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Isolation of Saponins and Sapogenins from Callus Tissue of *Panax ginseng*[1,2]

Tsutomu Furuya, Hisashi Kojima, Kunihiko Syono,[3a] Takafumi Ishii, Kazumichi Uotani, and Motohiro Nishio[3b]

School of Pharmaceutical Sciences, Kitasato University[3a] *and Pharmaceutical Product Development Laboratories, Meiji Seika Kaisha Ltd.*[3b]

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The callus derived from the petiole of *Panax ginseng* has been vigorously grown on Murashige and Skoog's agar medium (minus glycine) supplemented with 2,4-dichlorophenoxyacetic acid. Saponins such as ginsenosides Rb_1 (I) and Rg_1 (II) were obtained *via* the column of Sephadex LH-20 and silica gel, respectively. Sapogenins, such as panaxadiol (III), panaxatriol (IV) and oleanolic acid (V), were also isolated in crystalline form from the hydrolysates of crude saponins. The callus contains a considerable amount of saponins and the thin-layer chromatograms of the saponins are similar to those of ginseng root saponins.

Ginseng (*Panax ginseng* C. A. Meyer, Araliaceae, chôsen-ninjin in Japanese) is a herbaceous plant growing in eastern Asia and cultivated in north China, Korea, and Japan. Ginseng root used widely as a tonic in the Orient from ancient times is expensive because of long-term (4—6 years) and troublesome cultivation.

In order to apply the callus culture to the production of medicaments, the study on the ginseng callus culture has been undertaken several years ago and well-growing callus was obtained. We have reported on the isolation of panaxatriol from the ginseng callus in a preliminary communication[4] and now wish to report in detail on the isolation of two saponins and three sapogenins from the ginseng callus tissue.

β-glucose $\overset{6-1}{—}$ β-glucose (C-20 O); β-glucose $\overset{2-1}{—}$ β-glucose (C-3 O) — I

β-glucose (C-20 O); O—β-glucose (C-6) — II

III : R=H
IV : R=OH

V

The *n*-butanol soluble layer separated from the methanol extract of ginseng callus was evaporated to give the crude saponins. The separation of saponins was carried out on a

1) Part XVII in the series "Studies in Plant Tissue Cultures." Part XVI: K. Syono and T. Furuya, *Plant & Cell Physiol.*, **13**, 843 (1972).
2) A part of this work was presented at the 91st Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, April 1971.
3) Location: *a*) *Minato-ku, Tokyo*; *b*) *Kawasaki, Kanagawa.*
4) T. Furuya, H. Kojima, K. Syono, and T. Ishii, *Chem. Pharm. Bull.* (Tokyo), **18**, 2371(1970).

column of Sephadex LH-20 using $CHCl_3$–MeOH (1: 2). Ginsenoside Rb_1 (I)[5] was isolated from fractions 16—17 and ginsenoside Rg_1 (II)[5a,b,6] mainly from fractions 21—23. The results of Sephadex column chromatography support Shibata's paper[6] and Shoji's report[5c] that the molecular size of I is larger than that of II. Further, the presence of I and II was confirmed by comparison of nuclear magnetic reasonance (NMR) and mass spectra of their acetates with those of the authentic samples. At the same time, the presence of β-sitosterol glucoside[7] was detected by thin-layer chromatography (TLC). From the results of gas-liquid chromatography (GLC) described below, β-sitosterol glucoside is probably the main component of phytosterol glucosides.

Furthermore, the hydrolysates of crude saponins were chromatographed on a silica gel column to isolate three colorless needles. These compounds were identical with panaxadiol (III),[8] panaxatriol (IV)[9] and oleanolic acid (V),[10] respectively. Phytosterols obtained simultaneously were also shown to be a mixture of a large amount of β-sitosterol[7,10] and a small amount of campesterol and stigmasterol.

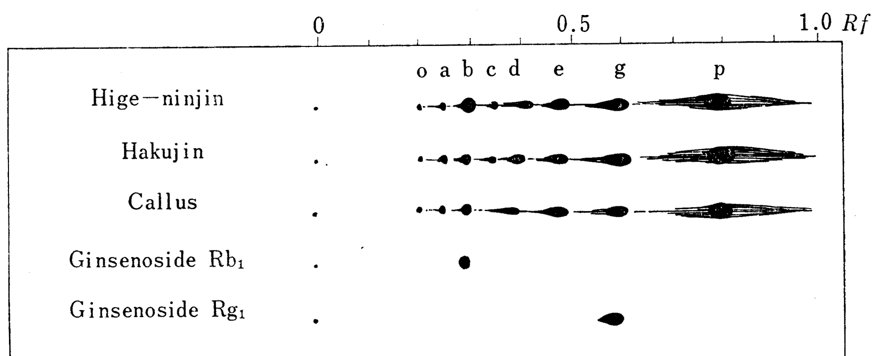

Fig. 1. Thin-Layer Chromatogram of Crude Saponins from the Ginseng Callus and The Ginseng Root

solvent: upper layer of BuOH–AcOH–H_2O (4:1:5)
o,a,b,c,d,e and g: ginsenosides Ro, Ra, Rb, Rc, Rd, Re and Rg
p: phytosterol glucosides

Though a comparatively large amount of IV was obtained in this experiment, the relative contents of III and IV seemed to vary with a change of culture period. Then, the quantitative determination of III and IV in the ginseng callus is now in progress. It is of great interest that the kind and amount of saponins in the callus are about the same as that in the ginseng root (see Fig. 1) and the pharmacological actions[11] of the water extract, the methanol extract and crude saponins of the callus are also almost in agreement with that of the roots.

5) *a*) S. Shibata, O. Tanaka, T. Ando, M. Sado, S. Tsushima, and T. Ohsawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 595(1966); *b*) S. Shibata, O. Tanaka, M. Nagai, T. Ando, Y. Ohmori, and Y. Iida, "Saponins and sapogenins of Panax ginseng and some related plant," The Government Press, Colombo, Ceylon, 1967, p. 1; *c*) S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, Abstract of Papers, 92nd Annual Meeting of Pharmaceutical Society of Japan, Osaka, April 1972, II, p. 248.
6) Y. Nagai, O. Tanaka, and S. Shibata, *Tetrahedron*, **27**, 881(1971).
7) M. Takahashi, K. Isoi, M. Yoshikura, and T. Osugi, *Yakugaku Zasshi*, **81**, 771(1961).
8) M. Fujita, H. Itokawa, and S. Shibata, *Yakugaku Zasshi*, **82**, 1634(1962); S. Shibata, M. Fujita, H. Itokawa, O. Tanaka, and T. Ishii, *Chem. Pharm. Bull.* (Tokyo), **11**, 759(1963).
9) S. Shibata, O. Tanaka, S. Soma, Y. Iida, T. Ando, and H. Nakamura, *Tetrahedron Letters*, **1965**, 207; G.B. Elyakov, A.K. Dzizenko, and Yu. N. Elkin, *ibid.*, **1966**, 141.
10) L. Hörhammer, H. Wagner, and B. Lay, *Pharmz. Ztg.*, **106**, 1307(1961).
11) K. Takagi, H. Saito, H. Nabata, and T. Murata, Abstracts of Papers, 92nd Annual Meeting of Pharmaceutical Society of Japan, Osaka, April 1972, III, p. 178.

Experimental

Melting points were determined on a Kofler block and are uncorrected. High resolution mass spectra were obtained on a JMS-OIS spectrometer with a direct inlet. The NMR spectra were run in a JNM-MH-100 spectrometer.

Tissue Culture of *P. ginseng*——The petiole of 2-year-old cultivated ginseng was transferred onto Murashige and Skoog's agar medium (minus glycine) containing 2,4-dichlorophenoxyacetic acid (1 ppm) in June, 1967. The callus has been subcultured on the same medium at about 25° in the dark and at 4—5 weeks intervals for about 3 years. The ginseng callus used for this experiment has showed the growth rate of 10—20 times in fresh weight per month.

Extraction and Separation of Crude Saponins——The callus harvested was homogenized with cold MeOH in a Waring blender, refluxed for 3 hr repeatedly and filtered. The combined filtrate was concentrated to a small volume *in vacuo*, which was treated with cold MeOH to remove insoluble substances. The MeOH soluble portion was again evaporated to dryness under vacuum. The residue was washed with ether. The ether insoluble portion was dissolved with H_2O and then extracted with *n*-BuOH. The BuOH layer was evaporated to give crude saponins.

Hakujin (white ginseng) and Hige-ninjin (lateral roots of white ginseng) were cut finely and extracted according to the same method describrd above to give crude saponins for TLC analyses.

Isolation of Saponins from Callus——1) Sephadex LH-20 (Pharmacia, 190.2 g) was allowed to swell in $CHCl_3$–MeOH (1: 2) and packed in the two columns (2.6×80 cm). The crude saponins (1.4 g) from the ginseng callus (208 g) were applied on the top of a column. Two columns were connected and eluted with $CHCl_3$–MeOH (1: 2) by two ways, that is, descending and ascending (flow rate *ca.* 0.1 ml/min and each fraction 7 ml). Fractions 16—17 were evaporated to give pale yellow powder, which gave *Rf* 0.34 [TLC, $CHCl_3$–MeOH–H_2O (65:35:10)] corresponding to ginsenoside Rb_1 (I) and fractions 21—23 gave pale yellow powder (111 mg), *Rf* 0.56 (II, main) and 0.60 (trace). 2) The crude saponins (17.6 g) obtained from the callus (fresh weight 25 kg) were chromatographed over silica gel eluted with $CHCl_3$–MeOH (9:1), (8: 2), then (6: 4). White powder (1.26 g) isolated from the fractions of $CHCl_3$–MeOH (8: 2) was identified with ginsenoside Rg_1 (II) by TLC developed with $CHCl_3$–MeOH–H_2O (65:35:10). Pale yellow powder isolated from fractions of $CHCl_3$–MeOH (6:4) mainly contained ginsenoside Rb_1 (I) by TLC. Further, it (0.37 g) was purified by silica gel column developed with $CHCl_3$–MeOH–H_2O (65: 35:10) to give pale yellow powder (40 mg) corresponding to I. The crude I (40 mg) and II (500 mg), respectively, were acetylated in a conventional way with Ac_2O in anhydrous pyridine at room temperature overnight. The NMR and mass spectra of the acetylated derivative of I (34 mg) were finely identified with the acetylation product of ginsenoside Rb_1 isolated from Hakujin. NMR $\delta_{TMS}^{CDCl_3}$: 0.82, 0.89, 1.00, 1.06, 1.29, C-C$\underline{H}_3$, 1.66, 1.74, C=C-C$\underline{H}_3$, 2.02, 2.06, 2.11, 2.14, O-CO-C$\underline{H}_3$. The acetylated derivative of II was recrystallized from $CHCl_3$–MeOH to give colorless leaflets (126 mg), mp 242.5—243° (lit,[6] mp 242—243°), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1752, 1227 (OAc) and no OH band, NMR $\delta_{TMS}^{CDCl_3}$: 0.96 (9H, s), 1.03 (3H, s), 1.05 (3H, s), 1.18 (3H, s) C-C$\underline{H}_3$, 1.59 (3H, s), 1.65 (3H, s), C=C-C$\underline{H}_3$, 1.97 (3H, s), 1.98 (6H, s), 2.00 (3H, s), 2,02 (9H, s), 2.04 (6H, s), 2.09 (3H, s) O-CO-C$\underline{H}_3$. Though its molecular peak could not record in mass spectrum, the fragmentation pattern was the same as that of an authentic sample. Therefore, each compound was shown to be acetylated derivatives of ginsenosides Rb_1 and Rg_1.

Isolation of Sapogenins from Callus——The crude saponins (2.8 g) obtained from the callus (fresh weight 500 g, dry weight 25 g) were hydrolyzed by refluxing with 5% H_2SO_4 in 50% aqueous EtOH. After working up in the usual way, the hydrolysates (0.8 g) were chromatographed on a silica gel column eluted (each 20 ml fraction) with C_6H_6 (fractions 1—5) and C_6H_6–AcOEt (2:1) (fractions 6—274).

To fractions 10—23 was added a small amount of acetone and the acetone insoluble portion was recrystallized from EtOH to give colorless needles (V, 1.5 mg), mp 293—294°, Mass Spectrum *m/e*: 456.358 (M^+) (*Anal.* Calcd. 456.360 for $C_{30}H_{52}O_3$), 248.180 (*Anal.* Calcd. 248.178 for $C_{16}H_{24}O_2$), 207.174 (*Anal.* Calcd. 207.174 for $C_{14}H_{23}O$), IR ν_{max}^{KBr} cm^{-1}: 3450—2850 (OH), 2950—2850, 1690 (COOH). V was identified with oleanolic acid in comparison with an authentic sample (mixed mp, mass and infrared (IR) spectra). The acetone soluble portion was found to be phytosterols by TLC and GLC.

To fractions 30—60 was also added a small amount of acetone and the acetone insoluble portion was recrystallized repeatedly from AcOEt to give colorless needles (III, 7 mg), mp 249—250°, Mass Spectrum *m/e*: 460.395 (M^+) (*Anal.* Calcd. 460.392 for $C_{30}H_{52}O_3$), 341.281 (*Anal.* Calcd. 341.284 for $C_{24}H_{37}O$), 127.113 (*Anal.* Calcd. 127.112 for $C_8H_{15}O$), IR ν_{max}^{KBr} cm^{-1}: 3420, 3250 (OH), 1120, 1066 (C–O–C). This compound was shown to be panaxadiol by comparison with an authentic sample (mixed mp, mass and IR spectra).

On recrystallization of fractions 90—170 from AcOEt, colorless needles (IV, 23 mg), mp 237—239° were obtained. Mass Spectrum *m/e*: 476.389 (M^+) (*Anal.* Calcd. 476.387 for $C_{30}H_{52}O_4$), 339.268 (*Anal.* Calcd. 339.269 for $C_{24}H_{35}O$), 127.113 (*Anal.* Calcd. 127.112 for $C_8H_{15}O$), IR ν_{max}^{KBr} cm^{-1}: 3665, 3365 (OH), 1123, 1068 (C–O–C). IV was corroborated to be panaxatriol in comparison with an authentic sample (mixed mp, mass and IR spectra).

TLC of Saponins and Sapogenins[12]——TLC was carried out on silica gel G developed with upper phase of *n*-BuOH–AcOH–H_2O (4:1:5) for saponins and C_6H_6–AcOEt (1:1) for sapogenins. The spots were detected by spraying 10% H_2SO_4 and 20% $SbCl_5$ in CCl_4. *Rf* values: ginsenoside Rb_1 (I) 0.30, ginsenoside Rg_1 (II) 0.59, β-sitosterol glucoside 0.80 and panaxatriol (IV) 0.17, panaxadiol (III) 0.29 g, oleanolic acid (V) 0.51, β-sitosterol 0.60.

GLC of Sapogenins——A Shimadzu Model GC-1C gas chromatograph equipped with a hydrogen flame inonization detector was used. A glass U-column (1.8 m×4 mm o.d.) containing 1.5% SE-30 on Gas-Chrom Q (80—100 mesh) was connected to gas chromatograph and operated under the following conditions. Temperatures of the injection port, detector block and column oven were 300°, 290° and 280°, respectively. The carrier gas was N_2, with a flow rate of 75.9 ml/min. Retention time (min): campesterol 4.5, stigmasterol 4.7, β-sitosterol 5.2, panaxadiol (III) 9.9, panaxatriol (IV) 14.9.

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12) T. Ando, O. Tanaka, and S. Shibata, *Syoyakugaku Zasshi*, **25**, 28 (1971).