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Spinacine from *Panax ginseng*

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Abstract □ An alkaloid was isolated from water-soluble fraction of *Panax ginseng* roots. It was characterized by spectroscopic data and synthesis as 4,5,6,7-tetrahydroimidazo(4,5-c) pyridine-6-carboxylic acid or spinacine, which was first isolated from the plant kingdom.

Keywords □ Spinacine, alkaloid, *Panax ginseng*.

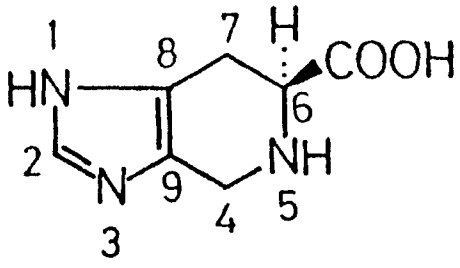
Some β-carboline alkaloids were isolated from an ether-soluble alkaloidal fraction of the roots of *Panax ginseng* C.A. Meyer.[1,2] They are also found in several plants.[1] In the present communication, we report the isolation of a water-soluble alkaloid from the ginseng root.

After removal of ether- and butanol-soluble constituents of fresh ginseng roots, the remaining water-soluble part did not show a positive color reaction by Dragen-dorff's reagent, but exhibited two positive spots by Pauly reaction on a TLC plate.

The water-soluble fraction was subjected to ion exchange chromatography on Dowex 50w × 8 and then Amberite CG-50 columns. After further purification through gel-filtration and silica gel column chromatography, we isolated compound I and histidine.

Compound I gave very weak color reaction by ninhydrin and was very stable by the treatment with 6N HCl at 100 °C for 4 hrs. It was optically active, $[\alpha]_D^{22}$ −165 °. Its UV spectrum showed an absorption peak at 208.5 nm, which did very resemble that of L-histidine. Its IR spectrum showed the presence of NH (3380 cm^{-1}) and α-amino acid (3100, 1630, 1500, 1400, 1250 cm^{-1}).

The ^{1}H-NMR spectrum of compound I resembled that of L-histidine except the absence of a signal due to one of two aromatic protons on imidazole ring and the presence of an additional ABq signal at δ4.33 (2H, J = 15 and 36Hz). Its ^{13}C-NMR spectrum showed seven carbon signals, *i.e.* $-CH_2-$ × 2, -CH- × 1, =CH- × 1, C = × 2, and COOH × 1. Secondary ion mass (SIMS) spectra of I in glycerol and glycerol plus NaI exhibited the peaks of *m/z* 168 $(M+1)^+$ and 190 $(M+Na)^+$, respectively, suggesting the molecular formula of I to be $C_7H_9N_3O_2$. These data indicated I to be spinacine.



spinacine

Assignment of ^{1}H- and ^{13}C-NMR signals (see "EXPERIMENTAL METHODS") also supported I as spinacine. The structure was finally identified by the direct comparison with the spectral data and TLC behaviour of the authentic sample, which was synthesized from L-histidine and formaldehyde.[3] Thus, I was established as 4,5,6,7-tetrahydroimidazo(4,5-c)pyridine-6-carboxylic acid or spinacine, which was first isolated from the liver of the shark, *Acanthias vulgaris*[4] and later from the crab, *Crangon vulgaris*.[4] It was first isolated from the plant kingdom.

EXPERIMENTAL METHODS

Gilford system 2600 UV/visible spectrophotometer was used for UV spectra. IR spectra were measured on Perkin-Elmer 281B IR spectrophotometer in KBr pellets. NMR spectra were determined on a Nicolet NT-360 spectrometer. Secondary ion mass (SIMS) spectra were measured on a Hitachi M-80 high resolution mass spectrometer.

Extraction and isolation of spinacine

Fresh ginseng radix(1.5 Kg, 6 years old) were crushed with a bladed mixer and were extracted with 50% MeOH (three times) on a boiling water

bath. The extract was freed from MeOH, and extracted with ether and then BuOH. The remaining aqueous solution was subjected to ion exchange chromatography on a Dowex 50w × 8 column (H^+, 3 × 20 cm). The resin column was washed with a large amount of water, and then eluted with 1 N-NH_4OH. Fractions showing positive Pauly reaction were pooled up and were concentrated under vacuum to obtain a syrupy residue.

The residue was subjected to ion exchange chromatography on a Amberlite CG-50 column (H^+, 3 × 10 cm). The Amberlite column was washed with a large amount of water, and then eluted with 4 N-acetic acid. Fractions showing positive Pauly reaction were pooled up and were concentrated under vacuum to obtain a viscous syrup.

The syrup was gel-filtered through a Sephadex G-10 column(2.8 × 92 cm) eluting with 5% EtOH. Fractions showing positive Pauly reaction were pooled up and were concentrated to obtain a residue. The residue was chromatographed on a silica gel column(2.8 × 45 cm) eluting with a solvent system of $CHCl_3$/MeOH/c-NH_4OH(17:10:2.5) to divide into two fractions. Concentration of each fraction yielded compound I (50 mg) and histidine (30 mg).

Compound I (spinacine)

Pure chromatographically ($CHCl_3$/MeOH/c-NH_4OH = 17:10:2.5 and 50% EtOH) and very soluble in water, but insoluble in alcohol, pyridine and other organic solvents. Very stable in 6N HCl at 100 °C for 4 hrs. Positive by Pauly reagent(red color) and weakly positive by ninhydrin; negative by Dragendorff's reagent. Amorphous powder (Literature[4], 264 °); $[\alpha]_D^{22}$ −165 °[Literature[4], $[\alpha]_D^{22}$ −169.9 °]; UV in water: 208.5 nm (ε = 15,000); IR in KBr (cm^{-1}): 3380 (br, -NH), 3100 (br), 1630 (br), 1500, 1400, 1250 (α-amino acid); ^{1}H-NMR (D_2O, 360 MHz): δ 3.01 (1H, dd, J = 10.5 and 16.5 Hz, one H on C_7-H_2), 3.31 (1H, dd, J = 5.5 and 16.5 Hz, the other H on C_7-H_2), 3.70 (1H, s, 1H of N1), 4.10 (1H, dd, α-H of C6), 4.33 (2H, ABq, J = 15 and 36 Hz, 2H of C4-H_2), 8.02 (1H, br. s., 1H of C2); ^{13}C-NMR (D_2O, DSS for reference): δ 136.8 (C2), 59.7 (C4), 56.7 (C6), 40.4 (C7), 124.3 (C8), 118.6 (C9), 173.2 (COOH); SIMS in glycerol: m/z 335 $(2M+1)^+$, 168 $(M+1)^+$; in glycerol plus NaI: m/z 357 $(2M+Na)^+$, 190 $(M+Na)^+$.

Synthesis of spinacine

The mixture of L-Histidine (0.3 g) and formaldehyde (35%, 1 m*l*) in water (3 m*l*) was heated at 55 °C for 4 hrs, and then at 98 °C for 2 hrs.[3] The reaction mixture was concentrated under vacuum to yield colorless residue. The residue was dissolved in water (3 m*l*), and then was adjusted to pH 7 with d-NH_4OH to give colorless powder. mp 260 ° [Literature[4], 264 °], $[\alpha]_D^{20}$ −170 °. Its IR, UV, ^{1}H-spectra and Rf value on silica gel plate (solvent, $CHCl_3$/MeOH/c-NH_4OH = 17:10:2.5, Rf = 0.12) were identical with those of compound I.

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