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# Synthesis of $^3H$-Labeled Dammarane Triterpene Glycosides of Korean Ginseng

Byung Hoon Han and Lin Keun Woo

*Natural Products Research Institute, Seoul National University, Seoul 110, Korea*

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**Abstract** □ A procedure of $^3H$-radio-labeling synthesis for the dammarane triterpene glycosides of Korean ginseng was established by using the ginsenoside $Rg_1$ as starting material. The protons in $C_{11}$ and $C_{13}$ of the aglycone moiety of the glycoside were exchanged with tritium by keto-enol tautomerization of 12-keto-ginsenoside $Rg_1$ which was prepared by partial acetylation, Sarett oxidation and saponification, producing nona-acetate, nonaside $Rg_1$. The acetyl-ketone and 12-keto-derivative of ginsenotritiated ketone was reduced by metallic sodium and isopropanol to produce the end product $^3H$-ginsenoside $Rg_1$ with 3% radio-chemical recovery in one experiment.

**Keyphrases** □ Dammarane triterpene–ginsenoside $Rg_1$–Korean ginseng-tritium labeling method–12–keto–ginsenoside $Rg_1$–Sarett oxidation–saponification

The triterpene glycosides of dammarane saponin series have been proposed as the candidate for the possible active principles of Korean ginseng as the results of extensive studies on the pharmacological activities[1~3] and the chemical nature[4,5] of the glycosides. It was shown to have adaptogenic activities[1], stimulating activities on nucleic acid and protein synthesis[2], hematopoietic activities[3], and many other papers have been concerned with various pharmacological activities of the components.

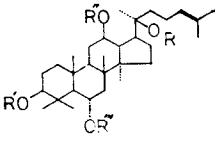
Nontheless, there are some scientists who hesitate to accept the various pharmacological activities of the glycosides. They believe, without experimental evidence, that the saponin is not absorbed in the gastro-intestinal tract so that it will not reach a corresponding receptor site to exhibit various pharmacological activities. Thus it has been desired to have data dealing with gastrointestinal absorption, distribution to the organs and subcellular distribution of the glycosides. For this purpose, we established a new procedure for $^3H$-labeling synthesis of dammarane triterpene glycosides, which is essential in the metabolic studies of the components. The present communication is concerned with this method of synthesis.

Ginsenoside $Rg_1$[5] (1), one of the dammarane triterpene glycosides of Korean ginseng known as a pharmacologically active substance, was chosen as the starting material for the synthesis. The overall process includes the preparation of the ketonic derivative, by five step chemical synthesis, tritiation by keto-

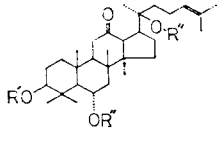
enol tautomerism and regeneration of $^3$H-ginsenoside $Rg_1$. Ginsenoside $Rg_1$ (I) produced decaacetate (II) mp 250–2° under ordinary acetylation condition. However, when acetylation was conducted at low temperature with limitted amount of reagent, a mixture of incompletely acetylated products consisting mainly of the nonaacetate (III) and a small amount of octaacetate was obtained.

The mixture was purified by column chromatography to give chrystalline nonaacetate (III) mp 143° with about 70% yield. Sarett oxidation of the nonaacetate (III) using chromic trioxide-pyridine complex produced ketoacetate (IV) mp 233–5° with almost quantitative yield. The ketoacetate (IV) was saponified by refluxing five hours with 5% sodium hydroxide in 5% aqueous alcohol to produce the ketone (V).

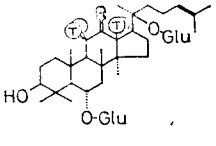
The steric conformation of the ketone (V) seemed to be very stable on alkali treatment, since no appreciable change in thin layer chromatogram was observed by the treatment. The ginsenoside $Rg_1$ -ketone (V) dissolved in 72ml alcohol was heated at 100°C for five hours with 0.5ml tritium oxide (3 Curie) and 100mg sodium hydroxide in a sealed tube to furnish tritiation of the ketone by keto-enol tautomerism.



[I] : R′=R″=H, R‴=Glu
[II] : R′=R″=Ac, R‴=Glu(Ac)$_4$
[III] : R′=Ac, R″=H, R‴=Glu(Ac)$_4$



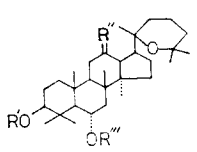
[IV] : R′=Ac, R″=Glu(Ac)$_4$
[V] : R′=H, R″=Glu



[VI] : R=O
[VII] : R=

The reaction mixture was extracted with butanol, washed with water and concentrated to dryness. Absolute dehydration was necessary for the next step reaction in the procedure. The tritiated ketone (VI) was reduced to produce $^3$H-ginsenoside $Rg_1$(VII) by the treatment with powdered metalic sodium and anhydrous isopropanol. The reaction mixture showed two spots on a thin layer plate, one of which is major component and superimposable with the starting material ginsenoside $Rg_1$. The other spot seemed to be C–12 epimer of the starting material. The epimeric mixture was subjected to silica-gel column chromatography to obtain pure $^3$H-ginsenoside $Rg_1$. Tritiation on hydroxyl protons should have occured by the above labeling process. In order to remove tritium on hydroxyl groups by exchange reaction, the purified product was refluxed for four hours in large volume of water and methanol mixture. Tritium atom bound on carbon atom was stable on the above treatment. Overal chemical yield for starting material was about 16%, and radio-chemical yield was about 2%. The low rate of radio chemical yield did not imply the low rate of exchange by keto-enol tautomerism, but seemed to be dependent on the low molecular ratio of ketone to the tritium oxide used.

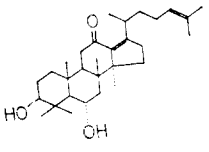
In order to assign the site of tritium labeling correctly, the location of ketone group in the ketonic substance was determined by a chemical process. Acid hydrolysis of the $Rg_1$-



[VIII] : R'=R''=H, R'''= 

[IX] : R'=R''=H, R'''=O

[X] : R'=R''=Ac, R'''= 



[XI]

ketone (V) produced the sapogenin which was chromatographically different from panaxatriol (VIII).

This fact implies that the location of the ketone group is in the aglycone part of $Rg_1$-ketone (V). The location of the ketone group in the $Rg_1$-ketone (V) should be either at C–3 or C–12, since C–6 and C–20 hydroxyl groups had already been bound by their glucosyl groups. Since ginsenoside $Rg_1$ (I) was known to produce the side chain cyclized sapogenin, panaxatriol (VIII), by acid hydrolysis, the sapogenin obtained by acid hydrolysis of the ginsenoside $Rg_1$-ketone (V) was at first compared by thin layer chromatography with 12:keto-panaxatriol (IX) which was synthesized from panaxatriol-3,6-diacetate (X). However, the TLC spot of the hydrolysate showed unexpectedly higher Rf-value than that of 12-keto-panaxatriol (IX). On the other hand, acid treatment on 12-keto-panaxatriol by boiling in 5%-sulfuric acid and acetic acid mixture produced a substance whose TLC-spot was superimposed correctly with that of sapogenin produced from the ginsenoside $Rg_1$-ketone (V) by acid treatment. This substance showed UV absorption maximum at 265 mu which seems to be attributable to s-cis- -unsaturated carbonyl group of compound (XI). The compound (XI) would have been produced from ginsenoside $Rg_1$-ketone (V) or 12-keto-panaxatriol (IX) by dehydration of C–20 hydroxyl group and migration of resulting double bond to form unsaturated carbonyl group as a result of acid treatment. Therefore it seems reasonable to assign C–12 position as the location of carbonyl group in ginsenoside $Rg_1$-ketone (V). This $^3H$-labeling process will be a useful method applicable to the other ginsenoside Rx group found in Korean ginseng.

## EXPERIMENTS

*Thin Layer Chromatography*

The following TLC solvents were used throughout the experiments unless otherwise stated and the solvents were also used as the elution solvents for the column chromatography.

Solbent A for glycosides: $CHCl_3$ : MeOH: $H_2O$—75 : 25 : 2. 5.

Solvent B for aglycones or glycoside acetates: Benz.: EtOAc—6:4.

Spots were visualized by spraying 5%-$H_2SO_4$ and heating.

*Isolation of Panax Saponin A (Ginsenoside $Rg_1$)* [1].

Eighteen kg of white ginseng were extracted by boiling in methanol and concentrated to give 2.5 kg syruppy residue. The residue was suspended in a small volume of water and extracted successively by ether and butanol. The butanol layer was concentrated to give 500 gm of a crude glycoside fraction. The glycoside fraction was chromatographed over silicagel column using solvent A to yield 18 gm pure ginsenoside $Rg_1$ [1].

*Peparation of Nona-acetate* [III].

To the solution of ginsenoside $Rg_1$ (9 gm) in 35 ml pyridine, the mixture of acetic anhydride (11 ml) and pyridine (15 ml) was added slowly and allowed to react in cold for three days. The mixture of incomplete acetylation products consisting mainly of decaacetate [II] (Rf 0.6), nonaacetate [III] (Rf 0.5) and octaacetate (Rf 0.4) was obtained by ordinary digestion process. The resulting mixture of acetates was chromatographed over silica gel column using solvent B and crystallized from MeOH to give 3.5 gm of pure nonaacetate, $C_{42}H_{72}O_{14}$. $(CH_2O)_9$, mp 143.

*Preparation of 12-Keto-ginsenoside $Rg_1$ [V]*

To five ml Sarett reagent, eight ml pyridine solution of 3.5 gm ginsenoside $Rg_1$-nonnacetate [III] was mixed and allowed to react at room temperature for 36 hours. The oxidation was terminated by the addition of methanol, diluted with water, and then extracted with ether. The ether extract was worked up in a usu al way to give 3.2 gm of ginsenoside $Rg_1$ nona-acetyl-ketone $C_{42}H_{70}O_{14}$. $(CH_2O)_9$ [IV] which gave fine needles from methanol, mp 233–5. The keto-acetate [IV] was saponified by refluxing with 100 ml of 2.5% sodium hydroxide in 50% ethanol for five hours. The reaction mixture was extracted with butanol and concentrated to give 2.1 gm ginsenoside $Rg_1$-12-ketone [V] which was amorphous but chromatographically pure on TLC using solvent A (Rf 0.16).

*Preparation of $^3H$-ginsenoside $Rg_1$ [VII]*

The ginsenoside $Rg_1$-12-ketone [V] (1.9 gm) was placed in a 20-ml glass ampoule and dissolved by addition of 7.2 ml ethanol. To this solution, 300 mg sodium ethoxide and 0.5 ml tritium oxide (3 curie) were added, and then the ampoule was sealed and heated in an ethanol bath for three hours to furnish tritiation of ketone by keto-enol tautomerism. The reaction mixture was concentrated to dryness, re-dissolved in 15 ml water and the acidity was adjusted to pH 7.0 by adding d-HCl. The tritiated ketone was extracted by butanol and the butanol extract was concentrated to dryness. In order to keep the absolute dehydration of the residue, azeotropic distillation process was repeated several times, finally yielding 1.6 gm amorphous powder of $^3H$-ginsenoside $Rg_1$-12-ketone [VI]. The $^3H$-ginsenoside-$Rg_1$-12-ketone [VI] (1.5 gm) was dissolved in anhydrous isopropanol (20 ml) and reduced by adding 3 gm powdered metallic sodium under nitrogen stream to produce $^3H$-ginsenoside $Rg_1$ [VII] with its 12-epimeric impurity. The reduction was terminated by adding 50 ml water and then it was extracted four times with 70 ml portions of butanol. The butanol layer was refluxed for four hours to remove loosely bound tritium atom on hydroxyl groups. The butanol layer was concentrated to dryness and the resulting residue was chromatographed over silica-gel column to produce 1.3 gm pure $^3H$-ginsenoside $Rg_1$ [VII] using solvent A. The resulting product was dissolved in 50 ml ethanol and stored in cool place to avoid radiolysis.

*Radioactivity Determination*

The Nuclear Chicago Liquid Scintillation Counter was used for the determination of radioactivity. The scintillation solution was prepared by dissolving 2.6-diphenyl-oxazole

(PPO) 6 gm. and 1.4-bis (2.5-phenyloxazolyl)-benzene (POPOP) 100 mg in one liter of toluol. In order to determine the radio-activity of final product ten microlitre of ethanol solution of $^3$H-ginsenoside $Rg_1$ was diluted quanititatively one hundred times by mixing ethanol and then spotted on a small strip of Whatmann paper. After drying the paper strip, it was dipped in the scintillation solution and counted for the radioactivity. Assuming the counting efficiency as about 10% in this experiment, the radioactivity of the final product of this synthesis was found to be about 55, 500 microcurie in total product.

*Preparation of 12-Keto-panaxatriol*

Panaxatriol [VIII] (100 mg) in three ml pyridine was acetylated by mixing one ml acetic anhydride and was allowed to stand at room temperature for 24 hours. Crystalline needles were deposited during reaction. After working up in usual ways, 110 mg of 3,6-panaxatriol [X]mp 267 C. was obtainedas fine needles from ethylacetate. The diacetyl-panaxatriol-diacetate [X] (80 mg) in seven ml acetic acid was oxidized by mixing anhydrous chromic acid (130 mg) solution in acetic acid. After completing the oxidation by standing at room temperature for 36 hours, the reaction mixture was diluted with a small amount of methanol and water. The diluted reaction mixture was extracted with ether and the ether layer was concentrated to give amorphous 12-keto-panaxatriol-diacetate (50 mg). The 12-keto-panaxatriol-diacetate was saponified by treatment with sodium ethoxide (10 mg) in absolute ethanol to produce 12-keto-panaxatriol [IX].

*Acid Treatment of 12-Keto-panaxatriol and 12-Keto-ginsenoside $Rg_1$*

The 12-keto-ginsenoside $Rg_1$ (10 mg) was dissolved in 0.5-ml volume of 5%-sulfuric acid in 50%-aqueous methanol and sealed in a small capirally tube and heated for five hours on a boiling water bath. The thin layer chromatogram of the reaction mixture (Benz: EtOAc, 6:4) showed a single sapogenin spot whose Rf value (0.6) was significantly different from those of panaxatriol (Rf 0.15) and 12-keto-panaxatriol (Rf 0.20). However, acid treatment on 12-keto-panaxatriol by the same reaction condition produced a modified sapogenin [XI] whose Rf-value was completely superimposable with that produced from 12-keto-ginsenoside $Rg_1$ by acid treatment.

## LITERATURE CITED

1) Brekhman, I.I., and Dardymov, I.D., New substances of plant origin which increase nonspecific resistance. *Ann. Rev. Pharmacol.* **9,** 419(1969).
2) Oura, H., Hiai, S., Nakashima, S., and Tsukada, K., Stimulating effect of the roots of Panax ginseng on the incorporation of labeled precursors into rat liver RNA. *Chem. Pharm. Bull.* **19,** 453 (1971).
3) Yamamoto, M., Biochemistry of panax ginseng. Biochemical effect on bone marrow, testes, and cancer cells. *Metabolism and Disease* **10,** 119 (1973).
4) Tanaka, O., Chemical constituents of ginseng. *Meatabolism and Disease* **10,** 86 (1973).
5) Iida, Y., Tanaka, O., and Shibata, S., Studies on saponins of ginseng (xxiv), the structure of ginsenoside $Rg_1$. *Tetrahedron Lett.* 5449 (1968).
6) Nagai, Y., Tanaka, O., and Shibata, S., Chemical studies on the oriental plant drugs, structure of ginsenoside $Rg_1$, a neutral saponin of ginseng root. *Tetrahedron* **27,** 881 (1971).