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Studies on the Alkaloidal Components of the Fruits of *Lycium chinense*

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Abstract □ N_9-Formylharman, 1-carbomethoxy-β-carboline and perlolyrine with an unidentified alkaloid ($C_{10}H_{13}NO_2$, M^+ 179.094) have been isolated from the fruits of *Lycium chinense* Miller (Solanaceae) and characterized on the basis of chemical and physical evidence.

Keywords □ *Lycium chinense* Miller, Solanaceae, Alkaloid, N_9-Formylharman, 1-Carbomethoxy-β-carboline, Perlolyrine.

Lycii Cortex, the root bark of *Lycium chinense* Miller (Solanaceae), has been shown to be clinically effective for hypertension,[1] and has been reported to exhibit hypotensive, hypoglycemic, antipyretic and antistress ulcer activity in animals.[1,2] On the chemical constituents of *Lycium chinense* Miller betaine, phytosterol, linolic acid, choline,[3] kukoamine-A[4] and recently a dipeptide, lyciumamide have been isolated from the cortex.[5] Nicotinamine,[6a] 3-hydroxy-7, 8-dehydro-β-ionone[6b] from the leaves, carotenoids, thirty six neutral volatile compounds[6c] including dihydroactinidiolide, sofranal, β-ionone, megastigmatrienone, β-hydroxy-β-ionone from the fruits have been identified. This paper describes the isolation and characterization of the alkaloidal components of Lycii fructus and the results of GLC-assay for alkaloid contents in the plant.

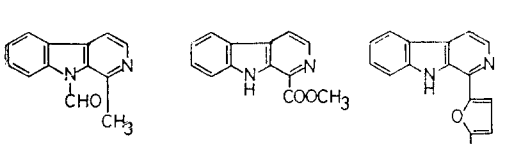

Fig. 1. The structures of the alkaloids isolated from the fruits of *Lycium chinense* Miller

EXPERIMENTAL METHODS

Melting points were taken on a Mitamura Riken Heat Block apparatus and uncorrected. UV spectrum was recorded on a Gilford system 2600 UV-VIS spectrophotometer and IR spectrum was recorded in KBr disk on a Perkin-Elmer 281B IR spectrophotometer. ^{1}H-NMR spectrum was determined with TMS as an internal standard using a Varian Model FT 80 A NMR spectrometer (80MHz). Mass spectrum was measured with Hewlett-Packard Model HP 5985B GC/MS system. Flash column chromatography was carried out on silica gel 60 (Merck Art. 7734). TLC and preparative TLC were performed on precoated silica gel 60 GF_{254} plates and spots were detected by Dragendorff reagent spray or by UV irradiation. GC analysis was carried out on SE-54 fused silica capillary column (0.2mm i.d. ×25m) under the temperature programing from 160°C to 280°C.

Isolation of Lycii-alkaloids

Lycii fructus, the fruits of *Lycium chinense* Miller (6 kg) was extracted with MeOH (24*l*)

under reflux for three hours, two times. The combined MeOH extracts were evaporated to yield MeOH ex. (750g). The MeOH ex. (750g) was dissolved in water to make 2.4*l* and extracted with Et_2O (3*l*×3). The combined Et_2O layer was evaporated to 1*l* volume and extracted with 5% HCl (0.5*l*×2). The aqueous layer was washed with Et_2O several times, made basic (pH 10) with c-NH_4OH addition and extracted with $CHCl_3$ (1.2*l*×3). The $CHCl_3$ layer was dried over Na_2SO_4 and evaporated to dryness to give alkaloid fraction (0.96g). The alkaloid fraction (0.74g) was subjected to silica gel flash column chromatography (2×27cm) and eluted with $CHCl_3$-MeOH (100 : 1, 250ml→70 : 1, 210ml→50 : 1, 300ml→30 : 1, 240ml) to yield fr. 1 (35mg), fr. 2 (25mg), fr. 3 (50mg) and fr. 4 (80mg).

Lycii-alkaloid-Ⅰ : Fr. 1 (35mg) was subjected to preparative TLC in hexane-EtOAc (5 : 2) and the plate was developed two times. The solid obtained from the band of Rf 0.63 was crystallized from $CHCl_3$-MeOH. Yellow needles, mp 177°C. IR $\nu_{max}^{KBr}cm^{-1}$; 1,670 (C=O), UV $\lambda_{max}^{MeOH}nm(log\varepsilon)$: 231 (4.1), 252 (5.86), 261 (5.84), 284 (4.02), 307.5 (5.71), 379 (5.68), ^{1}H-NMR ($CDCl_3$, δ_{ppm}) : 2.88 (3H, s, CH_3), 7.21 (1H, m, 7-H), 7.59 (2H, m, 6,8-H×2), 8.13 (1H, d, J=5Hz, 4-H), 8.16 (1H, d, J=8Hz, 5-H), 8.53 (1H, d, J=5Hz, 3-H), 10.26 (1H, br. s, CHO), Mass m/z (Rel. Int., %); 210 (M^+, 80.4), 195 (M^+-CH_3, 1.1), 182 (M^+-CO, 45.6), 168 (M^+-CO-CH_3+H, 100), 140 (54.1), 114 (18.4), 113 (19.5).

Lycii-alkaloid-Ⅱ : The oil (10mg) obtained from the band of Rf 0.38 of fr. 1 had molecular weight 179.094, $C_{10}H_{13}NO_2$ by high resolution mass spectrum.

Lycii-alkaloid-Ⅲ : The solid obtained from the band of Rf 0.14 of fr. 1 was crystallized from $CHCl_3$-MeOH. Needles, 2mg, 167°C, IR $\nu_{max}^{KBr}cm^{-1}$: 1,675 (C=O), UV $\lambda_{max}^{MeOH}nm(log\varepsilon)$: 245 (4.01), 258 (4.03), 280 (4.02), 302 (5.88), 371 (5.67), ^{1}H-NMR ($CDCl_3$, δ_{ppm}); 4.11 (3H, s, OCH_3), 7.23 (1H, m, 7-H), 7.52 (2H, m, 6,8-H×2), 8.11 (1H, d, J=5Hz, 4-H), 8.14 (1H, d, J=8Hz, 5-H), 8.56 (1H, d, J=5Hz, 3-H), 9.82 (1H, br. s, NH), Mass m/z (Rel. Int., %) : 226 (M^+, 38.5), 194 (10.1), 168 (92.6), 166 (10.0), 140 (45.8), 114 (20.0), 113 (19.2).

Lycii-alkaloid-Ⅳ : Fr. 4 was subjected to preparative TLC in $CHCl_3$-MeOH (10 : 1). The solid obtained from the band of Rf 0.42 was crystallized from $CHCl_3$, brown needles, 3mg, mp 165°C, IR $\nu_{max}^{KBr}cm^{-1}$: 3,380 (NH, OH), UV $\lambda_{max}^{MeOH}nm(log\varepsilon)$: 216 (3.50), 239 (3.50), 254 (3.40), 275 (3.39), 292 (3.41), 307 (3.30), 368 (3.21), 381 (3.25), ^{1}H-NMR ($CDCl_3$, δ_{ppm}) : 4.81 (2H, s, CH_2O-), 6.47 (1H, d, J=3.4Hz, 3′-H), 7.19 (1H, d, J=3.4Hz, 4′-H), 7.21-7.45 (1H, m, 7-H), 7.45 (2H, m, 6,8-H×2), 7.84 (1H, d, J=5.3Hz), 8.07 (1H, d, J=7.8Hz, 5-H), 8.42 (1H, d, J=5.4Hz, 3-H), 9.45 (1H, br. s, NH), Mass m/z (Rel. Int., %) : 264 (M^+, 78.6), 247 (72.3), 246 (50.1), 235 (13.5), 233 (11.6), 218 (42.4), 205 (68.5), 168 (52.4), 167 (100), 140 (90.8), 114 (40.1), 113 (40.5).

Determination of Alkaloid Contents by Gas Chromatography:

Alkaloid fraction (12mg) and standards were silylated with BSTFA (0.02ml) and then applied to GLC. (SE-54, 0.2mm i.d. ×25m, temperature programing from 160°C to 280°C, rate 4°C/min).

RESULTS AND DISCUSSION

Lycii-alkaloid-Ⅰ obtained as yellowish needles mp 177°C, showed M^+ at m/z 210 and its composition was suggested as $C_{13}H_{10}N_2O$ by its

mass spectrum.[7] The IR spectrum showed an absorption of carbonyl group and its UV spectrum was characteristics of a β-carboline type alkaloid.[8] The ^{1}H-NMR spectrum showed one methyl group at δ2.88 (3H, s) and six aromatic protons assignable as a β-carboline, and showed one singlet at δ10.26 (1H, s) which resisted on D_2O exchange reaction. Its mass spectrum showed prominent ion peak at m/z 182 and 168 corresponding to M^+-CO and M^+-CO-CH_3+H, respectiveley. From the above results, carbonyl group was assigned as N-formyl group and the suggested structure for Lycii-alkaloid-Ⅰ was N_9-formyl harman, which was finally identified by the fact that the alkaline hydrolysis[9] of this alkaloid yielded harman and by the direct comparison of physicochemical data of authentic sample. This alkaloid was previously isolated from *Codonopsis lanceolata*,[9] *Panax ginseng*,[10] *Polygala tenuifolia*.[11]

Lycii-alkaloid-Ⅲ obtained as colorless needles, mp 167°C, showed M^+ at m/z 226, $C_{13}H_{10}N_2O_2$. Its IR spectrum showed a conjugated carbonyl group at 1,675cm^{-1} and the UV spectrum was characteristic of a β-carboline.[8,12] Its ^{1}H-NMR spectrum showed one methoxy group at δ4.11 (3H, s.) and other protons assignable to a β-carboline. The above data including mass spectrum were compatible with those of 1-carbomethoxy-β-carboline. Lycii-alkaloid-Ⅲ was identified as 1-carbomethoxy-β-carboline by direct comparison of physicochemical data of authentic sample. This alkaloid was previously isolated from *Pleiocarpamutica*,[12] *Picrasma quassioides*,[13] *Codonopsis lanceolata*,[9] *Polygala tenuifolia*.[11]

Lycii-alkaloid-Ⅳ obtained as brown needles, mp 165°C, showed M^+ at m/z 264, $C_{16}H_{12}N_2O_2$. The ^{1}H-NMR spectrum showed aromatic protons assignable to a β-carboline. It also exhibited one methylene singlet at δ4.77 (2H, s) and AB quartet at 6.47 (1H, d), 7.19 (1H, d) with coupling constant J=3.4Hz, suggesting the presence of 5-hydroxymethyl-2-furyl group. This was supported by the mass ions at m/z 247, 246, 233, 205 and 167 corresponding to the elimination of OH, H_2O, CH_3O, $C_2H_3O_2$ and $C_5H_5O_2$ from M^+, m/z 264. The above data including UV spectrum were compatible with those of perlolyrine. Thus Lycii-alkaloid-Ⅳ was identified as perlolyrine[14] with the direct comparison of physicochemical data of authentic sample. This alkaloid was previously isolated from *Lolium pernne* L.,[14] *Codonopsis lanceolata*,[9] *Panax ginseng*[15] and *Polygala tenuifolia*.[11]

The molecular formula $C_{10}H_{13}NO_2$ (M^+, m/z 179.094) of *Lycii-alkaloid*-Ⅱ has been obtained on a high resolution mass spectrum, and it was found identical with the substance isolated previously in our laboratory from *Zizyphus jujuba var. inermis*.[16] However the structure determination is still under progress. The contents of N_9-formylharman, 1-carbomethoxy-β-carboline, perlolyrine and $C_{10}H_{13}NO_2$ (M^+ 179.094) determined by GLC after TMS derivatization were 1.5×10^{-5}%, 1.3×10^{-4}%, 2.8×10^{-4}% and 7.3×10^{-4}%, respectively.

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