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Alkaloidal Components of *Panax ginseng*

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Abstract□Over twelve alkaloids were delected in the roots of *Panax ginseng* C.A. Meyer. Among them three alkaloids were isolated and were identified as Nᵩ-formylharman, ethyl ß-carboline-1-carboxylate and perlolyrine on the basis of spectroscopic studies.

Keywords□*Panax ginseng* C.A. Meyer, Ginseng alkaloids, N_9-formylharman, Ethyl-ß-carboline-1-carboxylate, Perlolyrine, ß-carboline.

Ginseng radix (*Panax ginseng* C.A. Meyer) may be the most well known traditional medicine which has been most extensively studied. Many kinds of the constituents of ginseng were isolated; for examples, saponins, phytosterols, volatile oils, saccharides, phenolic acids and their glycosides, amino acids, peptides, proteins, peptide glycan, nucleosides, choline and some vitamines.[1,2] However, any alkaloid was not isolated from ginseng until now, although the presence of α-pyrrolidone was determined by GC/MS technique.[3]

In the present communication, we report the isolation and structure characterization of three ginseng-alkaloids, in addition to the detection of over twelve alkaloids in ginseng extracts.

A crude alkaloid fraction was prepared from the ethanol extracts of ginseng by the usual method of acid-base extraction. Its thin layer chromatogram over silica gel showed more than twelve spots being positive in Dragendorff's reagent. (Fig. 1) The alkaloidal fraction was subjected to silica gel column chromatography eluting with chloroform/ethyl acetate/methanol and then chloroform/methanol to afford Fr. 1 and Fr.2. Preparative TLC of each fraction yielded three crystalline ginseng-alkaloids, GA-4,-11 and -12.

GA-4 appeared as pale yellowish crystals. The mass spectrum showed the molecular weight of 210 in accord with $C_{13}H_{10}N_2O$, which was confirmed by elemental analysis. The UV spectrum showed the multiple absorption maxima, which was characteristic of ß-carboline.[4] The ^{1}H-NMR spectrum of GA-4 resembled that of harman except the presence of an additional singlet at δ10.25 (1H, br.s.), whose proton resisted exchange with D_2O. The singlet was attributed to the proton of an aldehyde, since the IR spectrum of GA-4 showed a carbonyl band at 1670cm^{-1}. This was also supported by the mass fragments at m/z 182 (M^+-CO) and 168 (M^+-CO-

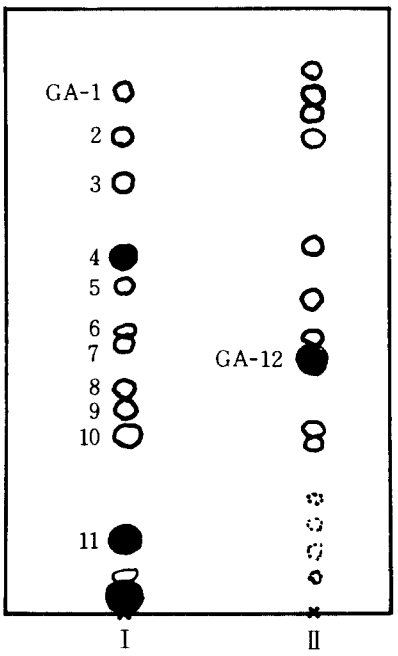

Fig. 1. Thin layer chromatograms of the alkaloidal fraction of ginseng extracts. GA, ginseng alkaloid.

Developing solvents; I:hexane/ethylacetate (2:1), II:chloroform/methanol (10:1). Detection; Dragendorff's reagent.

CH_3 + H), and by the production of harman on alkaline hydrolysis of GA-4. By these results GA-4 was identified as N_9-formylharman. GA-4 was finally characterized by the direct comparison of the spectral data of N_9-formylharman, which was previously isolated by us from *Codonopsis lanceolata*,[5] *Polygala tenuifolia*,[6], and *Lycium chinense*.[7]

GA-11 was obtained as needle crystals. Its molecular formula was determined as $C_{14}H_{12}N_2O_2$ (M^+ at m/z 240) by the mass spectrum, and the fragment ions at m/z 211, 198, 168 and 167 could be ascribable to the loss of C_2H_5, C_2H_5O, $COOC_2H_5$ + H and $COOC_2H_5$, respectively. The UV spectrum was characteristic of ß-carboline type alkaloid[4], and the IR spectrum showed an absorption band due to the conjugated carbonyl group at 1660 cm^{-1}. The ^{1}H-NMR spectrum showed the typical quartet-triplet pattern of ethyl group at δ4.45 (2H, q, J = 7.2Hz) and 1.37 (3H, t, J = 7.2Hz). Aromatic protons at the range of δ7.15-8.42 were similar to those of norharman.[5,6,8] While N_9-H of norharman is appeared at δ9.03 (1H, br.s), that of GA-11 was shown at δ9.84 (1H, br.s). The down-field shift may be due to the carboethoxy group in the *peri* position, suggesting that the group was attached to C_1. These data supported the structure of GA-11 as ethyl ß-carboline-1-carboxylate. The structure was finally identified by the direct comparison of the spectral data, mp. and TLC behavior of the authentic sample, which was previously isolated by us from *Polygala tenuifolia*.[6] Ethyl ß-carboline-1-carboxylate has already been isolated from *Picrasma quassioides* Bennet as one of inhibitors of c-AMP phosphodiesterase[9,10] and an antibacterial substance.[11]

GA-12 was obtained as brownish yellow needles. The mass spectrum exhibited them molecular weight of 264 in accord with $C_{16}H_{12}N_2O_2$. Its ^{1}H-NMR signals at the aromatic region of δ7.27-8.37 were very similar to those of ethyl ß-carboline-1-carbolxylate (GA-11). Signals at δ 6.42 (1H, d, J = 3.3Hz), 7.17 (1H, d, J = 3.3Hz) and 4.77 (2H, s) suggested the presence of 5-hydroxymethyl-2-furyl group. Acetylation of GA-12 with acetic anhydride/pyridine yielded monoacetate. These data were consisted with those of perlolyrine, which was already isolated from *Codonopsis lanceolata*[5], *Polygala tenulfolia*[6] and *Lycium chinensis*[7] by us. This alkaloid was first isolated from *Lolium perenne*. L.[12]

EXPERIMENTAL METHODS

Melting points were determined on Mitamura Riken heat block Model-MRK and were uncorrected. Gilford system 2600 UV/VIS spectrophotometer was used for UV spectra. IR spectra were measured on Perkin-Elmer 281 B IR spectrophotometer in KBr pellets. ^{1}H-NMR spectra were determined on Varian Model FT 80A NMR spectrometerwith TMS as internal reference. Mass spectra were measured on Hewlett-Packard Model HP 5985B GC/MS system. Elemental analysis was carried out on Carlo Erba strumentazione Model 1106. Column chromatography was carried out over silica gel 60 (Merck Art. 7734). TLC and preparative TLC were performed on precoated silica gel 60 GF254 plates and spots were detected with Dragendorff's reagent or UV irradiation.

Extraction and isolation of ginseng alkaloids

Twenty eight Kg of powdered dried-roots (white ginseng) of *Panax ginseng* C.A. Meyer was extracted with 70% EtOH (5 times). The extract was concentrated to yield 7.4kg of syrup, which was suspended in water (30 *l*) and extracted with ethyl ether (30 *l* × 2). The ethereal extract was evaporated to 3 *l*/volume and was extracted with 5% HCl (1*l* × 2 times). The 5% HCl layer was exhaustively washed with ethyl ether, and basified to pH 9 with c-NH_4OH. The alkaline solution was extracted with $CHCl_3$ (3*l* × 2 times). The $CHCl_3$ extract was dried over Na_2SO_4 and freed from solvent to yield 0.8g of alkaloid fraction. The $CHCl_3$ extract was fractionated on a silica gel column (2 × 20 cm) using $CHCl_3$-EtOAc-MeOH (50: 10:1, 300ml) and then $CHCl_3$-MeOH (10: 1, 330ml) to give fr. 1 (50mg) and fr. 2 (260mg). Fraction 1 was subjected to preparative TLC with hexane-EtOAc (4: 1). The plate was developed two times, and bands of Rf values 0.4 and 0.1 were scrapped off and extracted with $CHCl_3$-MeOH (10: 1). respectively, giving GA-4 and GA-11 Fraction 2 was subjected to preparative TLC with $CHCl_3$-MeOH (10: 1). The plate was developed two times and the band of Rf 0.5 was scrapped off and extracted with $CHCl_3$-MeOH (3: 1), giving GA-12.

N_9-Formylharman, GA-4

Recrystallization from $CHCl_3$ -MeOH yielded pale yellow needles (3.7mg). yield 1.3×10^{-5} %. mp 178-179°C. UV λ_{max}^{MeOH} nm (log ε) : 212.5, 251.5, 261, 284, 307.5, 379 (4.12, 5.85, 5.85, 4.00, 5.70, 5.69). IR ν_{max}^{KBr} cm^{-1} 1670 (C = 0) ^{1}H-NMR $CDCl_3$ δ : 2.89 (3H, s, CH_3), 7.18-7.37 (1H, m, 7-H), 7.55-7.63 (2H, m, 6,8-H), 8.13 (1H, d, J = 5Hz, 4-H), 8.15 (1H, d, J = 7.4Hz, 5-H), 8.53 (1H, d, J = 5Hz, 3-H), 10.25 (1H, br.s, CHO). MS m/z (%): 210 (M^+, 28.6), 182 (M^+-CO,17.1), 168 (M^+-CO-CH_3 + H, 37.1), 140 (17.1), 114 (8.6), 113 (14.3), Anal. Calcd. for $C_{13}H_{10}N_2O$: C, 74.27; H, 4.80; N, 13.32, Found: C, 73.01; H,5.10; N, 13.08.

Ethyl ß-carboline-1-carboxylate, GA-11

Recrystallization from $CHCl_3$-MeOH yield needles (4.2mg). yield 1.5×10^{-5}%. mp 122-123°C. IR ν_{max}^{KBr} cm^{-1}: 1660 (C = O), UV λ_{max}^{MeOH} nm (log ε): 246.5, 258, 275, 301, 370 (4.0, 4.0, 4.02, 3.91, 3.71). ^{1}H-NMR $CDCl_3$ δ : 1.37 (3H, t, J = 7.2Hz, $C\underline{H}_3CH_2O$), 4.45 (2H, q, J = 7.2Hz, $CH_3C\underline{H}_2O$), 7.15 (1H, m, 7-H), 7.34 (2H, m, 6,8-H), 7.85 (1H, d, J = 5Hz, 4-H), 7.89 (1H, d, J = 7.5Hz, 5-H), 8.42 (1H, d, J = 5Hz, 3-H), 9.84 (1H, br.s, NH). MS m/z (%): 240 (M^+, 21.2), 211 (M^+-C_2H_5, 0.5), 195 (M^+-C_2H_5O, 1.6), 168 (M^+ -$COOC_2H_5$ + H, 100), 167 (M^+-$COOC_2H_5$,

16.3), 166 (40.0), 140 (20.5), 114 (9.5), 113 (7.4).

Perlolyrine, GA-12

Recrystallization with $CHCl_3$ yielded yellow needles (46mg). yield 1.5×10^{-4}%. mp 166°C. UV λ_{max}^{MeOH} nm (logε): 216, 238.5, 253.5, 274, 292, 307, 368, 381 (3.51, 3.49, 3.41, 3.38, 3.41, 3.28, 3.21, 3.24). IR ν_{max}^{KBr} cm^{-1}: 3370 (NH, OH), ^{1}H-NMR $CDCl_3$ δ: 4.77 (2H, s, -CH_2O), 6.42 (1H, d, J=3.3Hz, 4′-H), 7.17 (1H, d, J=3.3Hz, 3′-H), 7.27 (1H, m, 7-H), 7.53 (2H, m, 6,8-H), 7.78 (1H, d, J=5.2Hz, 4-H), 8.05 (1H, d, J=7.7Hz, 5-H), 8.37 (1H, d, J=5.2Hz, 3-H), 9.65 (1H, br. s, NH). MS m/z(%): 264 (M^+, 100), 247 (M^+-OH, 68.4), 246 (M^+-H_2O, 47.4), 235 (10.5), 233 (M^+-CH_2OH, 5.5), 218(15.8), 205(M^+-$C_2H_3O_2$, 21.1), 168(35.8), 167 (M^+-$C_5H_5O_2$, 20.1), 140 (21.1), 114 (15.8).

Acetylation of GA-12 (5mg) with acetic anhydride-pyridine yielded its acetate, which was crystallized from EtOAc to give needles (4mg). mp 160°C. ^{1}H-NMR $CDCl_3$ δ: 2.15 (3H, s, $COCH_3$), 5.24 (2H, s, -CH_2O-), 6.60 (1H, d, J=3.4 Hz, 3′-H), 7.18 (1H, d, J=3.4Hz, 4′-H), 7.24-7.36 (1H, m, 7-H), 7.63-7.55 (2H, m, 6,8-H), 7.85 (1H, d, J=5Hz, 4-H), 8.10 (1H. d, J=8Hz, 5-H), 8.43 (1H, d, J=5Hz, 3-H), 9,93 (1H, br. s, NH). MS m/z (%): 306 (M^+, 17.7), 263 (M^+-$COCH_3$, 16.9), 247(M^+-$OCOCH_3$, 80.3), 246 (M^+-CH_3COOH, 100), 218 (17.7), 205 (8.9), 168 (8.9), 167 (28.2), 140 (20.2), 114 (7.3), 113 (9.7).

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