5—162° as colorless plates. The identification with the authentic sample prepared from betulafolianetriol (IX) was established by mixed melting point and the comparison of IR spectra.

Smith's Degradation of the Saponins——A mixture of saponins (GNS[4]) (3.0 g) which was mainly consisted of ginsenosides-Rb_1, $-Rb_2$ and -Rc was oxidized with $NaIO_4$ (13 g) in water (250 ml) under stirring for 4 hr 40 min. The resulted precipitate was collected by filtration, washed with dil. H_2SO_4 and water successively, and dissolved in a mixture of EtOH (30 ml) and water (15 ml). To this solution was added $NaBH_4$ (1.0 g) and the mixture was allowed to stand at room temperature for 17 hr. After dilution with water (45 ml), the reaction mixture was acidified to pH 1.8—2.0 with 2N H_2SO_4 (about 11 ml) and kept at room temperature overnight. The mixture was extracted with ether and the ether layer was washed with water, dried, and concentrated to dryness. The crude product was chromatographed on silica gel to give colorless needles (from benzene), mp 197—200°, which was identified to be V (mixed melting point and comparison of IR spectra). Absence of III in the above crude product was rigorously established by gas chromatography: Condition; column: SE 30 on chromosorb W (glass column), sample chamber temp.: 290—297°, detector temp.: 268°, column temp.: 239°, carrier gas N_2 27 ml/min × 2.8 kg/cm², detector: hydrogen flame ionization detector. Retention time (min.): 20(*S*)-protopanaxadiol (V) 49.30—49.76, 20(*R*)-protopanaxadiol (III): 56.23. The broadness of the peaks of both compounds would be due to the partial dehydration of the tertiary OH at $C_{(20)}$.

Ginsenoside-Rc (230 mg) was oxidized with $NaIO_4$ (1.1 g) in water (20 ml) for 4 hr in the same way as above. The product was reduced with $NaBH_4$ (0.1 g) in EtOH (2.5 ml) and water (1.5 ml) at room temperature for 24 hr, and then the pH of the reaction mixture was adjusted to 1.8—2.0 with 2N H_2SO_4, and allowed to stand overnight at room temperature. The reaction mixture was poured into water saturated with NaCl and extracted with ether. The ether layer was washed with water, dried, and concentrated to dryness. The presence of V and the absence of III in this residue was confirmed by thin-layer and gas chromatography. (TLC: solvent: EtOAc(10 ml)–$CHCl_3$(10 ml)–EtOH(12 drops), on silica gel. V and III showed similar *Rf* values).

Ginsenosides-Rb_1 and -Rb_2 were also subjected to Smith's degradation in the same way. The same results as in the case of ginsenoside-Rc were obtained.

Degradation of the Prosapogenin (XI)——XI (0.3 g) was oxidized with $NaIO_4$ (1.0 g) in a mixture of MeOH (120 ml), EtOH (20 ml), and water (60 ml) under ice cooling and stirring for 116 hr. The precipitate was filtered off and the filtrate was diluted with water and extracted with *n*-BuOH three times. The BuOH layer was washed with aqueous $NaHCO_3$ and water successively, and evaporated to dryness *in vacuo*. A solution of the residue and KOH (2.5 g) in a mixture of EtOH (15 ml) and H_2O (35 ml) was refluxed under the stream of N_2 for 1 hr. The reaction mixture was neutralized with 2N H_2SO_4 under ice cooling and extracted with a mixture of ether–EtOH (5:1) four times. The organic layer was washed with water, dried, and concentrated to dryness. The residue was purified by chromatography on silica gel followed by recrystallization from AcOEt affording 20(*R*)-protopanaxadiol (III) (27 mg), mp 248—248.5° which was identified with the authentic sample by mixed melting point and IR spectra.

Acetylation of the Saponins——Ginsenoside-Rc[4] (70 mg) was acetylated with acetic anhydride (1.5 ml) and pyridine (3 ml) at room temperature for 65 hr. After working up in the usual way, reprecipitation of the product from its MeOH solution by dilution with water afforded the acetate as colorless powder which was proved to be homogeneous by thin-layer chromatography and showed no OH band in its IR spectrum. Ginsenosides-Rb_1 and -Rb_2 were also acetylated in the same way as above to yield the corresponding acetates as colorless powder, which also showed no OH band.

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