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The Synthesis of ^{14}C-Labeled Dammarane Glycoside of Ginseng

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Abstract—Panax Saponin A (isopropyl-^{14}C) was prepared from natural saponin in good yield by the processes; acetylation, osmium tetroxide oxidation, periodic acid oxidation, Wittig process, and saponification.

Some of the saponins (protopanaxatriol series) showed central nervous system (CNS) stimulant action and some of them(protopanaxadiol series) showed CNS-depressant activity in neuropharmacological observation[1] using mice. Also, the stimulant action was proved by many authors[2] through the experiments with mice swimming in water and more clearly with a special device, in which mice were made to run up an endless rope. Numerous data[2] on restoration of blood albumin after massive bleeding, and on stimulation of immune body production, suggested a peculiar anabolic action of the saponins.

Recently, the saponins (protopanaxadiol series, prostisol) have been demonstrated to increase the incorporation rate of labeled precursor into cytoplasmic polysomal RNA of rat by Oura[3], and one of purified saponins (protopanaxatriol series, Panax Saponin A) has been proved to have a stimulating activity on the incorporation of ^{14}C-leucine into serum and liver protein of mice in our laboratory[4].

Although the saponins have assumed to be considerably important substances as the medicinal candidates due to their physiological activities, a major drawback in pharamacodynamic studies such as absorption, secretion and accumulation, is the lack of labeled saponins. For instance, our previous work has made an example of necessity of the labeled saponin to illustrate a mechanism of long-lasting biological activities of a ginseng saponin. Panax saponin A (protopanaxatriol diglucoside) has been found to have delayed and long-lasting anti-inflammatory activity, of which the time course tendency is closely related with that of stimulating activity of the saponin on the ^{14}C-leucine incorporation.

This consideration prompted us to synthesize ^{14}C-labeled Panax Saponin A[5], one of genuine glycosides of Korean ginseng. The synthesis was performed by the procedures presented in scheme I-III. As the starting material, deca-acetate(**II**) of Panax saponin A, $C_{42}H_{72}O_{14}$, was used, which was obtained from Panax saponin A(**I**), $C_{42}H_{72}O_{14}$, by the

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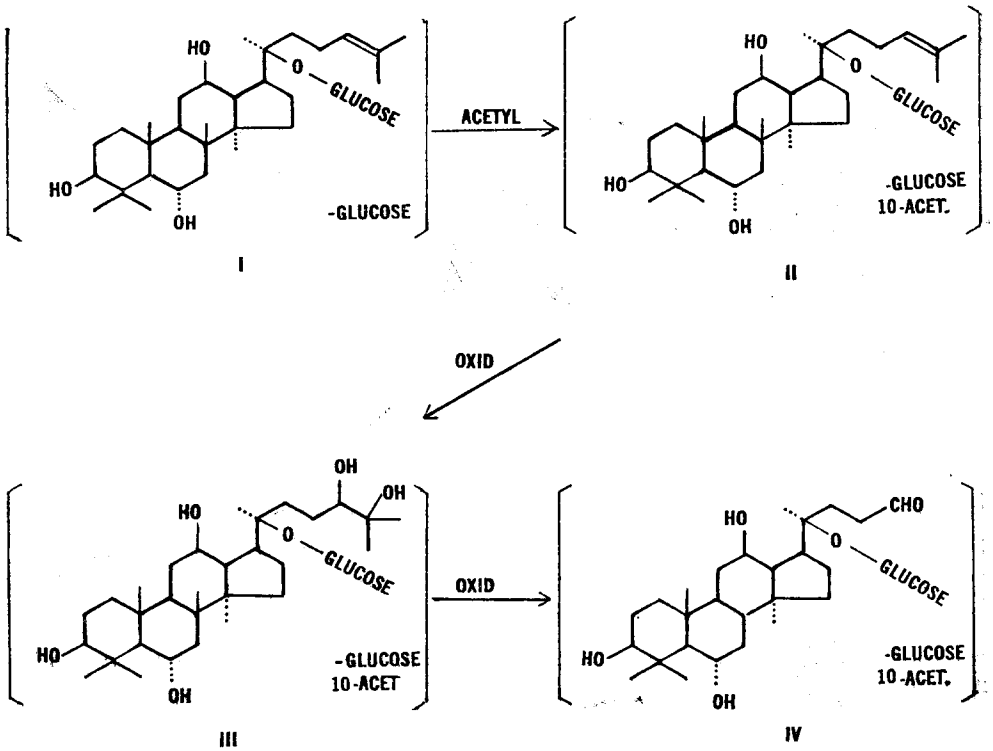
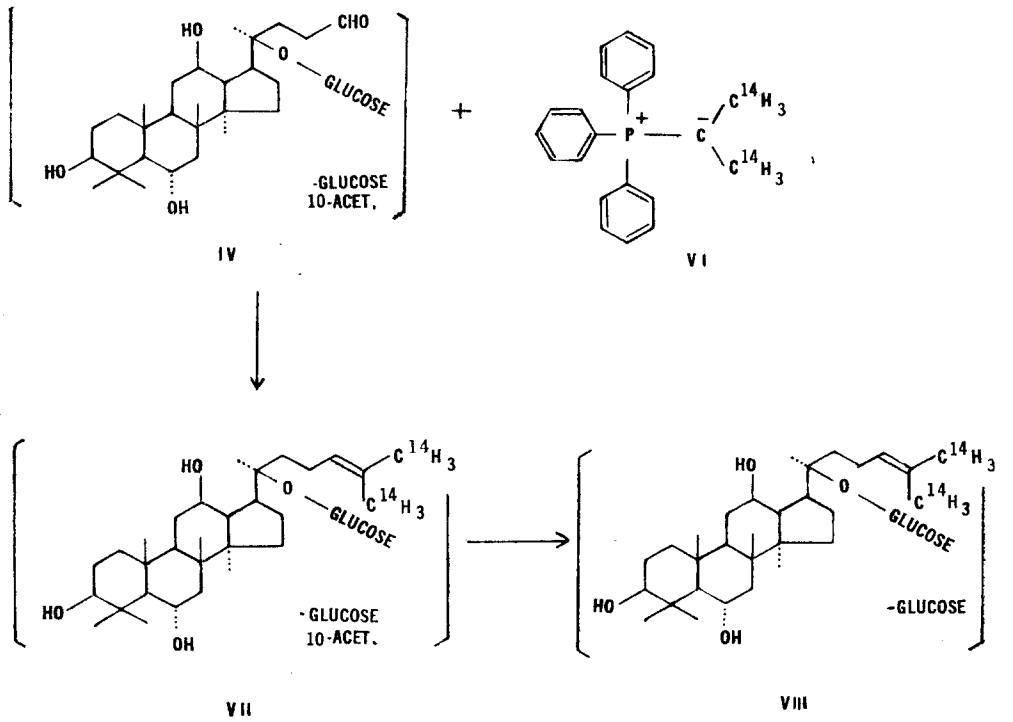
usual method.

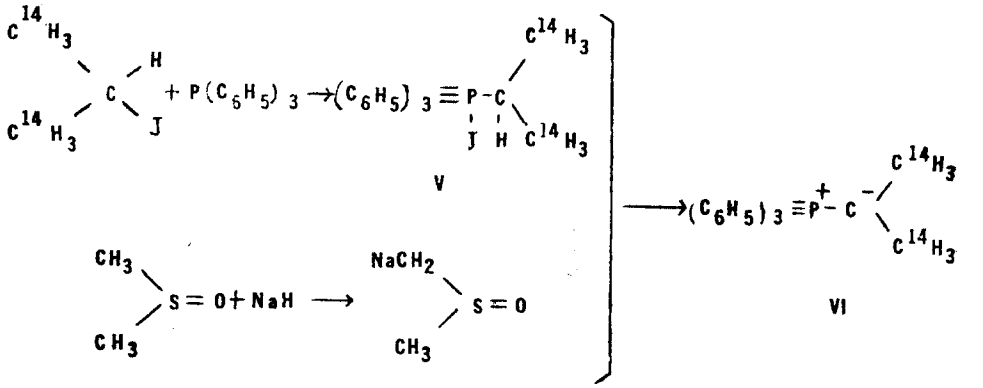
II was oxidized to 24,25-glycol (**III**) by the treatment with OsO_4-pyridine in C_6H_6 at room temperature, and fine needles, $C_{42}H_{72}O_{14}$ $(OH)_2 \cdot 10(C_2H_2O)$, was crystallized from MeOH after being chromatographed on silica-gel. Infra-red spectrum of **III** revealed a strong absorption of hydroxyl peak at 3542 cm^{-1} due to newly generated-glycol. Three carbon fragment (C_{25}-C_{27}) of **III** was broken down by subsequent HIO_4 oxidation (overnight treatment at room temperature in dioxane solvent), to form trisnoraldehyde(**IV**) in fine needles from MeOH, $C_{39}H_{66}O_{15} \cdot 10(C_2H_2O)$. On the other hand, ^{14}C-isopropyl iodide was condensed with triphenyl phosphine by boiling in a sealed tube on a water bath to give ^{14}C-isopropyl-triphenyl-phosphonium iodide (**V**), bitetragonal prisms from C_6H_6, mp 192° (decomp.). Halogen content of **V** was consistent with the theoretical value for $C_{21}H_{22}PI$ and ir (KBr) of **V** showed additional absorption due to isopropyl fragment at 2860, 1470, 1398, 1380cm^{-1} and due to additional P-C bond at 725,715cm^{-1}. An isopropylidene-triphenyl-phosphorane (**VI**), $(C_6H_5)_3P=C(CH_3)_2$, was obtained by the treatment of theoretical amount of sodium methylsulfinyl carbanion[6] in DMSO solvent under the N_2 stream. Compound **IV** and **VI** were condensed to produce a ^{14}C-labeled derivative **VII** of **II**, whose



Scheme 1—Preparation of Panax A acetate saponin-trisnor-aldehyde.



Scheme 2—Synthesis of (^{14}C) Panax saponin A.



Scheme 3—Synthesis of (^{14}C) isopropylidene-triphenylphosphorane.

thin layer chromatogram and radio-autogram is consistent with that of **II**, fine needles from MeOH, mp 250° in yield of over than 40%. Refluxing the acetate with dil-NaOH in 50% MeOH for 5 hr ^{14}C-Panax saponin A was obtained in a quantitative yield.

DISCUSSION

Although various routes for the organic synthesis[7] of ^{14}C labeled compounds have been known in the field of triterpene chemistry, the methods have not been applicable to the triterpene glycosides due to interferences of sugar moiety. Present study established a new method for the synthesis of the ^{14}C-labeling genuine glycosides of dammarane series.

The method is composed of following five steps of chemical reaction; 1) protection of hydroxyl function by acetylation, 2) transformation of double bond to glycol function by OsO_4 oxidation, 3) break down of side chain by HIO_4 oxidation producing terminal carbonyl function, 4) resynthesis of C=C double bond by Wittig process introducting three carbon fragment as the ^{14}C-carrier, and 5) saponification of acetyl function giving the labeled dammarane glycoside. Considering the above chemical steps, it will be said that the method established will be applicable as the general method for the labeling synthesis of triterpene glycosides, when chemical structures fall in following categories; 1) the glycosides should have double bond in side chain, no double bond in polycyclic ring, no glycol function which is composed with two tertiary hydroxyl function, no ester function, and no carbonyl function.

EXPERIMENTAL

Isolation of Panax Saponin A—The mixture of Panax saponins (50gm), an anti-inflammatory glycoside fraction of dammarane series obtained from Korean ginseng by our previous work[5], was chromatographed over silica-gel column (500gm, 5×75cm, particle size<0.08mm) to separate a crude Panax saponin A (yield; ca. 10gm) (solvent; $CHCl_3$: MeOH : H_2O=75 : 25 : 2.5).

Deca-acetate of Panax Saponin A (II)—The crude Panax saponin A (10gm) was acetylated by boiling 2hr with Ac_2O(20ml) and pyridine (40ml). The reaction mixture was poured into ice-water and the resulting precipitate was collected and recrystallized from MeOH giving deca-acetate of Panax saponin A (**II**) (10.5gm) as colorless needles, mp 252°. ir(KBr); strong acetyl absorption and no hydroxyl band.

Glycol-derivative of Panax Saponin A deca-acetate(III)—Deca-acetate of Panax saponin A(**II**) (210gm) and OsO_4 (600gm) in absolute C_6H_6 (20ml) and pyridine (3ml) was left standing in the dark for 4 days at room temperature. The reaction mixture was treated with H_2S to give precipitate. After the precipitates were filtered and then washed with Me_2CO, the combined filtrate was evaporated in vacuum to give dark brown crystalline residue. The residue was chromatographed over silica-gel column(20gm, 1.5×30cm). After exhaustive elution with C_6H_6, the black band, formed on the top of column, was eliminated mechanically. Subsequent elution with C_6H_6 : Et_2O(1 : 1) gave the glycol derivative (**III**) in a chromatographically single state. The glycol derivative(**III**) was recrystallized from MeOH to give fine needles (yield, 1.5gm), mp 263°, $[\alpha]_D^{20}=+5°$, ir(KBr); strong hydroxyl absorption at 3542cm^{-1} due to glycol.

Trisnor-aldehyde of Panax Saponin A deca-acetate (IV)—Glycol derivative **III** (1.2gm) was oxidized by overnight treatment with HIO_4 (450gm) at room temperature in 83%-dioxane solvent (30ml) in order to break down the three carbon fragment (C_{25}—C_{27}) from the side chain of aglycone.

The reaction mixture was diluted with the addition of H_2O, and the resulting precipitate was collected in peroxide free Et_2O. The Et_2O layer was washed with $NaHCO_3$ solution, and then with H_2O successively and dried over Na_2SO_4. Removal of the solvent gave crystalline mass. The crystalline mass was recrystallized from absolute MeOH to give fine needles, **IV** (0.9gm, 89% yield), mp 231°, $[\alpha]_D^{15}=-13.1$, strongly positive to Tollens' reaction, ir (KBr), a weak absorption at 2821cm^{-1} due to CH-streching of CHO, branching of carbonyl absorption at 1750 and 1725cm^{-1} due to acetoxyl and carbonyl function, and no OH band. Nmr spectrum of **IV** showed methylene adjacent carbonyl proton at $\delta_{CDCl_3}^{TMS}$ 9.775 (triplet, 1H, J=3cps).

Isopropyl (^{14}C)-triphenyl-phosphonium-iodide (V)—A mixture of triphenyl-phosphine (100mg) and ^{14}C-isopropyl-iodide (1.0mc) in dry C_6H_6 (4ml) was condensed by heating at 100° for 5 hr in a sealed tube to produce (^{14}C)-isopropyl-triphenyl-phosphonium iodide. In order to complete the reaction, the condensing reaction was repeated after the addition of excess amount of isopropyliodide (2ml). During the condensation reaction, **V** was crystallized out as the bitetragonal prisms, mp 193°. The halogen content of the substance, assayed by Fajans procedure, was consistent with the theoretical result for the expected formula **V**. $C_{21}H_{22}PI$. ir-spectrum of **V** showed additional absorption at 2860, 1470, 1398, 1380cm^{-1} due to isopropyl fragment and at 725, 715cm^{-1} due to additional P-C bond. Radioautographic result (X-ray film, 14days exposure) was superimposed with the TLC-spot of **V** (solvent; petr. ether: C_6H_6=4 : 1, colour development: I_2-vapour).

Wittig Reagent (^{14}C-isopropylidene-triphenylphosphorane) (VI)—All experiments were performed in a strictly anaerobic and anhydrous conditions as possible. NaH (500mg) was washed twice with n-pentane to remove mineral oil. Anhydrous DMSO(5ml) was introduced to the washed NaH and heated at 75-80° to produce sodium methyl sulfinyl carbanion under N_2 gas evolution. The resulting solution of sodium methyl sulfinyl carbanion (0.5ml) was cooled and **V** (100mg) was mixed to produce **VI** in a small closed tube, the air of which was sufficiently displaced by N_2. Resulting dark red colour of **VI** was stable only in the condition of strictly anaerobic and anhydrous state.

Synthesis of ^{14}C-Panax Saponin A (VIII)—To the dark red solution of **VI** in the closed tube, **III** (0.9gm) in DMSO (1.5ml) was introduced via syringe untill the dark red color was faded. The reaction mixture was poured into d-HAc, extracted with $CHCl_3$. $CHCl_3$ layer was washed with H_2O several times to remove DMSO and dried over Na_2SO_4. After working up on usual way, **VII** was crystallized as fine needles. Thin layer chromatogram of the subtance showed the spots of **II**, unreacted trisnor-aldehyde derivative and some other unidentified substances. The recovery of **VII** from **III** seems to be exceeding 40%. Deca-

acetate was saponified by heating with 5%-NaOH (3ml) for 5hr in a sealed tube. The reaction mixture was deionized by Amberite-ir-120 (5 g dry wt.). The deionized solution was evaporated at reduced pressure to give amorphous powder of ^{14}C-Panax Saponin A. Radio-autogram of **VII** and **VIII** were superimposed with the thin layer chromatograms of **I** and **II**.

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