Arch. Pharm. Res. 8(4), 257~260 (1985)

Isolation of Isomaltol-α-D-glucopyranoside[1] and Ketopropyl-α-D-glucopyranoside[2] from Korean Red Ginseng

Byung Hoon Han, Myung Hwan Park and Yong Nam Han

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

(Received October 10, 1985)

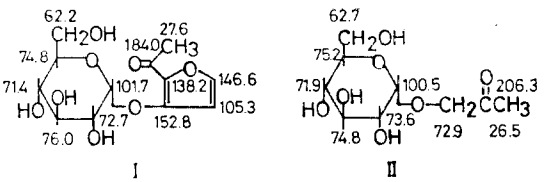
Abstracts: □ Based on spectral and chemical evidences, two glycosides isolated from Korean red ginseng are characterized as isomaltol-α-D-glucopyranoside and ketopropyl-α-D-glucopyranoside. These compounds are not found in Korean white ginseng.

Keywords □ isomaltol-α-D-glucopyranoside, ketopropyl-α-D-glucopyranoside, Korean red ginseng, *Panax ginseng* C.A. Meyer

In our previous papers, it was reported that both of ether-soluble acidic and butanol-soluble fractions of Korean ginseng (*Panax ginseng* C.A. Meyer) exhibited the potent antioxidant activities against the lipid peroxidation of the ethanol-intoxicated mouse liver, and also that maltol, salicylic acid and vanillic acid were isolated as components of the active principles of the ether-soluble acidic fraction.[1,2]

This paper describes that the purification of UV-absorbing substances from the butanol fraction which has remained unidentified is carried out to yield three compounds in crystalline states. They are identified by chemical and spectrometrical evidences to be isomaltol-α-D-glucopyranoside, ketopropyl-α-D-glucopyranoside and adenosine. The latter two compounds were also reported by Tanaka O., *et al.*,[3] concomitant to this research finding.

[1,2] Reported at the 31st Annual Convention of the Pharmaceutical Society of Korea held in Kwangjoo (1982), D_{24} and D_{23}.



I II

Fig. 1. The structure and ^{13}C-NMR data of compounds I and II isolated from Korean red ginseng.

In order to isolate UV-absorbing substances from the butanol fraction, the methanol solution of crude ginseng saponins was treated with an equal volume of ethyl acetate, and resultant precipitates were removed. Purification of the ethyl acetate/methanol soluble fraction over silica gel yielded three compounds, Compounds I, II and III, in respective crystalline states.

Compound I, $C_{12}H_{16}O_8 \cdot H_2O$, M^+ m/z 288, mp 141°, $[\alpha]_D^{23}+203°$ (in methanol) was positive in Molish test, suggesting it as a glycoside. It showed absorption bands of hydroxy (3,400 cm^{-1}), α,β-unsaturated ketone and olefin (1650, 1590cm^{-1}) and furan ring (1590, 885, 800cm^{-1}) in its IR spectrum, and showed the absorption peak at 280nm in its UV spectrum which was not shifted by addition of NaOH. The proton NMR spectrum of compound I exhibited a signal for one acetyl group at δ 2.40 (3H), signals for one anomeric proton at δ5.54 (1H, d, J=3.5Hz), and signals for two protons at δ6.29 and 7.28 (each d, J=2Hz) on the furan

ring. Acetylation of compound I with acetic anhydride and pyridine yielded a tetraacetate. On hydrolysis, compound I gave an aglycone which exhibited red-purple color by ferric ion. The aglycone showed absorption bands of hydroxyl ($3,410cm^{-1}$), α,β-unsaturated ketone and olefin ($1,590\sim1,570cm^{-1}$), and the furan ring (875, $800cm^{-1}$). Its proton NMR spectrum exhibited signals for one acetyl at δ2.40 (3H, s), two aromatic protons at δ6.29 and 7.28 (each d, J=2Hz) and one hydroxyl group at δ4.64 (1H, br. s). These data of the aglycone was identical with those of isomaltol.[5] The TLC and GLC of the aqueous portion of the hydrolysate disclosed the composition as D-glucose.

The coupling constant (J=3.5Hz) of the anomeric proton at δ5.54 and the chemical shift of the anomeric carbon at δ101.7 in addition of the assignment of total carbon atoms as shown in Fig. 1 indicated that compound I was identified as isomaltol-α-D-glucopyranoside. To confirm the proposed structure, isomaltol-α-D-glucopyranoside was prepared from maltose according to the Hodge's method.[4] The mp, $[\alpha]_D$, UV, IR and NMR data of the synthesized compound were identical to those of compound I isolated and also they showed the same retention time in GLC of their TMS-derivatives. Isomaltol -α-D-glucopyranoside was found only in red ginseng, and the content in red ginseng and the total saponin fraction of its methanolic extract were 0.11 and 1.35%, respectively. The isolation of the compound I from white ginseng was in failure.

Compound II, $C_9H_{16}O_7$, mp 177$\sim$178°, $[\alpha]_D^{23}$ +178.8°, showed positive reactions in Molish test and iodoform test, suggesting it as a glycoside possessing such acetyl group as acetone, which was further confirmed by absorption bands of hydroxyl ($3,400cm^{-1}$) and ketone ($1,710cm^{-1}$) in its IR spectrum, and by signals for one acetyl group at δ2.10 (3H, s), one methylene group at δ4.38 bearing the acetyl group (-O-$\underline{CH_2}COCH_3$) and an anomeric proton at δ5.30 (1H, d, J=3.5Hz) in its PMR spectrum. Acid hydrolysis of compound II gave D-glucose, as judged from TLC and GLC of the hydrolysate. The coupling constant (J=3.5 Hz) of the anomeric proton at δ5.54 and the chemical shift of the anomeric carbon at δ100.5 in addition of the assignment of total nine carbon atoms as shown in Fig. 1 indicated that compound II was identified as ketopropyl-α-D-glucopyranoside. In the mass spectrum, fragment ions at m/z 163 (glycosyl), 73 (OCH_2-$COCH_3$) and 57 (CH_2COCH_3) supported the proposed structure. The isolation of ketopropyl-α-D-glucose from white ginseng was also in failure.

Compound III, mp 232$\sim$233°, was positive in nitrogen and Molish test. Acid hydrolysis of compound III yielded adenine and ribose. The physical properties of compound III including mixed mp, $[\alpha]_D$, UV, IR and PMR data were identical to those of standard adenosine (E. Merck). The content of adenosine in red ginseng and the total crude saponin were 0.04 and 0.5 %, respectively. Adenosine was isolated from white ginseng.

EXPERIMENTAL METHODS

Melting points were determined by a Mitamura Heat Block Model-MRK apparatus and were uncorrected. UV, IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra and optical rotations were taken on Shimadzu MPS 50L, Perkin-Elmer 283B, Varian EM-360 60MHz NMR, Jeol-PET-100 NMR, Jeol 01-SG-2 spectrometers and Autopol III automatic polarimeter, respectively. GLC

was carried out on Pye Unicam 104.

Isolation of Compounds I to III

Korean red ginseng was refluxed with 70% ethanol for 6 hrs in a boiling water bath. The extraction was repeated four times. All the extracts were combined and ethanol was removed. The syrupy extracts were suspended into water, then extracted with benzene and chloroform. The aqueous layer was partitioned into butanol for preparing crude saponins. The crude saponins (500g) were dissolved in methanol (2*l*), and then ethylacetate (2*l*) was added by stirring. The methanol/ethyl-acetate soluble fraction was freed from solvent to yield brownish powders (54g). And then they were subjected to column chromatography over silica gel, eluting with chloroform/hexane/methanol/acetic acid(5:1:1:0.2). Three sub-fractions showing UV-absorption were obtained, Fraction A, 2.2g, Rf 0.45; Fraction B, 4.5g, Rf 0.40; Fraction C, 5.2g, Rf 0.20.

Compound I : Fraction B was rechromatographed on a silica gel column using an eluent of chloroform/methanol/water (75:25:2.5). UV-absorbing and saponin-free fraction was obtained. (890mg) It was crystallized from methanol to yield compound I. (394mg) colorless needles, mp 141°, $[\alpha]_D^{23}$+203° (c=0.33, methanol), UV λ_{max} in methanol (log ε): 280nm (4.15), no bathchromic shift with NaOH, IR ν_{max}^{KBr} cm^{-1}: 3400, 1110 (OH); 1650, 1590 (α, β-unsaturated ketone and olefin); 885, 800 (furan), ^{1}H-NMR (DMSO-d_6, δppm): 2.40(3H, s, $COCH_3$), 4.55 (1H, t, C_6'-OH), 5.10 (2H, d, J=4Hz, C_2'-OH, C_3'-OH), 5.28 (1H, d, J=5Hz, C_4'-OH), 5.54 (1H, d, J=3.5Hz, C_1'-H), 6.80 (1H, d, J=2Hz, C_4-H), 7.82(1H, d, J=2Hz, C_5-H), ^{13}C-NMR(C_5D_5N, δppm): Fig. 1, Mass; m/z 288(M^+), 273 (M^+-15), 163, 126, Anal. Calcd. for $C_{12}H_{16}O_8 \cdot H_2O$: C47.10, H5.92, Found: C47.12, H5.90. Acetylation of compound I with acetic anhydride and pyridine yielded a tetraacetate. IR ν_{max}^{KBr} cm^{-1}: 1750, 1240, 1220 (acetyl), 1670, 1590, 1040, ^{1}H-NMR ($CDCl_3$, δppm): 2.08 (12H, s, 4×CH_3CO), 2.53 (3H, s, CH_3CO), 5.68(1H, d, J=3.5Hz), 6.51(1H, d, J=2Hz), 7.44 (1H, d, J=2Hz).

Compound II : Fraction C was subjected to column chromatography over silica gel, using an eluent of chloroform/methanol/water (70:30:4). UV-absorbing and saponin-free fractions were collected and were freed from solvent. The residue (600mg) was crystallized from methanol. Compound II was obtained as colorless needles. (252mg) mp177~178°, $[\alpha]_D^{23}$+178.8° (c=0.33, methanol), IR ν_{max}^{KBr}cm^{-1}:3400(OH), 1710 (C=O), ^{1}H—NMR(C_5D_5N, δppm): 2.10(3H, s, CO-$C\underline{H}_3$), 4.38(2H, s, -O$C\underline{H}_2$COCH$_3$), 4.02~4.68 (6H, m), 5.30 (1H, d, J=3.5Hz, anomeric proton), ^{13}C-NMR (C_5D_5N, δppm): Fig. 1, Mass m/z(%); 218(M^+-H_2O, 2), 205(M^+-CH_2-OH, 3), 163(13.3), 145(31), 127(13.7), 103 (39.1), 73(100), 61(24.7), 57(72.1), 43(59), Anal. Calcd. for $C_9H_{16}O_7$: C45.76, H6.78; Found: C45.77, H6.86. Acetylation of compound II with acetic anhydride and pyridine as usual gave a tetraacetate. mp 168°, $[\alpha]_D^{20}$+158.9 (c=0.3, chloroform), IR ν_{max}^{KBr}cm^{-1}: 1750, 1220 (acetyl), ^{1}H-NMR ($CDCl_3$, δppm): 2.06(6H, s, 2×CH_3CO), 2.12, 2.16, 2.18 (3×3H, each s, 3×CH_3CO), 4.24 (2H, s).

Compound III: Fraction A was rechromatographed on a silica gel column, using an eluent of chloroform/methanol/water (75:25:2.5). UV-absorbing and saponin-free fraction (Rf 0.45) was crystallized from methanol to yield colorless needles. (120mg) mp232~233°, $[\alpha]_D^{20}$ -60.4° (c=0.5, water), UV λ_{max} in methanol (log ε):260.5(4.13), ^{1}H-NMR(DMSO-d_6, δppm):

3.6(2H, C'_5-H_2), 4.10(2H, m, $C'_{3,4}$-H×2), 4.50(1H, q, C'_2-H), 5.10 (1H, s, C_2'-OH), 5.40 (2H, s, $C'_{3,5}$-OH×2), 5.85 (1H, d, J=5.8Hz, C'_1-H), 7.30 (2H, s, NH_2), 8.10(1H, s, C_2-H), 8.30 (1H, s, C_8-H).

Indentification of D-*Glucose from Compound I*

Compound I (3mg) was dissolved in dioxane (150 μl) and 10% H_2SO_4 (50μl). The mixture was heated for 45 minutes in a boiling water bath. After cooling, water (100μl) and chloroform were added. After extraction, the aqueous layer was neutralized with 5% $Ba(OH)_2$ and the supernatant was taken by centrifugation.

D-Glucose was identified by TLC (silica gel, chloroform / methanol / water=15:10:2.5) and GLC (TMS-derivative, column 3% SE-30 4mm×1.5m, Tc 180°, T_D 200°, N_2 300m/min, t_R 3.2, 4.6min).

Isomaltol as Aglycone of Compound I

Compound I (60mg) was dissolved in 0.1N HCl and heated for 20 min in a 94°C water bath. The reaction mixture was extracted with $CDCl_3$(1ml). The $CDCl_3$ layer was dried over sodium sulfate anhydrous and was subjected to ^{1}H-NMR measurement. ^{1}H-NMR ($CDCl_3$, δppm): 2.42 (3H, s, $COCH_3$), 4.64 (1H, br. s, OH), 6.29 (1H, d, J=2Hz), 7.28 (1H, d, J=2Hz), UVλ_{max}: 281.5nm, IRν_{max}^{KBr}cm^{-1}: 3410 (OH), 1590~1570, 1480, 875, 863, 800, 770.

*Preparation of Isomaltol-α-*D-*glucopyranoside from Maltose*

Isomaltol-α-D-glucopyranoside was prepared by the Hodge's method.[4] Maltose (14.4g) and piperidine (14ml) were added to absolute ethanol (12ml), and then glacial acetic acid (2.5ml) was dropped into the mixture with stirring. The mixture was kept in 78°C water bath, and trimethylamine (2ml) was added. After 10 hrs, trimethylamine (2ml) was added again. And the reaction mixture was further heated at 78° for 10hrs. The reaction mixture was concentrated under reduced pressure and was subjected to column chromatography, eluting with chloroform/methanol/water (75:25:2.5). UV-absorbing fractions were collected and crystallized from methanol. Colorless needles, 1.1g yield, mp 142°. ^{1}H-NMR(DMSO-d_6) was superimposed with that of compound I. The synthesized compound was identical with compound I by GLC. (TMS derivative with TMS-imidazole, column OV-1 4mm×1.5m, Tc 210°, T_D 240°, N 240ml/min, t_R 11.4 min.)

Identification of D-*glucose from Compound II*

A solution of compoud II (2mg) in 0.4ml of 2.5% H_2SO_4 (dioxane: 10% H_2SO_4=3:1) was heated for 45 min. The reaction mixture was diluted with water (1ml) and extracted with chloroform. The aqueous layer was neutralized by 5% Ba $(OH)_2$ and the supernatant was taken by centrifugation. D-Glucose was detected by TLC and GLC.

LITERATURE CITED

1) Han, B.H., Park, M.H., Woo, L.K., Woo, W.S. and Han, Y.N.: Studies on the antioxidant components of Korean ginseng (I). *Korean Biochem. J.* **12**, 33(1979).

2) Han, B.H., Park, M.H. and Han, Y.N.: Studies on the antioxidant components of Korean ginseng (III). Identification of phenolic acids. *Arch. Pharm. Res.* **4**, 53 (1981).

3) Matsuura, H., Hirao, Y., Yoshida, S., Kunihiro, K., Fuwa., T., Kasai, R. and Tanaka, O.: Study of red ginseng: New glucosides and a note on the occurence of maltol. *Chem. Pharm. Bull.* **32**, 4674 (1984).

4) Hodge, J.E. and Nelson, E.C.: Preparation and properties of galactosyl-isomaltol and isomaltol. *Cereal Chemistry* **38**, 207(1961).

5) Fisher, B. E. and Hodge, J.E.: The structure of isomaltol. *J. Org. Chem.* **29**, 776 (1964).