[Chem. Pharm. Bull. 24(12)3212—3213(1976)]

UDC 547.918.02.05 : 581.192

Saponins of Buds and Flowers of *Panax ginseng* C.A. Meyer. (1). Isolation of Ginsenosides-Rd, -Re, and -Rg_1

Shoji Yahara,[1a] Kiyoko Matsuura, Ryoji Kasai, and Osamu Tanaka[1b]

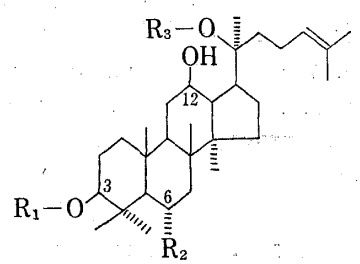
Faculty of Pharmaceutical Sciences, Kyushu University[1a] and Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine[1b]

(Received April 19, 1976)

From the dried mixture of buds and flowers of *Panax ginseng* C.A. Meyer, there were isolated and identified ginsenosides-Rd(I), -Re(II), and -Rg_1(III), all of which were already isolated from Ginseng roots. The high yield (*ca.* 2.5%) of II from the buds and the flowers is significant with respect to the source of this saponin and its aglycone, 20 (*S*)-protopanaxatriol.

In continuing the studies on active principles of Ginseng, the study on dammarane-type saponins of leaves of *Panax ginseng* C.A. Meyer was reported previously.[2] The present paper deals with the chemical investigation of buds and flowers of this medicinal plant, reporting the isolation and the identification of ginsenosides-Rd(I),[3] -Re(II),[4] and -Rg_1(III)[5] all of which have already been isolated from Ginseng roots.

Thin-layer chromatography (TLC) of the methanolic extract of the mixture of dried buds and flowers demonstrated the presence of numerous saponins. When an aqueous suspension of the methanolic extract was shaken with ether, a crystalline compound was deposited from the aqueous layer in a yield of *ca.* 2.5%, which was identified as ginsenoside-Re(II).[4] The isolation of II in such a high yield without chromatography indicates the significance of the buds and the flowers as important sources of II and its sapogenin, 20(*S*)-protopanaxatriol.[5,6] The aqueous layer was then extracted with *n*-butanol and after evaporation, the butanolic extract was subjected to dialysis. The dialyzed fraction was chromatographed to afford II and III. The non-dialyzed fraction was separated by droplet counter current chromatography (DCCC) followed by column chromatography giving I and several new saponins, named ginsenosides-Mx. The respective identification of known saponins, I, II, and III was furnished by comparisons of TLC, ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR,[7] and mass spectra of their trimethylsilyl ethers[8] with those of authentic roots saponins and by the results of the enzymatic hydrolysis.[6]



I: R_1=β-glucopyranose---β-glucopyranose (2 1)
R_2=H
R_3=β-glucopyranose

II: R_1=H
R_2=O-β-glucopyranose---α-rhamnopyranose (2 1)
R_3=β-glucopyranose

III: R_1=H
R_2=O-β-glucopyranose
R_3=β-glucopyranose

Chart 1

1) Location: *a*) *Maedashi, Higashi-ku, Fukuoka-shi*; *b*) *Kasumi, Hiroshima-shi.* Correspondance should be addressed to O. Tanaka.
2) S. Yahara, O. Tanaka, and T. Komori, *Chem. Pharm. Bull.* (Tokyo), **24**, 2204 (1976).
3) S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **22**, 421 (1974).
4) S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **22**, 2407 (1974).
5) Y. Nagai, O. Tanaka, and S. Shibata, *Tetrahedron*, **27**, 881 (1971).
6) H. Kohda and O. Tanaka, *Yakugaku Zasshi*, **95**, 246 (1975).
7) R. Kasai, J. Asakawa, and O. Tanaka, *Tetrahedron*, accepted.
8) R. Kasai, K. Matsuura, O. Tanaka, S. Sanada and T. Shoji, to be published.

TLC of the methanolic extract of fruits of this plant showed the similar pattern suggesting the presence of the same type of dammarane-saponins in the fruits as those of the buds and the flowers. The structural elucidation of new saponins of the buds and the flowers are in progress.

Experimental

NMR spectra were taken on JEOL PS-100 FT spectrometer in C_5D_5N; ^1H NMR at 100 MHz and ^{13}C NMR at 25 MHz. Mass spectra were determined on JEOL 01 SG2 spectrometer at 75 eV.

Enzymatic Hydrolysis and Identification of the Resulted Aglycone and Monosaccharides——By the procedure reported in the previous papers.[2,6]

TLC of the Saponins——On silica gel. Solvent $CHCl_3$: MeOH: H_2O (13: 8: 2, homogeneous). Detection H_2SO_4.

Trimethylsilylation for Mass Spectra-Determination[8]——A solution of a small amount of a sample (1—3 mg) in trimethylsilylimidazole (*ca.* three drops) in a stoppered micro-tube was heated at 90° for 1 hr. After dilution with a few drops of H_2O, the reaction mixture was extracted with *n*-C_6H_{14}. After washing with H_2O several times, *n*-C_6H_{14} layer was concentrated to dryness and subjected to determination of mass spectrum.

Extraction and Separation of Saponins——The dried mixture of the buds and the flowers (700 g) (collected at Daikon-Jima Ginseng farm, Shimane-Ken at the end of May) was extracted with hot MeOH and the MeOH solution was concentrated to dryness. A suspension of the MeOH-extract in H_2O (*ca.* 500 ml) was washed with ether several times and the aqueous layer was allowed to stand at room temperature to deposit a crystalline saponin (yield 2.5%), which was recrystallized from aqueous MeOH to give colorless needles, mp 170—171°, $[\alpha]_D^{24}$ +0.5° (c=0.2, MeOH) being proved to be identical with ginsenoside-Re(II).

After separating crystals of II, the aqueous solution was extracted with *n*-BuOH and the BuOH-layer was concentrated to dryness. An aqueous solution of the residue was subjected to dialysis against H_2O. The non-dialyzed fraction was separated by DCCC(solvent system $CHCl_3$: MeOH: H_2O: *n*-PrOH (5: 6: 4: 1), stationary phase: lower layer and moving phase: upper layer) followed by column chromatography on silica gel (elution with $CHCl_3$: MeOH: H_2O (300: 110: 17 homogeneous)) to furnish isolation of several saponins, one of which, white powder, $[\alpha]_D^{24}$ +17.8° (c=0.19, MeOH) (yield 0.5%) was proved to be identical with ginsenoside-Rd(I).

The dialyzed fraction was chromatographed on polyamide (elution with H_2O) to remove free sugars and phenolic glycosides etc. and the saponin-fraction was further chromatographed on silica gel. Elution with $CHCl_3$: MeOH: H_2O (300: 110: 17 homogeneous) afforded two saponins, white powder, $[\alpha]_D^{24}$ +19.5° (c=0.27, MeOH) and colorless needles from aqueous MeOH, mp 170—171°, $[\alpha]_D^{24}$ +0.5° (c=0.2, MeOH). The former (yield 0.3%) was proved to be identical with ginsenoside-Rg_1(III) and the latter (yield 0.3%) was found to be identical with II.

Acknowledgement The authors are grateful to Mr. Y. Ikeda, Matsue-Shi, Shimane-Ken for his kind donation of the buds and the flowers of *Panax ginseng* cultivated at Daikon-Jima. Thanks are also due to the financial support by a Grant-in-Aid for scientific research from the Ministry of Education, Science and Culture. They are indebted to Prof. I. Nishioka, Kyushu University for his encouragement.