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# Preparation of 20(*R*)- and 20(*S*)-Ginsenoside $Rh_1$ from Ginsenoside Re

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**Abstract**□The mild hydrolysis of ginsenoside Re with 50% acetic acid gave a prosapogenin mixture, 20(*R*)- and 20(*S*)-ginsenoside $Rg_2$. The products were acetylated to give the peracetates, which were further converted into 20(*R*)- and 20(*S*)-ginsenoside $Rh_1$ by the alcoholic alkaline treatment.

**Keywords**□*Panax ginseng*, 20(*R*)-ginsenoside $Rh_1$, 20(*S*)-ginsenoside $Rh_1$, ginsenoside Re, prosapogenin.

## Introduction

Ginseng saponins isolated from the root of *Panax ginseng* C.A. Meyer have been for long regarded as important principles manifesting the pharmacological and biological activities. Most of these studies on ginsenosides were conducted by using the major components, that is, ginsenosides $Rb_1$, $Rb_2$, Rc, Rd and $Rg_1$ but little is known about the minor components, ginsenosides $Rg_2$, $Rg_3$, $Rh_1$ and $Rh_2$ which are produced during the process of the preparation of red ginseng (steam processing).[1,2] Recently some researchers have tried to investigate on the chemistry and pharmacological actions of these minor components. It was found by Kitagawa *et al.*[3] that ginsenoside $Rh_2$ exhibited cytotoxic activities on 3LL (Lewis lung cancer cell), $MH_1C_1$ (Morris hepatoma cell), B16 (Melanoma cell) and HeLa cell. Matsuda *et al.*[4] also reported that 20(*R*)- and 20(*S*)-ginsenoside $Rg_3$ inhibited collagen and ADP-induced platelet aggregation, and that 20(*S*)-ginsenoside $Rg_3$, 20(*R*)- and 20(*S*)-ginsenoside $Rh_1$ inhibited the conversion from fibrinogen to fibrin induced by the thrombin. Ota *et al.*[5] reported ginsenosides $Rh_1$ and $Rh_2$ affected the growth of B16 melanoma cells, the expression of their melanotic phenotype and the control of phenotypic expression in different ways.

Korean red ginseng is manufactured by steam heating the six-year-old fresh ginseng root. Therefore, it seems likely that various chemical transformation such as epimerization and hydroxylation took place during the processing. Especially, partial hydrolysis of the crude ginseng saponins afforded prosapogenins named 20(*R* & *S*)-ginsenosides $Rg_2$, $Rg_3$, $Rh_1$ and $Rh_2$.[1,2,6] The present report deals with the preparation of 20(*R*)-ginsenoside $Rh_1$ and its epimer from ginsenoside Re.

## Experiment

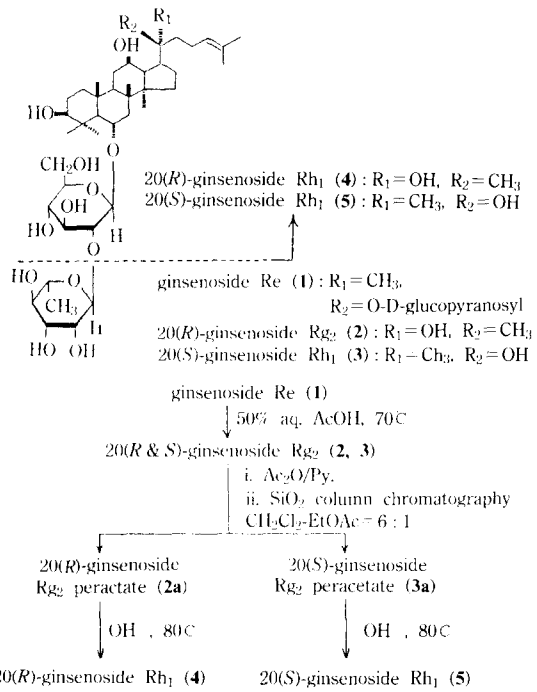
NMR spectra were obtained on a BRUKER Model AC 300 F in $C_5D_5N$ using TMS as an internal standard ($^1$H-NMR at 300 MHz and $^{13}$C-NMR at 75 MHz).

**Preparation of 2 and 3**

A solution of **1** (3g) in 50% acetic acid (100 m*l*) was heated at 70℃ for 2 hrs. The reaction mixture was diluted with water (100 m*l*) and extracted three times with n-butanol. After evaporation of the solvent the residue was chromatographed over silica gel column using $CHCl_3$ : MeOH : $H_2O$ (10 : 3 : 1) as eluent to provide the mixture of prosapogenins **2** and **3** (1.22g) which was identified by comparison with the authentic samples.

**Preparation of 2 and 3 peracetates, 2a and 3a**

A mixture of **2** and **3** (1g) was acetylated with acetic anhydride-pyridine (1 : 1, 50 m*l*) at room temperature for 20 hrs. The reaction mixture was extracted three times with ethylacetate and washed with 5% aq. HCl, satd. aq. $NaHCO_3$ and saline suc-




**Scheme 1.** Degradation of ginsenoside Re.

cessively, and then dried over magnesium sulphate anhydrous. The obtained solution was evaporated and chromatographed over silica gel column using $CH_2Cl_2$ : EtOAc (6 : 1) as eluent to give **2a** (420 mg) and **3a** (370 mg), respectively.

**Preparation of 4 and 5**

**2a** and **3a** (each 0.2g) were deacetylated with 5% NaOH-n-butanol (40 ml) at 80℃ for 6 hrs to cleave the glycosidic bond, respectively. After the reaction mixture was washed with water and evaporated, the residue was purified by a combination of silica gel column chromatogrphy using $CHCl_3$ : MeOH : $H_2O$ (10 : 3 : 1) as an eluent and semi-preparative HPLC column ($CH_3CN$ : $H_2O$=90 : 10, Altech-$NH_2$ 1×30 cm, Detector RI) to give **4** (37 mg) and **5** (33 mg).

## Results and Discussion

20(*R*)- and 20(*S*)-ginsenoside $Rh_1$ were obtained by chemical transformation of 20(*R* & *S*) ginsenoside $Rg_2$ formed by mild acidic hydrolysis from ginsenoside Re as shown in Scheme 1.

It is well known that 20-O-glycosyl moiety of ginsenoside is readily hydrolyzed and epimerized by treatment with aqueous acid.[7,8] **1** was treated with 50% aq. acetic acid at 70℃ for 2 hrs, and n-butanol layer of the reaction mixtures was chromatographed on silica gel column to yield **2** and **3**, which were identified by direct comparison of TLC with authentic samples. A mixture of **2** and **3** was acetylated

**Table 1.** $^{13}$C-NMR chemical shift of **4**, **5**, 20(*R*)-$Rh_1$ and 20(*S*)-$Rh_1$ (75 MHz, $d_5$-Py., δc)

| Compound | 20(*R*)-$Rh_1$[1] | **4** | 20(*S*)-$Rh_1$[8] | **5** |
|---|---|---|---|---|
| Aglycon | | | | |
| C- 1 | 39.6 | 39.4 | 39.4 | 39.4 |
| C- 2 | 27.8 | 27.7 | 27.9 | 27.2 |
| C- 3 | 78.5 | 78.6 | 78.6 | 78.6 |
| C- 4 | 40.2 | 40.3 | 40.3 | 40.3 |
| C- 5 | 61.3 | 61.4 | 61.4 | 61.4 |
| C- 6 | 77.9 | 78.1 | 78.0 | 78.0 |
| C- 7 | 45.0 | 45.1 | 45.2 | 45.2 |
| C- 8 | 41.0 | 41.0 | 41.1 | 41.1 |
| C- 9 | 50.1 | 50.1 | 50.2 | 50.2 |
| C-10 | 39.6 | 39.6 | 39.6 | 39.5 |
| C-11 | 32.0[a] | 32.1[a] | 32.0[a] | 32.0[a] |
| C-12 | 70.8 | 70.9 | 71.0 | 71.0 |
| C-13 | 48.7 | 48.7 | 48.2 | 48.2 |
| C-14 | 51.6 | 51.7 | 51.6 | 51.6 |
| C-15 | 31.6[a] | 31.3[a] | 31.1[a] | 31.2[a] |
| C-16 | 26.6 | 26.6 | 27.2 | 27.0 |
| C-17 | 50.4 | 50.5 | 54.7 | 54.7 |
| C-18 | 17.3[b] | 17.3[b] | 17.4[b] | 17.4[b] |
| C-19 | 17.6[b] | 17.6[b] | 17.6[b] | 17.5[b] |
| C-20 | 73.0 | 73.0 | 73.0 | 73.0 |
| C-21 | 22.6 | 22.5 | 26.8 | 26.8 |
| C-22 | 43.1 | 43.2 | 35.8 | 35.8 |
| C-23 | 22.6 | 22.7 | 23.0 | 23.0 |
| C-24 | 125.9 | 126.0 | 126.3 | 126.3 |
| C-25 | 130.7 | 130.7 | 130.6 | 130.7 |
| C-26 | 25.8 | 25.8 | 25.8 | 25.8 |
| C-27 | 17.6 | 17.7 | 17.6 | 17.7 |
| C-28 | 31.6 | 31.7 | 31.7 | 31.5 |
| C-29 | 16.3[b] | 16.3[b] | 16.4[b] | 16.4[b] |
| C-30 | 17.0[b] | 17.0[b] | 16.8[b] | 16.8[b] |
| β-D-gluco-pyranosyl | | | | |
| C-1 | 105.7 | 105.9 | 105.9 | 106.0 |
| C-2 | 75.3 | 75.4 | 75.4 | 75.4 |
| C-3 | 80.0[c] | 80.0[c] | 80.0[c] | 80.0[c] |
| C-4 | 71.7 | 71.7 | 71.8 | 71.9 |
| C-5 | 79.5[c] | 79.6[c] | 79.5[c] | 79.6[c] |
| C-6 | 62.9 | 63.0 | 63.1 | 63.1 |

[a],[b],[c] Assignments may be interchangeable within the same vertical column.

δ of methyl groups in **4** and **5**

| | **4** | **5** | difference |
|---|---|---|---|
| 1 | 0.86 | 0.79 | 0.07 |
| 2 | 1.05 | 1.00 | 0.05 |
| 3 | 1.22 | 1.16 | 0.06 |
| 4 | 1.37 | 1.37 | 0.00 |
| 5 | 1.58 | 1.58 | 0.00 |
| 6 | 1.62 | 1.61 | 0.01 |
| 7 | 1.65 | 1.64 | 0.01 |
| 8 | 2.11 | 2.11 | 0.00 |

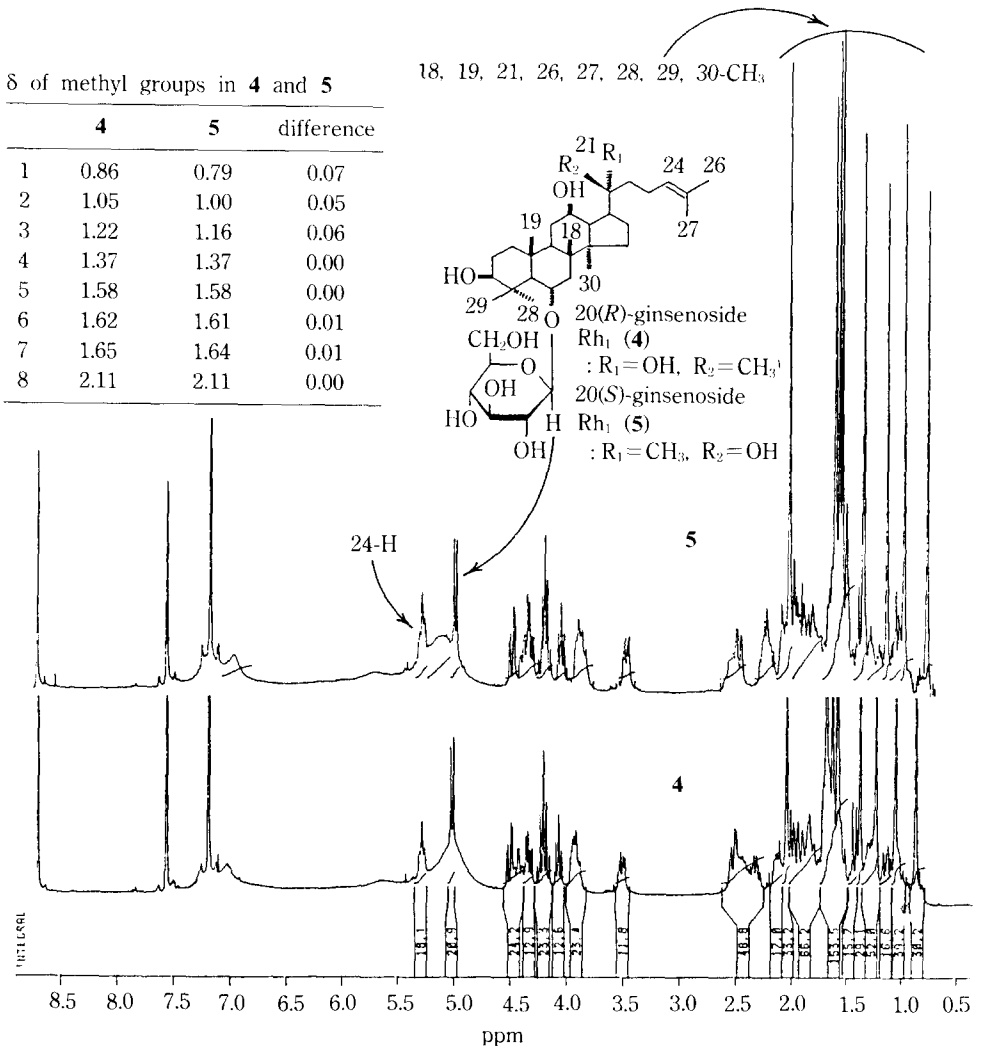



**Fig. 1.** Comparison of $^1$H-NMR (300 MHz, $d_5$-Py., δ) between **4** and **5**.

with acetic anhydride-pyridine to give their peracetates, **2a** and **3a**, which were separated by silica gel column chromatography.

The products were treated with 5% NaOH-n-butanol to give **4** and **5**, respectively, which have been further purified by semi-preparative HPLC in order to confirm the structure of **4** and **5**.

While C-20 epimers in the dammarane series are difficult to be distinguished by TLC, IR, mass and proton NMR spectra, the comparison of chemical shift difference of the C-17, C-21 and C-22 signals in the $^{13}$C-NMR spectra of 20-epimers of ginsenoside $Rh_1$ is of great significance. These differences arise from the γ-gauche effect associated with the conformation around C-17 and C-20 linkage which is fixed to C-12 by strong hydrogen bonding.[9] As shown in Table 1, the $^{13}$C-NMR spectrum of **5** was assigned by referring to that of ginsenoside $Rh_1$ which was already isolated from the root of *Panax ginseng*.[8] The structure of **4** could be determined by direct comparison with the $^{13}$C-NMR spectrum of 20(*R*)-$Rh_1$ which was isolated from red ginseng.[1] The configuration of *R* and *S* forms could be easily determined from the fact that the chemical shift of C-17, C-21 and C-22 were shifted upfiled (4.2, 4.3 ppm) and downfield (7.4 ppm), respectively. This

fact confirmed that the absoulte configurations of C-20 chirality of two compounds are different.

The $^1$H-NMR spectrum (Fig. 1) of the sugar mioety of **4** was quite similar to that of **5** except the chemical shift of three methyl proton signals of the aglycone at δ 0.86, 1.05, 1.23 and 0.79, 1.00, 1.16, respectively. The above evidence suggested that the protons of each methyl group in *R* and *S* forms of **4** and **5** have slightly different magnetic environments as the results of the conformation around the C-17, C-20 linkage.

## 요 약

Ginsenoside Re를 50% 초산으로 가수분해하여 prosapogenin인 20(*R*)-ginsenoside $Rg_2$ 및 20(*S*)-ginsenoside $Rg_2$ 혼합물을 얻었다. 이것을 acetyl화한 후 각각을 분리하였고, 분리된 물질을 alkali 처리하여 20(*R*)-ginsenoside $Rh_1$과 20(*S*)-ginsenoside $Rh_1$을 조제하였다.

## References

1. Kitagawa, I., Yoshikawa, M., Yoshihara, M., Hayashi, T. and Taniyama, T. : *Yakugaku Zasshi* **103**, 612 (1983).
2. Kitagawa, I., Taniyama, T., Shibuya, H., Noda, T. and Yoshikawa, M. : *Yakugaku Zasshi* **107**, 495 (1987).
3. Kitagawa, I. : Proc. 4th. Int. Ginseng Symp. 159 (1984).
4. Matsuda, H., Kubo, M., Tani, T., Arichi, S. and Kitagawa, I. : *Shoyakugaky Zasshi* **39**, 123 (1985).
5. Ota, T., Yamamoto, K., Zong, Z., Yamazaki, M., Odashima, S., Kitagawa, I., Abe, H. and Arichi, S. : *Cancer Res.* **47**, 3863 (1987).
6. Kim, S.I., Baek, N.I., Kim, D.S., Lee, Y.H., Kang, K.S. and Park, J.D. : *Yakhak Hoeji* **35**, 432 (1991).
7. Shibata, S., Ando, T. and Tanaka, O. : *Chem. Pharm. Bull.* **14**, 1157 (1966).
8. Han, B.H., Park, M.H., Han, Y.N., Woo, L.K., Sankawa, U., Yahara, S. and Tanaka, O. : *Planta Medica* **44**, 146 (1982).
9. Beierbeck, H. and Saunders, J.K. : *Cand. J. Chem.* **54**, 2985 (1976).