of the bronchodilator activity of *l*-isoproterenol and had very weak effect on the heart rate. These results indicate that **4b** is a potent and highly selective β-adrenoceptor stimulant, and may be practically useful as a bronchodilator.

Experimental[10]

5-(2-Allylamino-1-oxopropyl)-8-hydroxycarbostyril (3b)——Allylamine (50 ml) was added to 50.0 g of crude 5-(2-bromo-1-oxopropyl)-8-hydroxycarbostyril with shaking. After 3 hr, the resulting solution was evaporated to dryness and the residue was dissolved in iso-PrOH. The solution was adjusted to pH 1—2 with concentrated hydrochloric acid and evaporated to dryness. The residual solid was collected, washed with iso-PrOH and dissolved in MeOH. The solution was made alkaline with KOH–MeOH solution, adjusted to pH 1 with concentrated hydrochloric acid under cooling in ice-water and evaporated to dryness. The residue was recrystallized from MeOH–acetone to give 7.60 g (22% from 8-hydroxycarbostyril) of **3b**.

5-(2-Allylamino-1-hydroxypropyl)-8-hydroxycarbostyril (4b)——A suspension of 1.20 g (0.0038 mol) of **3b** in 50 ml of MeOH was adjusted to pH 9, then 1 g of sodium borohydride was added in small portions with stirring and cooling in ice-water. After completion of reduction, the reaction mixture was adjusted to pH 1 and the precipitate was filtered off. The filtrate was evaporated to dryness. The residue was dissolved in MeOH and evaporated to dryness to remove boron as methyl borate. The residue was recrystallized from H_2O to give 0.60 g (47%) of **4b**. NMR (Me_2SO-d_6–D_2O) δ: 5.63 (1H, d, J=4.0 Hz, =C<u>H</u>–OH). Compound **4c**: NMR (D_2O) δ: 5.75 (1H, d, J=3.0 Hz, =C<u>H</u>–OH).

Acknowledgement We thank Dr. M. Umezato of the Laboratories of Biological Research, Otsuka Pharmaceutical Co. Ltd., for the pharmacological data.

10) Melting points (uncorrected) were determined by the capillary method. Elemental microanalyses were done in a Yanagimoto MT-2 CHN recorder. NMR spectra were recorded with a Hitachi R-20B spectrometer.

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Application of High-performance Liquid Chromatography to the Isolation of Ginsenoside-Rf, -Rg_2, and -Rh_1 from a Crude Saponin Mixture of Ginseng[1]

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(Received July 8, 1980)

The minor components of ginseng saponins, ginsenoside-Rf, -Rg_2, and -Rh_1, were isolated from the crude saponin fraction by a high-performance liquid chromatography (HPLC) procedure involving preparative HPLC on silica gel followed by semi-preparative HPLC on a column packing of Carbohydrate Analysis. This method was rapid and convenient.

Keywords——*Panax ginseng* C.A. Meyer; ginsenoside-Rf; ginsenoside-Rg_2; ginsenoside-Rh_1; high-performance liquid chromatography; isolation

In our previous paper, an improved method for the isolation of ginsenoside-Rb_1, -Rb_2, -Rc, -Rd, -Re, and -Rg_1 by high-performance liquid chromatography (HPLC) was reported.[1] Now, in order to improve the separation and efficiency of isolation of ginsenoside-Rf, -Rg_2,

1) T. Nagasawa, T. Yokozawa, Y. Nishino, and H. Oura, *Chem. Pharm. Bull.*, **28**, 2059 (1980). This work was presented at the 100th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April, 1980.
2) Location: *a) 2630 Sugitani, Toyama, 930-01, Japan*; *b) 112 Ini-Dong, Chongro-Ku, Seoul, Korea.*

and -Rh_1, which are minor components in ginseng saponins, an HPLC procedure for the isolation of these components has been developed.

Experimental

Apparatus and Column——Liquid chromatography was performed on ALC 201 and PrepLC/System-500 machines (Waters Associates, Inc., Milford, Mass., U.S.A.), using a refractometer (RI detector). A stainless steel column (30 cm × 3.9 mm) packed with Carbohydrate Analysis (Waters Associates, Inc.), a stainless steel column (30 cm × 7.8 mm) with the same packing, and a PrepPAK-500/Silica cartridge (30 cm × 5.7 cm) for the PrepLC/System-500 (Waters Associates, Inc.) were used.

Materials——Ginsenoside standards used were provided by Professor Osamu Tanaka, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, and by Professor Junzo Shoji, School of Pharmaceutical Sciences, Showa University.

The preparation of the crude ginseng saponin fraction for the isolation of ginsenoside-Rf and -Rg_2 was described in a previous report.[1)]

The crude ginseng saponin fraction for the isolation of ginsenoside-Rh_1 was prepared as noted in Chart 1. The sample of 70% ethanol extract was provided by the Korea Ginseng Research Institue.

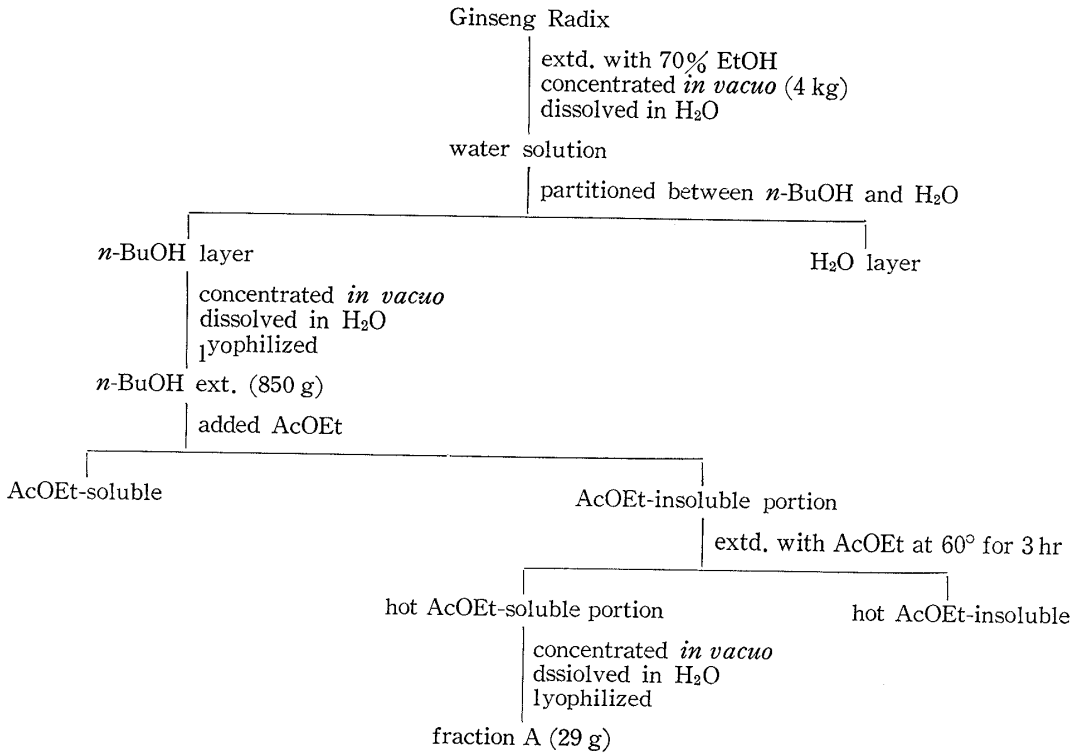

Chart 1. Extraction and Fractionation of Crude Ginseng Saponins for the Isolation of Ginsenoside-Rh_1

Identification Procedure——Each of the ginsenosides and crude ginseng saponins was dissolved in MeOH to *ca.* 10—20 mg/ml. All the samples or standard solutions were filtered through a TM-2P membrane filter (Toyo Roshi Co., Tokyo; pore size, 0.45 μm) before injection.

Fractionation Procedure——The fractionation procedure for ginsenoside-Rf and -Rg_2 was previously reported.[1)] In the fractionation procedure for ginsenoside-Rh_1, fraction A was dissolved in a carrier solvent (BuOH: AcOEt: H_2O=4: 1: 2, v/v, upper phase). The concentration of the solution was *ca.* 240 mg/ml.

Isolation Procedure——Each fractionated ginseng saponin was dissolved in a mixture of the carrier solvent and a little methanol. Sample solutions were filtered through a TM-2P membrane filter before injection.

HPLC Conditions for Identification——Liquid chromatography was performed on the ALC 201 machine. A stainless steel column (30 cm × 3.9 mm) packed with Carbohydrate Analysis was used. The mobile phase was a mixture of acetonitrile (AcCN) and water (H_2O) (86: 14 or 92: 8, v/v). The RI detector was used to determine each ginsenoside. The RI attenuation was set at 8×, and the flow rate was 2 ml/min.

HPLC Conditions for Fractionation——Liquid chromatography was carried out with the PrepLC/System-500. Two PrepPAK-500/Silica cartridges were used. The mobile phase was a mixture of BuOH: AcOEt:

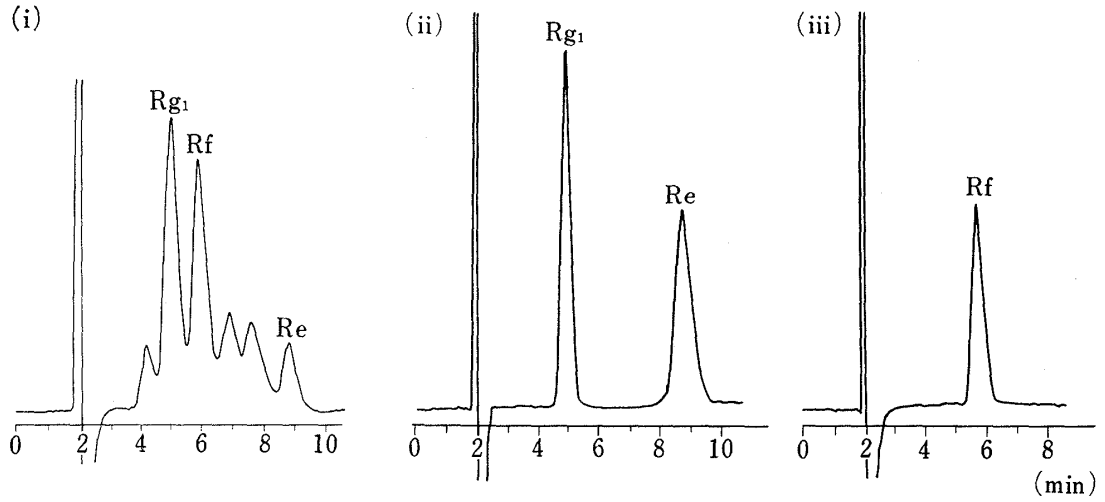

Fig. 1. Chromatograms of Fraction I-6,[1] Ginsenoside–Rg_1, Ginsenoside–Rf, and Ginsenoside–Re

Conditions: column, 30 cm × 3.9 mm; packing, carbohydrate analysis; mobile phase, AcCN: H_2O=86: 14 (v/v); flow rate, 2 ml/min; RI detector, attenuation, 16×.
(i); fraction I-6.
(ii); ginsenside-Rg_1 and ginsenoside-Re.
(iii); authentic sample and isolated ginsenoside-Rf.

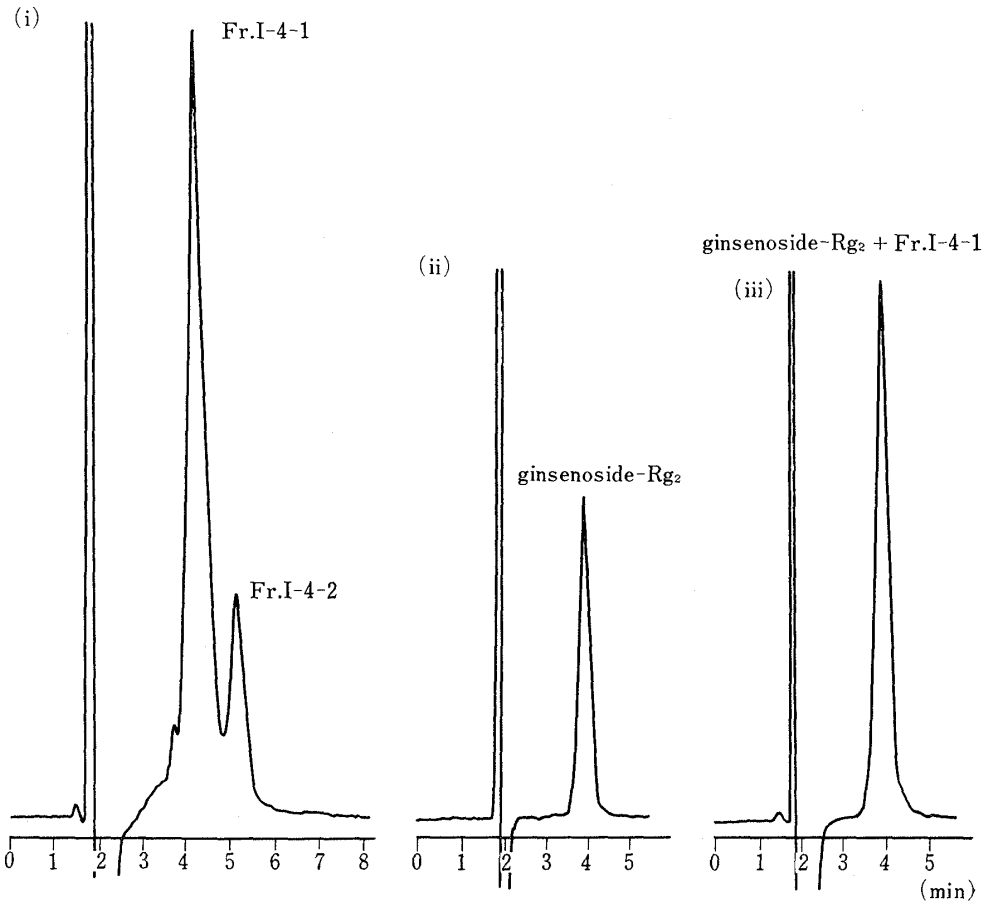

Fig. 2. Elution Profile of Fraction I-4[1] and Chromatogram of Ginsenoside-Rg_2 and Isolated Ginsenoside–Rg_2 (Fr. I-4-1)

Conditions: column, 30 cm × 3.9 mm; packing, carbohydrate analysis; mobile phase, AcCN: H_2O=86: 14 (v/v); flow rate, 2 ml/min; RI detector, attenuation, 16×.
(i); fraction I-4.
(ii); authentic sample.
(iii); authentic sample and isolated ginsenoside-Rg_2 (Fr. I-4-1).

H_2O=4: 1: 5 or 4: 1: 2 (v/v, upper phase). The flow rate was 50 ml/min.

HPLC Conditions for Isolation——Liquid chromatography was performed on the ALC 201 machine. A stainless steel column (30 cm×7.8 mm or 30 cm×3.9 mm) packed with Carbohydrate Analysis was used. The mobile phase is specified in "Results and Discussion."

Results and Discussion

Isolation of Ginsenoside-Rf by Semi-preparative HPLC

As reported previously, crude ginseng saponins were divided into 10 fractions that each contained 2 or 3 ginsenosides by preparative HPLC, and fraction I was further separated into seven subfractions (Fr. I-1—Fr. I-7) by semi-preparative HPLC.[1] Each of the subfractions was subjected to analytical HPLC with a mixture of AcCN and water (86; 14, v/v) as a mobile phase.

As shown in Fig. 1, ginsenoside-Rf was contained in fraction I-6. It was isolated from Fr. I-6 by semi-preparative HPLC, which was carried out by repeated fractionation on a semi-preparative column (30 cm×7.8 mm) with a mixture of AcCN: H_2O=86: 14 (v/v) as a mobile phase at the flow rate of 8 ml/min. No impurities in the isolated ginsenoside-Rf were detected by analytical HPLC.

Isolation of Ginsenoside-Rg_2 by Anaiytical HPLC

Ginsenoside-Rg_2 was isolated from Fr. I-4[1] by analytical HPLC. Fig. 2 shows the elution profile when Fr. I-4 was chromatographed on an analytical column (30 cm×3.9 mm) with a mixture of AcCN: H_2O=86: 14 (v/v) as a mobile phase at the flow rate of 2 ml/min.

As shown in Fig. 2, Fr. I-4 was further separated into 2 fractions. In cochromatography with an authentic sample, Fr. I-4-1 was identified as ginsenoside-Rg_2. No impurities were detected in the isolated ginsenoside-Rg_2 by analytical HPLC.

Fractionation of Fraction A for the Isolation of Ginsenoside-Rh_1 by Preparative HPLC

Figure 3 shows the elution profile of fraction A (prepared according to Chart 1) upon analytical HPLC. Fraction A was used as the crude saponin fraction for the isolation of

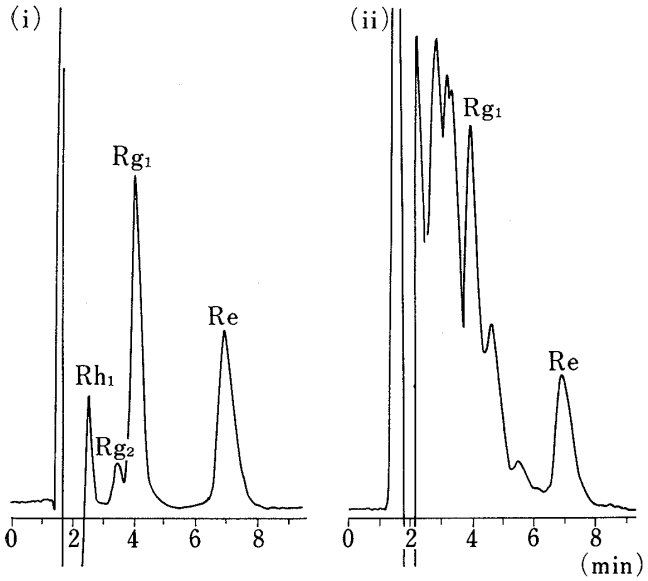

Fig. 3. Chromatogram of Ginsenoside-Rh_1, -Rg_2, -Rg_1, and -Re and Elution Profile of Fraction A

Conditions: column, 30 cm×3.9 mm, packing, carbohydrate analysis; mobile phase, AcCN: H_2O=86: 14 (v/v); flow rate, 2 ml /min; RI detector, attenuation, 16×.
(i); authentic samples.
(ii); fraction A.

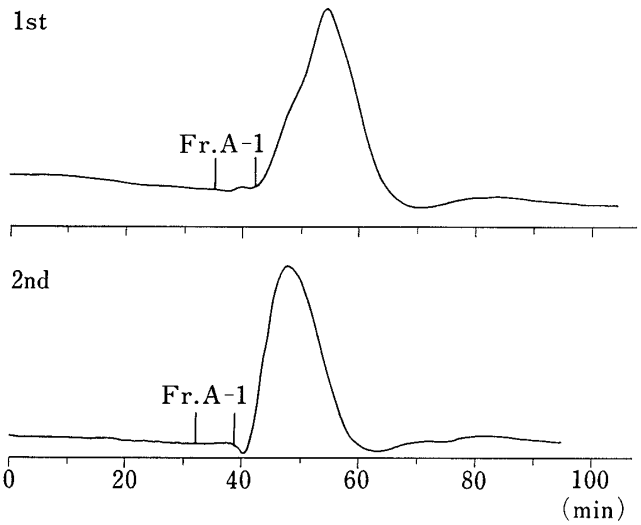

Fig. 4. Elution Profiles of Fraction A on Preparative HPLC

Conditions: instrument, PrepLC/System-500; column, PrepPAK-500/Silica cartrige×2; mobile phase, BuOH: AcOEt: H_2O=4: 1: 2 (v/v, upper phase); flow rate, 50 ml/min, RI detector (setting 2); sample load, 14 g/60 ml/injection (28 g in total).

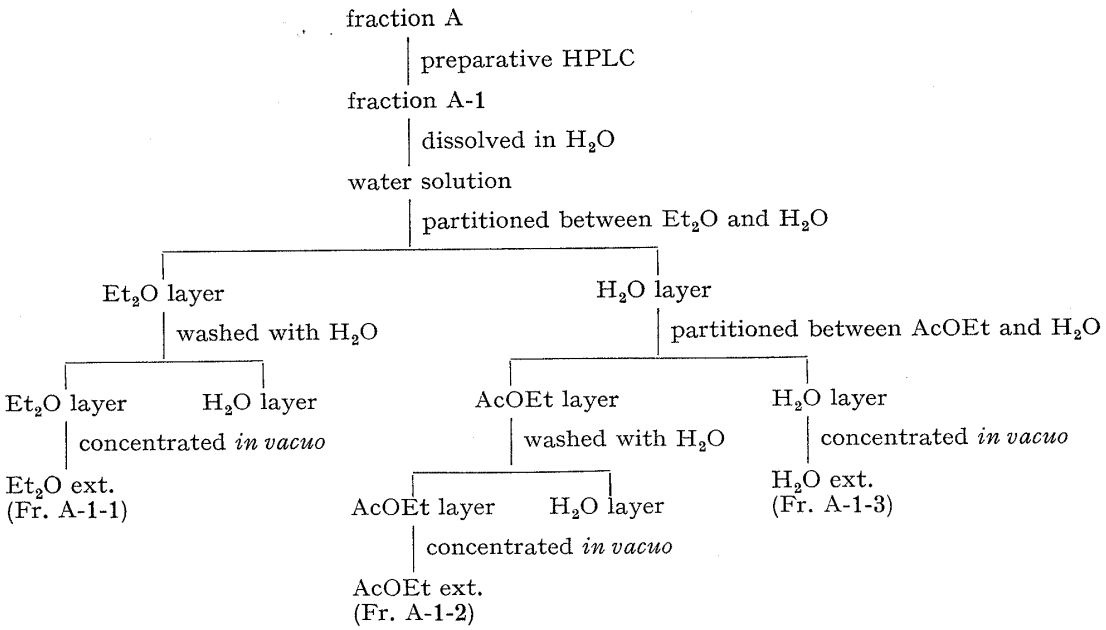

Chart 2. Further Fraction of Fraction A-1

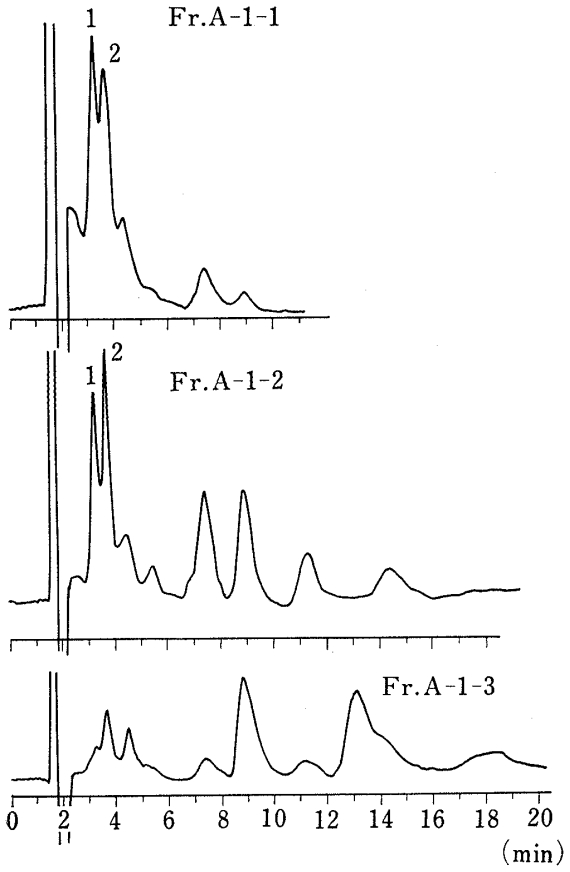

Fig. 5. Elution Profies of Fractions A-1-1, A-1-2, and A-1-3

Conditions: column, 30 cm×3.9 mm; packing, carbohydrate analysis; mobile phase, AcCN: H_2O=92: 8 (v/v); flow rate, 2 ml/min; RI detector, attenuation, 16×. peak 2; ginsenoside-Rh_1

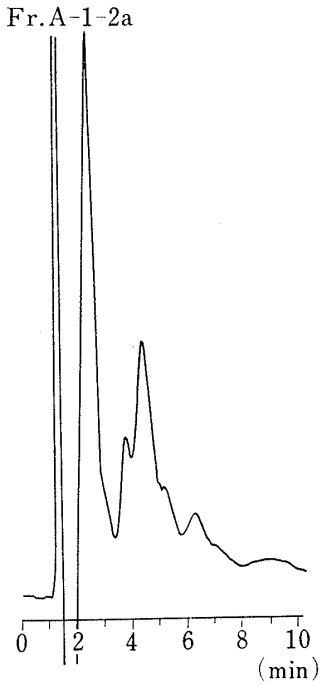

Fig. 6. Elution Profile of Fraction A-1-2 on Semi-preparative HPLC

Conditions: column, 30 cm×7.8 mm; packing, carbohydrate analysis; mobile phase, AcCN: H_2O =89: 11 (v/v); flow rate, 8 ml/min; RI detector, attenuation, 16×.

ginsenoside-Rh_1. An improved isolation procedure for ginsenoside-Rh_1 from fraction A by HPLC was developed.

Preparative HPLC employing a mixture of BuOH: AcOEt: H_2O=4: 1: 2 (v/v, upper phase) was carried out twice successively for 4 hr with repeated used of the cartridges, applying 28 g of fraction A in total. Fig. 4 shows the elution profiles. Deactivation of silica packed in the cartridges by water in the carrier solvent clearly affected the resolution and retention of saponins. Furthermore, the elution times of saponins were delayed compared with those obtained in a previously reported preparative HPLC procedure.[1] In each HPLC run, fraction A was fractionated, and then each Fr. A-1 (noted in Fig. 4) which contained ginsenoside-Rh_1 as determined by analytical HPLC, was pooled and concentrated *in vacuo*.

Fraction A-1 obtained by preparative HPLC procedure was further fractionated by partition between ether, ethyl acetate and water according to Chart 2. Fig. 5 shows the elution profiles of fractional A-1-1, A-1-2, and A-1-3. Peak 2 (illustrated in Fig. 5) was identified as ginsenoside-Rh_1 on the basis of the retention time and cochromatography with an authentic sample.

Isolation of Ginsenoside-Rh_1 by Semi-preparative HPLC

In analytical HPLC of fractions A-1-1, A-1-2, and A-1-3, it was observed that ginsenoside-Rh_1 was contained in Fr. A-1-2 and Fr. A-1-1. Thus, for the isolation of ginsenoside-Rh_1, fraction A-1-2 was further subjected to repeated fractionation by semi-preparative HPLC, eluting with a mixture of AcCN: H_2O=89: 11 (v/v) at a flow rate of 8 ml/min. The elution profile is shown in Fig. 6. The fraction corresponding to ginsenoside-Rh_1 (Fr. A-1-2a in Fig. 6) from semi-preparative HPLC was concentrated *in vacuo*. In this procedure, ginsenoside-Rh_1 was enriched in fraction A-1-2a. Accordingly, ginsenoside-Rh_1 was isolated from fraction A-1-2a by semi-preparative HPLC by repeated fractionation with a mixture of AcCN:

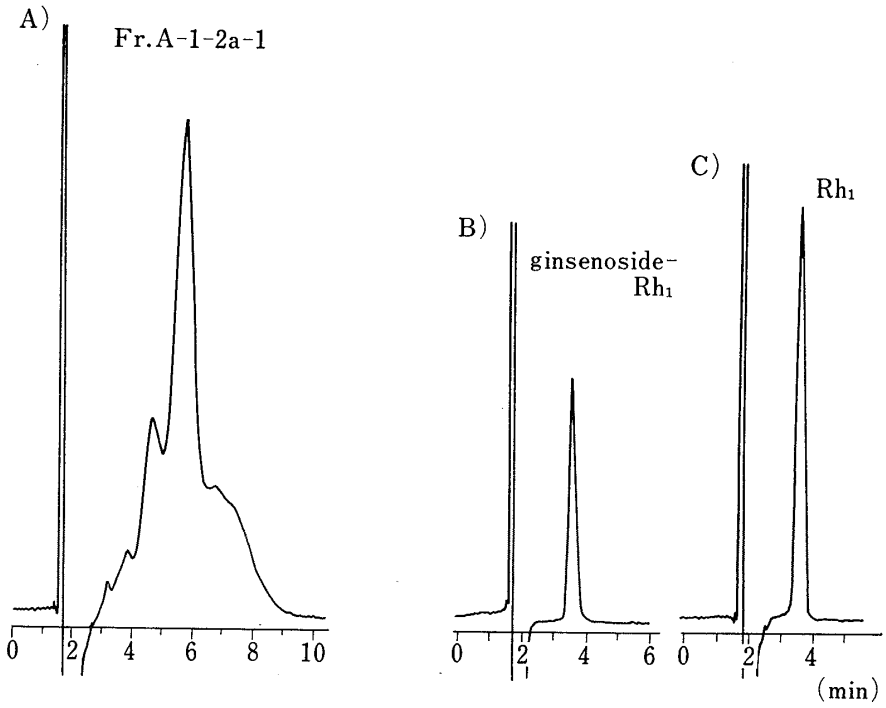

Fig. 7. A) Elution Profile of Fraction A-1-2a on Semi-preparative HPLC. B, C) Chromatograms of Ginsenoside–Rh_1 and Fraction A-1-2a-1 (Isolated Ginesenoside–Rh_1) on Analytical HPLC

Conditions: A) column, 30 cm×7.8 mm; packing, Carbohydrate Analysis; mobile phase, AcCN: H_2O=94: 6 (v/v); flow rate, 8 ml/min; RI detector, attenuation 16×, B, C) column, 30 cm×3.9 mm; packing, Carbohydrate Analysis; mobile phase, AcCN: H_2O=90: 10 (v/v); flow rate, 2 ml/min; RI detector, attenuation 8x.
A) fraction A-1-2a.
B) authentic sample.
C) Isolated ginsenoside-Rh_1 (Fr. A-1-2a-1) and authentic sample.

H_2O=94: 6 (v/v) at the flow rate of 8 ml/min. The elution profile is shown in Fig. 7. As illustrated in Fig. 7, Fr. A-1-2a-1 was identified as ginsenoside-Rh_1 by cochromatography with an authentic sample. No impurities were detected by analytical HPLC.

Acknowledgement The authors are grateful to Professor Osamu Tanaka, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, and to Professor Junzo Shoji, School of Pharmaceutical Sciences, Showa University, for their kind gifts of the ginsenosides used as standards, and to Dr. Hyo Won Bae, Korea Ginseng Research Institute, for his kind gift of the 70% ethanol extract from ginseng radix used in this work.

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Studies on Sulfenanilides. V.[1] Anodic Oxidation of 4′-Substituted 2-Nitrobenzenesulfenanilides at a Reticulated Vitreous Carbon Electrode

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Constant current electrolysis of 4′-substituted 2-nitrobenzenesulfenanilides (4′-OMe (**1a**), 4′-Me (**1b**), 4′-Cl (**1c**), 4′-COOEt (**1d**)) was carried out in acetonitrile containing 0.1 M ethyltributylammonium trifluoromethanesulfonate (ETBT), 1% trifluoroacetic acid, and 1% trifluoroacetic anhydride at a reticulated vitreous carbon (RVC) electrode. The quantity of electricity to be fed into the electrolytic cell was determined from the anodic potential-time curves. The yields of 2,7-disubstituted phenazines (di-OMe (**2a**), di-Me (**2b**), di-Cl (**2c**), di-COOEt (**2d**)) were 56%, 24%, 42%, and 33%, respectively. The RVC anode was found to be useful for preparative electrolysis of **1a**—**d**, since the considerable yields of phenazines were obtained within several minutes without the use of an expensive potentiostat.

Keywords——anodic oxidation; 2-nitrobenzenesulfenanilides; 2,7-disubstituted phenazines; constant current electrolysis; reticulated vitreous carbon; oxidation of sulfenamides; RVC electrode

Reticulated vitreous carbon (RVC) is a glassy carbon with a porous structure having a free void volume of about 97% and a surface area of about 65 cm^2/cm^3.[3] A relatively small piece of it can provide an electrode with a substantial surface area for its low electrical resistance and physically continuous structure. So far, RVC has been used as an optically transparent electrode,[4] a rotated porous carbon disk electrode,[5] and a flow-through electrode in voltammetric analysis.[6] An RVC electrode should be very useful for preparative electrolysis because of the high ratio of the porous electrode surface to solution volume, wide operating voltage range, chemical inertness, high electrical conductivity, and relatively reproducible performance of RVC. Therefore, we have attempted to utilize RVC for the anodic oxidation of 4′-substituted 2-nitrobenzenesulfenanilides (4′-OMe (**1a**), 4′-Me (**1b**), 4′-Cl (**1c**), 4′-COOEt

1) Part IV: H. Sayo, K. Mori, and T. Michida, *Chem. Pharm. Bull.*, **27**, 2316 (1979).
2) Location: *Ikawadani-cho, Tarumi-ku, Kobe 673, Japan.*
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