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Chemical Studies on the Ether-Soluble Alkaloidal Fraction of *Panax ginseng*. Isolation of 1-carbobutoxy-β-carboline and 1-carbomethoxy-β-carboline

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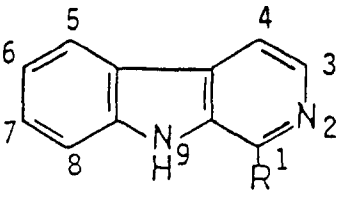
Abstract□Two alkaloids were isolated from an ether-soluble alkaloidal fraction of *Panax ginseng*. They were identified as 1-carbobutoxy-β-carboline and 1-carbomethoxy-β-carboline.

Key words□*Panax ginseng*, Araliaceae, β-carboline, alkaloid, 1-carbobutoxy-β-carboline, 1-carbomethoxy-β-carboline.

Alkaloidal fraction of *Panax ginseng* was reported to have various biological and pharmacological activities such as anticancer effect in HeLa cells[1], increase of enzyme activities of succinate, serum lactic and glutamic dehydrogenases[2] and decrease of total cholesterol in serum and liver tissue of rats[3]. But the presence of alkaloids in ginseng has been very uncertain until recently, three minor β-carboline alkaloids reported by Han's group[4] in 1986. Further chemical study on the alkaloidal fraction has resulted in the isolation of two unidentified β-carboline alkaloids together with a known β-carboline alkaloid. This paper deals with the structural elucidation of these β-carboline alkaloids.

Alkaloidal fraction of ginseng was subjected to silica gel column chromatography and preparative thin layer chromatography(TLC) to yield compounds II, III and IV in the order of increasing polarity. Compounds II, III and IV showed positive Dragendorff reaction and UV spectrum of each compound exhibited the typical absorption pattern of β-carboline skeleton[5].

Recrystallization of compound IV gave palely yellowish needle, mp 164°. Its IR spectrum showed absorption bands due to amine (3375 cm^{-1}) and conjugated carbonyl (1680 cm^{-1}) groups. ^{1}H-NMR spectrum of this compound exhibited a carbomethoxy signal at δ 4.11 (3H, s) along with signals of aromatic protons similar to those of β-carboline[6] at δ 7.18-8.54, suggesting that a carbomethoxy group was attached to C_1 and also did a broad singlet of



II R= $COOC_4H_9$

III R= $COOC_2H_5$

IV R= $COOCH_3$

indolic NH at δ 9.82 which disappeared by treatment with D_2O. It was further supported by its mass spectrum showing a prominent ion peak at *m/z* 168 corresponding to the elimination of carbomethoxy group. On the basis of these data, compound IV was identified as 1-carbomethoxy-β-carboline, which was first isolated from ginseng. This alkaloid has already been isolated from *Picrasma quassioides*[7], *Polygala tenuifolia*[8], *Lycium chinense*[9], *Codonopsis lanceolata*[10].

Compound II was recrystallized from acetone to give palely yellowish crystal, mp 92-94°. Its ^{1}H-NMR spectrum exhibited typical carbobutoxy signal at δ 0.97 (3H, t, J=6.9Hz), 1.38 (2H, quin, J=6.7Hz), 1.75 (2H, sex, J=6.8Hz) and 4.50 (2H, t, J=6.9Hz) and also signals of aromatic protons

similar to those of β-carboline at δ 7.21-8.56, suggesting that one carbobutoxy group was located at C_1. It was also supported by its mass fragments at *m/z* 240, 213, 196 and 168 produced by degradation of a carbobutoxy group from the molecular ion and its mass spectral data were also identical with those of a product synthesized from compound IV with p-toluenesulfonic acid in n-butanol by transesterification. On the basis of these data, compound II was identified as 1-carbobutoxy-β-carboline, which was first isolated from ginseng. This alkaloid has already been isolated from *Polygala tenuifolia*[8].

Compound III was recrystallized from acetone to give palely yellowish needle, mp 121°. Its IR spectrum showed amine and conjugated carbonyl groups at 3400 and 1670 cm^{-1}, respectively. The presence of a carboethoxy group in its structure was confirmed by the signals of ethyl group at δ 1.50 (3H, t, J = 7.3Hz) and 4.48 (2H, q, J = 7.3Hz) in the ^{1}H-NMR spectrum. It was further supported by its mass fragments at *m/z* 211, 196 and 168 produced by degradation of carboethoxy group from the molecular ion peak. Therefore, compound III was identified as 1-carboethoxy-β-carboline, which was previously isolated from ginseng[4].

EXPERIMENTAL METHODS

The melting points were determined on Gallenkamp melting point apparatus and are uncorrected. IR spectra were recorded in KBr disc by a Perkin-Elmer 599B spectrophotometer. UV spectra were run with a Shimadzu Model UV-200S double beam spectrophotometer. ^{1}H-NMR spectra were recorded on a Varian FT-80A spectrometer operating at 80 MHz in $CDCl_3$ and chemical shift values are quoted in ppm (δ) downfield from TMS as an internal standard. Mass spectra were taken on a Varian Model MAT 212 GC/MS system equipped with direct inlet system and operating at 70eV. Column chromatography was carried out on silica gel (Merck Art. 9385, 230-400 mesh). TLC and preparative-TLC were performed on precoated silica gel $60F_{254}$ plate (Merck) and detected by Dragendorff's reagent or by UV illumination.

Plant material

The roots of *Panax ginseng* (white ginseng with skin) were taken in September, 1984 from Jungpyoung Experiment Station, Jungpyoung, Korea.

Extraction and fractionation

Fifty kg of dried ginseng containing about 10% moisture was extracted with MeOH under reflux for 6 hr three times. Removal of the solvent under reduced pressure gave a MeOH extract (9.8 kg). The MeOH extract was suspended in water (20L) and then extracted with ether (20L × 4). Removal of the solvent under reduced pressure provided an ether extract (770g). The ether extract was dissolved in ether (7L) and the solution was partitioned into 5% aqueous HCl. After the aqueous layer was exhaustively washed with ether (7L × 4) and basified to pH 9 with c-NH_4OH, it was extracted with $CHCl_3$ (8L × 3). The $CHCl_3$ extract was dried over anhydrous sodium sulfate and filtered, and removal of the solvent under reduced pressure gave crude alkaloidal fraction (2.6g, 5×10^{-3}%).

Isolation of alkaloids

The alkaloidal fraction (2.5g) was applied to a column of silica gel (3 × 30 cm) and eluted successively with $CHCl_3$ and $CHCl_3$/MeOH (100:1, 70:1, 50:1, 30:1, and 10:1) to give fractions I (128 mg), II (890 mg), III (254 mg), IV (272 mg) and V (188 mg). Fraction I was further purified by preparative-TLC on silica gel with hexane/ethylacetate (4:1) to yield compound II (5.3 mg), which was recrystallized from acetone. Fraction II was repeatedly chromatographed on silica gel column with $CHCl_3$/MeOH (50:1) to give fractions II-1 (102 mg) and II-2 (380 mg). Fraction II-1 was further purified by preparative-TLC on silica gel with hexane/ethylacetate (2:1) to yield compounds III (6.8 mg) and IV (18 mg), which were recrystallized from acetone.

Compound II

mp: 92-94°; UV λmax in MeOH (log ε): 245 (4.02), 256 (4.03), 272 (4.08), 300 (3.86), 368 (3.66); MS *m/z* (rel. int. %): 268 (M^+, 15.1) 240 (M^+-C_2H_5 + H, 0.9), 213 (M^+-C_4H_9 + 2H, 2.7), 196 (M^+-OC_4H_9 + H, 10.7), 195 (M^+-OC_4H_9, 5.4), 182 (3.6), 168 (M^+-$COOC_4H_9$ + H, 100), 167 (M^+-$COOC_4H_9$, 17.8), 140 (16.0), 139 (12.1), 114 (7.2), 113 (4.4); ^{1}H-NMR: 9.83 (1H,br,s,NH, exchanged with D_2O), 8.56(1H,d,J = 5.2Hz, C_3-H), 8.14 (1H,d,J = 8Hz, C_5-H), 8.12 (1H,d,J = 5Hz, C_4-H), 7.60-7.45 (2H,m, $C_{6,8}$-H), 7.30-7.21 (1H,m, C_7-H), 4.50 (2H,t,J = 6.9Hz, -OCH_2), 1.75 (2H, sex, J = 6.8Hz,-$CH_2CH_2\mathbf{CH_2}CH_3$), 1.38 (2H,quin, J = 6.7Hz,-$CH_2\mathbf{CH_2}CH_2CH_3$), 0.97 (3H,t, J = 6.9Hz,-$CH_2CH_2CH_2\mathbf{CH_3}$)

Compound III

mp: 121°; UV λmax in MeOH (log ε): 211 (4.04), 245 (4.03), 257 (4.03), 271 (4.05), 300 (3.85),

368 (3.62); IR (cm^{-1}): 3400 (-NH), 1670 (C=O), 1630 (aromatic C=C), 1210, 1190, 1075; MS *m/z* (rel. int. %): 240 (M^+, 30.5), 211 (M^+-C_2H_5, 0.4), 196 (M^+-OC_2H_5+H, 4.8), 195 (M^+-OC_2H_5, 1.3), 168 (M^+-$COOC_2H_5$+H,100), 167 (M^+-$COOC_2H_5$, 17.1), 140 (20), 114 (8.2), 113 (5.2); ^{1}H-NMR: 9.85 (1H,br,s,-NH, exchanged with D_2O), 8.50 (1H,d,J=5Hz, C_3-H), 8.10 (1H,d,J=7.5Hz, C_5-H), 8.08 (1H,d,J=5Hz, C_4-H), 7.54-7.44 (2H, m,$C_{6,8}$-H), 7.36-7.15 (1H,m,C_7-H), 4.48 (2H, q, J=7.3Hz,-**CH_2**CH_3), 1.50 (3H, t,J=7.3Hz,-CH_2 **CH_3**)

Compound IV

mp: 164°; UV λmax in MeOH (log ε): 216 (4.18), 245 (4.02), 258 (4.02), 274 (4.04), 300 (3.83), 371 (3.51); IR (cm^{-1}): 3375 (NH), 1680 (C=O), 1630 (aromtic C=C), 1490 (indole C=C), 1250, 1070; MS *m/z* (rel. int. %): 226 (M^+, 46.0), 194 (M^+-CH_3OH, 8.2), 168 (M^+-$COOCH_3$+H, 100), 166 (M^+-CH_3OH-CO, 80.1), 140 (18.2), 139 (23.4), 114 (14.0), 113 (10.8); ^{1}H-NMR: 9.82 (1H, br, s,NH, exchanged with D_2O), 8.54 (1H,d, J=5Hz, C_3-H), 8.12 (1H,d,J=8Hz, C_5-H), 8.10 (1H,d, J=5Hz, C_4-H), 7.58-7.43 (2H,m,$C_{6,8}$-H), 7.35-7.18 (1H,m, C_7-H), 4.11 (3H,s,OCH_3)

Conversion of IV to II by transesterification

According to Rehberg 's method[11], ten mg of IV was refluxed with p-toluenesulfonic acid in n-butanol (5 m*l*) for 6 hr. After cooling, the reaction mixture was neutralized with saturated sodium carbonate solution and extracted with ether (10 m*l*×3). The ethereal solution was dried over anhydrous sodium sulfate, filtered and evaporated to dryness *in vacuo*. Preparative-TLC of the residue on silica gel with hexane/ethylacetate (2:1) gave palely yellowish crystal (4 mg) from acetone. mp: 91°;MS *m/z* (rel. int. %): 268 (M^+, 7.3) 196 (M^+-OC_4H_9+H, 7.3), 195 (M^+-OC_4H_9, 4.8), 182 (3.2), 168 (M^+-$COOC_4H_9$+H,100), 167 (M^+ -$COOC_4H_9$, 23.7), 140 (26.5), 114 (11.3), 113 (7.4)

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