Alkaloids from *Panax Ginseng*

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Abstract: Over twelve alkaloids were detected in the ether-soluble alkaloidal fraction of *Panax ginseng* roots. Among them, three alkaloids were identified as N_9-formylharman, ethyl β-carboline-1-carboxylate and perlolyrine on the basis of chemical and spectroscopic studies. And also spinacine was isolated from the water-soluble fraction of the roots.

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 of three ether-soluble alkaloids and one water-soluble alkaloid from the roots of *Panax ginseng*.

Ether-Soluble Alkaloids

A crude ether-soluble 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 β-carboline[4]. The ^{1}H-NMR spectrum of GA-4 resembled that

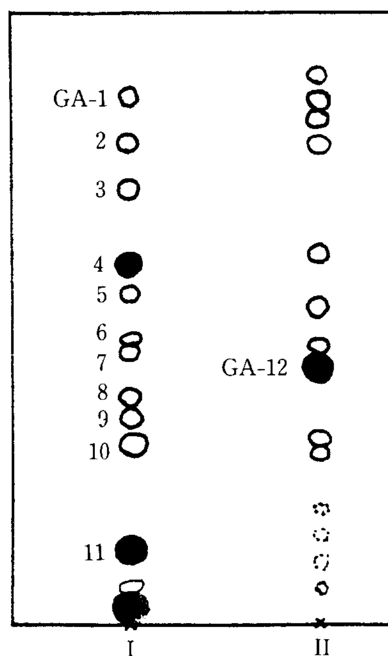


Fig.1 *Thin layer chromatograms of the ether-soluble alkaloidal fraction of ginseng roots. GA, ginseng alkaloid; developing solvents, I: hexane/EtOAc(2:1), II: $CHCl_3$/MeOH(10:1); detection, Dragendorff's reagent; plate, silica gel.*

of harman except the presence of an additional singlet at δ 10.25(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 1670 cm^{-1}. This was also supported by the mass fragments at m/z 182(M^+-CO) and 168(M^+-$COCH_3$+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-fromylharman, which was previously isolated by us from *Codonopsis lanceolata*[5], *Polygala tenuifolia*[6], and *Lycium chinese*[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 β-carboline type alkaloid[4], and the IR spectrum showed 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 δ 4.45(2H, q, J=7.2Hz) and 1.37(3H, t, J =7.2Hz). Aromatic protons at the range of δ 7.15-8.42 were similar to those of norharman[5,6,8]. While N_9-H of norharman is appeared at δ 9.03(1H, br.s), that of GA-11 was shown at δ 9.84(1H, br.s). The downfield shift may be due to the carboethoxy group in the *peri* position, suggesting that the group was attached to Cl. These data supported the structure of GA-11 as ethyl β-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 β-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 the molecular weight of 264 in accord with $C_{16}H_{12}N_2O_2$. Its ^{1}H-NMR signals at the aromatic region of δ 7.27-8.37 were very similar to those of ethyl β-carboline-1-carboxylate(GA-11). Signals at β 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 tenuifolia*[6] and *Lycium chinensis*[7] by us. This alkaloid was first isolated from *Lolium perenne*. L[12].

The chemical structures and isolation yields of the β-carboline alkaloids isolated from *P. ginseng*, and other plants are summarized in Table I. Recently, Park *et al.* isolated more three alkaloids from ginseng radix; butyl β-carboline-1-carboxylate [13], methyl β-carboline-1-carboxylate[13] and 4-methyl-5-thiazol ethanol[14]. Their isolation yields were in the ranges of 1.3-15×10^{-5}%.

Water-Soluble Alkaloid

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 Dragendorff's reagent, but exhibited three spots positive by Pauly reaction on a TLC plate(Fig.2).

The water-soluble fraction was subjected to ion exchange chromatography by Dowex 50w×8 and then Amberlite CG-50. After further purification through gel-filtration and silica gel chromatography, we isolated compound I, and compound II plus histidine. Compound II and histidine could not be separated from each other, until now.

Compound I gave positive Pauly reaction and very weak color reaction by ninhydrin, but was negative by Dragendorff's reagent. I was very stable by the treatment with 6N HCl at 100℃ for 4 hrs. Its UV spectrum showed an absorption peak at 208.5 nm(ε=15,000), which did very resemble that of L-histidine. IR spectrum

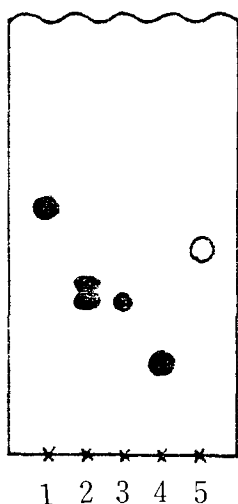
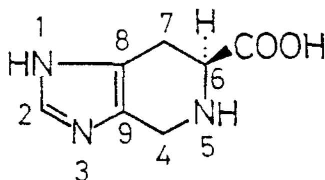


Fig.2 *Thin layer chromatogram of the water-soluble alkaloids(compound I and II) of ginseng roots. 1, compound I; 2, compound II+His; 3, His; 4, carnosine; 5, anserine; developing solvent, $CHCl_3$/MeOH/c-NH_4OH(17:10:3);, positive spot by Pauly diazonium test.*

of I showed the presence of NH(3380 cm^{-1}) and α-amino acid(3100, 1630, 1500, 1400, 1250 cm^{-1}).

^{1}H-NMR spectrum of I resembled that of L-histidine except the absence of a signal due to one aromatic proton on imidazole ring and the presence of an additional ABq signal at δ 4.33(2H, J=15 and 36Hz). Its ^{13}C-NMR spectrum showed seven carbon signals, *i.e.* -CH_2-x2, CH-x1, =CH-x1, -C=x2, COOH x1. 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 $C_7H_9N_3O_2$, and I to be spinacine.



spinacine

Assignment of ^{1}H-and ^{13}C-NMR signals(see "Experimental Methods") supported I as spinacine. The structure was finally identified by the direct comparison of the spectral data and TLC behaviour of the authentic sample, which was syntesized from L-histidine and formaldehyde[16]. Thus, compound 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*[15] It was later found in the Crab *Crangon vulgaris*[15].

Experimental Methods

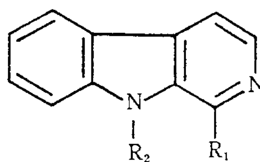
Melting points were determined on Mitamura Riken heat block Model-MRK and were uncorrected. Gilford system 2600 UV/VIS spectrophotometer was used for UV spectra. IR spectra were measured on Perkin-Elmer 281B IR spectrophotometer in KBr pellets. ^{1}H-NMR spectra were determined on Varian Model FT 80A NMR spectrometer or a Nicolet NT-360 spectrometer with TMS as internal reference. Electron impack mass(EIMS) spectra were measured on Hewlett-Packard Model HP 5985B GC/MS system or secondary ion mass(SIMS) spectra on a Hitachi M-80 high resolution mass spectrometer. 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

Ether-soluble 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 7.4 Kg 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 c-NH_4OH. The alkaline solution was extracted with $CHCl_3$ (3l×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, 300 ml) and then $CHCl_3$-MeOH(10:1, 330 ml) to give fr.1(50 mg) and fr. 2(260 mg). 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

Table I. *β-Carboline alkaloids*



Alkaloids	R_1	R_2	Botanical origin	Yield 10^{-5}%	Ref.
Norharman	H	H	*Polygala tenuifolia*	15	6
			Codonopsis lanceolata	3.5	5
			Ailanthus malabarica	—	4
			Picrasma quassioides	—	10
Harman	CH_3	H	*P. tenuifolia*	10	6
N^9-Formyl harman*	CH_3	CHO	*Panax ginseng*	1.3	**
			C. lanceolata	4.5	5
			Lycium chinese	1.5	7
			P. tenuifolia	7	6
1-Carbomethoxy β-carboline	$COOCH_3$	H	*Panax ginseng*	3.6	13
			L. chinese	17	7
			P. tenuifolia	4	6
			P. quassioides	—	10
			A. malabarica	—	4
			Pleiocarpa mutica	—	17
1-Carboethoxy β-carboline	$COOC_2H_5$	H	*P. ginseng*	1.5	**
			C. lanceolata	6	5
			P. tenuifolia	0.8	6
			P. quassioides	—	10
1-Carbobutoxy β-carboline*	$COOC_4H_9$	H	*P. tenuifolia*	6.3	6
			Panax ginseng	1.1	13
Perlolyrine	(furan ring) CH_2OH	H	*Panax ginseng*	15	**
			P. tenuifolia	30	6
			C. lanceolata	18	5
			L. chinese	28	7
			Lolium perenne	—	12

*New compounds isolated from the plant by us **This paper

times and the band of Rf 0.5 was scrapped off and extracted with $CHCl_3$-MeOH(3:1), giving GA-12.

Water-soluble alkaloid: 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 aquous 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(17:10:3) to divide into two fractions. Concentration of each fraction yielded compound I(50 mg) and compound II plus histidine(30 mg). Compound II and histidine were not separated from each other until now.

N_9-Formylharman, GA-4

Recrystallization from $CHCl_3$ -MeOH yieled pale yellow needles(3.7 mg). yield 1.3×10^{-5}%. mp 178-179℃. UVλ_{max}^{MeOH} nm(log ε): 212.5, 251.5, 261, 284, 307.5, 379(4.12, 5.85, 4.00, 5.70, 5.69). IRν_{max}^{KBr}cm^{-1}:1670(C=0) ^{1}H-NMR $CDCl_3$ m: 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 β-carboline-1-carboxylate, GA-11

Recrystallization from $CHCl_3$-MeOH yield needles(4.2 mg). yield 1.5×10^{-5}%, mp 122-123℃. IRν_{max}^{KBr}cm^{-1}: 1660(C=0). UVλ_{max}^{MeOH}(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, $\underline{CH_3}CH_2O$), 4.45(2H, q, J=7.2Hz, $CH_3\underline{CH_2}O$), 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(46 mg). yield 1.5×10^{-4}%. mp 166℃. UV λ_{max}^{MeOH} nm (log ε) : 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(5 mg) with acetic anhydride/pyridine yielded its acetate, which was crystallized from EtOAc to give needles(4 mg). mp 160℃. ^{1}H-NMR $CDCl_3$ δ: 2.15(3H, s, $COCH_3$), 5.24(2H, s, -CH_2O-), 6.60(1H, d, J=3.4Hz, 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_3-COOH,

100), 218(17.7), 205(8.9), 168(8.9), 167(28.2), 140(20.2), 114(7.3), 113(9.7).

Compund I

Pure chromatographically and very soluble in water, but insoluble in alcohol, pyridine and other organic solvents. Very stable in 6N-HCL at 100℃ for 4 hrs. Positive by Pauly reagent(red color); negative by Dragendorff's reagent. amorphous powder[Literature[15], 264°]; $[\alpha]_D^{22}$ -165° [Literature[15], $[\alpha]_D^{20}$ -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 16.5Hz, one H on C_7-H_2), 3.31(1H, dd, J=5.5 and 16.5Hz, 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 36Hz, 2H of C4-H_2), 8.02(1H, br. s, 1H of C2); ^{13}C-NMR(D_2O, DSS for reference): m136.8(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(0.3g) and formaldehyde(35%, 1 m*l*) in water(3 m*l*) was heated at 55℃ for 4 hrs, and then at 98℃ for 2 hrs. The reaction mixture was concentrated under vacuum to yield colorless residue. The residue was dissolved in water(3 ml), and then was adjusted to pH 7 with d-NH_4OH to give colorless powder. mp 260°[Literature[15], 264°]. $[\alpha]_D^{20}$ -170°. Its IR, UV, ^{1}H-NMR spectra and Rf value on silica gel plate(solvent, $CHCl_3$/MeOH/c-NH_4OH=17:10:3, Rf=0.12) were identical with those of compound I.

References

1. Han, B.H., Han, Y.N. and Park, M.H.: *Advances in Chenese Medicinal Materials Research*, World Scientific Publ. Co., Singapore. Philadelphia, p.485(1985).
2. Shoji, J.: *ibid*, p.455(1985).
3. Woo, L.K., Suh, C.S., Chang, J.J. and Shin, K.H.: Presence of α-pyrrolidone in ginseng extracts. *Yakhak Hoeji*, **13**, 121(1969).
4. Joshi, B.S., Kamat, V.N. and Gawad, D.H.: Some β-carboline alkaloids of *Ailanthus malabaric* DC, *Heterocycles*, **7**, 193(1977).
5. Chang, Y.K., Kim, S.Y. and Han, B.H.: Chemical studies on the alkaloidal constituents of *Codonopsis lanceolata*. *Yakhak Hoeji*, **30**, 1(1986).
6. Han, B.H., Park, J.H., Park, M.H. and Han, Y.N.: β-Carboline alkaloids of *Polygala tenuifolia*. *Arch. Pharm. Res.*, **8**, 243(1985).
7. Han, B.H., Park, J.H., Park, M.H. and Han, Y.N.: Studies on the alkaloidal components of the fruits of *Lycium chinese*. *Arch. Pharm. Res.*, **8**, 249(1985).
8. Pouchert, C.J. and Campbell, J.R.: *The Aldrich library of NMR spectra* U.S. Aldrich Chemical Company, **8**, 78(1974).
9. Sung, Y.I., Koike, K., Nikado, T., Ohmoto, T. and Sankawa, U.: Inhibitors of cyclic AMP phosphodiesterase *in Picrasma quassioides* Bennet, and inhibitory activities of related β-carboline alkaloids, *Chem. Pharm. Bull.*, **32**, 1872(1984).
10. Ohmoto, T. and Koike, K.,: Studies on the constituents of *Picrasma quassioides* Bennet. III., The alkaloid constituents, *Chem. Pharm. Bull.*, **32**, 3579(1984).
11. Yang, J.S., Luo, S.R., Shen, X.Y. and Li, Y.X.: *Yao Hsueh Hsueh Pao*, **14**, 167(1979).
12. Jeffreys, J.A.D.: The alkaloids of perennial ryegrass(*Loliuum Perenne L.)* Part IV. Isolation of a new base, perlolyrine; The crystal structure of its hydrobromide dihydrate, and the synthesis of the base, *J. Chem. Cos. (C), 1091(1970).*
13. Park, J.D., Kim, M.W., Yoo, S.J. and Wee, J.J.: Chemical studies on the ether-soluble alkaloidal fraction of *Panax ginseng* I. Isolation of 1-carbobutoxy-β-carboline and 1-carbomethoxy-β-carboline. *Arch. Pharm. Res.*, **10**(3), 197(1987).
14. Park, J.D., Kim, M.W. *et al.*: private communication
15. Hill, R.K.: The imidazole alkaloids. *Alkaloids* vol. 2, 858, John Wiley & Sons(1984); and references cited therein.
16. Harvey, D.G., Miller, E.J. and Robson, W.: The adamkiewicz, Hopkins and Cole, and Rosenheim test. *J. Chem. Soc.* p.953(1941).
17. Achenbach, H. and Biemann, K.: Two new alkaloids isolated from Pleiocarpa mutica Benth. *J. Am. Chem. Soc.*, **87**, 4177(1965).