Arch. Pharm. Res. 8(4), 243~247 (1985)

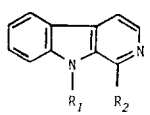
β-Carboline Alkaloids of *Polygala tenuifolia*

Byung Hoon Han, Jeong Hill Park, Myung Hwan Park and Yong Nam Han
Natural Products Research Institute, Seoul National University, Seoul 110 Korea
(Received September 16, 1985)

Abstract □ A new β-carboline alkaloid, 1-carbobutoxy-β-carboline as well as N_9-formylharman, 1-carboethoxy-β-carboline, 1-carbomethoxy-β-carboline, perlolyrine, harman and norharman were isolated from the rhizoma of *Polygala tenuifolia* Willdenow. The structures were elucidated on the basis of spectroscopic studies and chemical evidence.

Keywords □ *Polygala tenuifolia* Willdenow, Alkaloid, β-Carboline, 1-Carbobutoxy-β-carboline, N_9-Formylharman, 1-Carboethoxy-β-carboline, 1-Carbomethoxy-β-carboline, Perlolyrine, Harman, Norharman.

The rhizoma of *Polygala tenuifolia* Willdenow (Polygalaceae) is a well known Chinese medicine used as an expectorant, tonic and sedative. The chemical constituents of this crude drug have been investigated and the presence of polygalitol, N-acetyl-D-glucosamine, glucose, fructose[1], 3, 4, 5-trimethoxycinnamic acid[2], three xanthone derivatives[2], onjisaponins A, B, C, D, E, F and G have been reported[3,4]. Kim suggested presence of alkaloid but didn't isolated them[5]. This paper describes the isolation of seven alkaloids, all belonging to the β-carboline series, from the rhizoma of *Polygala tenuifolia* Willdenow. Those compounds were designated tentatively Wonji-X (X; 1, 2, 3, ···7).



		R_1	R_2
Wonji-1	N_9-Formylharman	CHO	CH_3
Wonji-2	1-Carbobutoxy-β-crboline	H	$COOC_4H_9$-n
Wonji-3	1-Carboethoxy-β-carboline	H	$COOC_2H_5$
Wonji-4	1-Carbomethoxy-β-carboline	H	$COOCH_3$
Wonji-5	Perlolyrine	H	
Wonji-6	Norharman	H	H
Wonji-7	Harman	H	CH_3

Chart: β-Carboline Alkaloids isolated from the Rhizoma of *Polygala tenuifolia* Willdenow.

EXPERIMENTAL METHODS

All melting points were taken on Mitamura Riken Heat Block Medel-MRK and uncorrected. UV spectrum was recorded by Gilford system 2600UV-VIS spectrophotometer and IR spectrum was measured in KBr disk on Perkin-Elmer 281 B IR spectrophotometer. NMR spectrum was determined with TMS as internal standard using Varian Model FT 80A NMR spectrometer (80 MHz). 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 prepararative TLC were performed on precoated silica gel 60 GF_{254} plates and spots were detected with Dragendorff reagent or by UV lamp.

Isolation of Wonji-alkaloid:

Polygalae Radix (12kg, radix of *Polygala tenuifolia*) was extracted with hot MeOH (50*l* ×2) for three hours. The methanolic extract (3.26kg) was suspended in water (7*l*) and extracted with Et_2O(8*l*×4). The Et_2O layer was concentrated to 3*l* volume and extracted with 5% HCl (1.5*l*×2) and the aqueous layer

was washed with Et_2O several times and was made basic to pH 10 with c-NH_4OH and then extracted with $CHCl_3$ ($3l \times 3$). The $CHCl_3$ layer was dried over Na_2SO_4 and evaporated to give alkaloid fraction (1.15g). The alkaloid fraction (1.14g) was subjected to silica gel flash column chromatography (2×25cm) and eluted with $CHCl_3$ -MeOH (20 : 1 → 10 : 1) to yield four fractions by monitoring with Dragendorff reagent, fr. 1 (130mg), fr. 2 (240mg), fr. 3 (79mg), fr. 4 (70mg). Fr. 1 (130mg) was subjected to preparative TLC in hexane -EtOAc (2 : 1) to yield four crystalline substances.

Wonji-1: The solid obtained from the band of Rf 0.4 was crystallized from $CHCl_3$-MeOH to give yellow needles. 8 mg, 7×10^{-5}%, mp 178°C, IR ν_{max}^{KBr}cm^{-1}: 1670 (C=O), UVλ_{max}^{MeOH} nm(logε): 212.5 (4.12), 251.5(5.85), 261(5.85), 284 (4.0), 307.5(5.70), 379(5.69), ^{1}H-NMR ($CDCl_3$, δppm): 2.89 (3H, s, CH_3), 7.37–7.18 (1H, m, 7-H), 7.63–7.55(2H, m, 6,8-H), 8.14 (1H, d, J=5Hz, 4-H), 8.17(1H, d, J=8Hz, 5-H), 8.54 (1H, d, J=5Hz, 3-H), 10.25 (1H, br. s., CHO), Mass m/z (Rel. Int., %): 210 (M^+, 79.6), 195(M^+-CH_3, 0.9), 182 (M^+-CO, 44.5), 168(M^+-CO-CH_3+H, 100), 140 (43.4), 114(14.6), 113(13.2).

Wonji-2: The solid obtained from the band of Rf 0.3 was crystallized from dimethyl sulfoxide to yield plates. 7mg, 6.3×10^{-5}%, mp 95°C, IRν_{max}^{KBr}cm^{-1}: 1670(C=O), UVλ_{max}^{MeOH} nm (logε): 246.5(4.05), 258(4.05), 275.5(4.09), 301(3.92), 370.5 (3.71), ^{1}H-NMR ($CDCl_3$, δppm): 0.99(3H, t, J=6.8Hz, CH_3), 1.39 (2H, sextet, J=6.8Hz, CH_2), 1.74 (2H, sextet, J=6.8Hz, CH_2), 4.54(2H, t, J=6.7Hz, -OCH_2-), 7.31–7.24 (1H, m, 7-H), 7.59–7.47 (2H, m, 6,8-H×2), 8.15 (1H, d, J=8.1Hz, 5-H), 8.13 (1H, d, J=5Hz, 4-H), 8.59 (1H, d, J=5.1Hz, 3-H), 9.85 (1H, br. s., NH). Mass m/z (Rel. Int.,%): 268(M^+, 13.4), 240(2.1), 213(2.1), 196(9.2), 182(4.2), 168(100), 167(19), 140 (17.6), 114(6.3), 113(6.3).

Wonji-3: The solid obtained from the band of Rf 0.15 was crystallized from $CHCl_3$-MeOH to yield needles. 2mg, 0.8×10^{-5}%, mp 123°C, IRν_{max}^{KBr} cm^{-1}: 1670(C=O), UVλ_{max}^{MeOH} nm (logε): 246.5(4.0), 258(4.0), 275(4.02), 301(3.91), 370(3.71), ^{1}H-NMR ($CDCl_3$, δppm): 1.37 (3H, t, J=7.2Hz, $C\underline{H}_3CH_2$), 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×2), 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). Mass m/z(Rel. Itn., %): 240(M^+, 21.2), 211(0.5), 195(1.6), 168(100), 167(16.3), 166(40.0), 140(20.5), 114(9.5), 113(7.4).

Wonji-4: The solid obtained from the band of Rf 0.1 was crystallized from $CHCl_3$ -MeOH to yield needles. 5mg, 4×10^{-5}%, mp 166°C, IR ν_{max}^{KBr} cm^{-1}: 3380(NH), 1680 (C=O), UVλ_{max}^{MeOH}nm (log ε):246(4.03), 258(4.02), 275.5(4.05), 301 (5.88), 371(5.66), ^{1}H-NMR ($CDCl_3$, δppm): 4.12(3H, s, OCH_3), 7.40–7.20 (1H, m, 7-H), 7.60–7.45 (2H, m, 6,8-H), 8.10 (1H, d, J=5Hz, 4-H), 8.13(1H, d, J=8Hz, 5-H), 8.55 (1H, d, J=5Hz, 3-H), 9.58(1H, br. s, NH), Mass m/z (Rel. Int.,%): 226(M^+, 42.1), 194 (9.5), 168(100), 166(9.26), 140(26.3), 114 (15.8), 113(15.6).

Wonji-5: Fr. 2 (240mg) was rechromatographed on silica gel (1.8×18cm) with eluent of $CHCl_3$-MeOH (20 : 1) to give Dragendorff reaction positive fraction (50mg) and then was subjected to preparative TLC in $CHCl_3$-MeOH(10 : 1). The plate was developed two times and the solid obtained from the band of Rf 0.5 was crystallized from $CHCl_3$ to yield needles. 34mg, 3×10^{-4}%, mp 165°C, IRν_{max}^{KBr}cm^{-1}:3370(NH, OH), UVλ_{max}^{MeOH} nm(log ε):216 (3.51), 238.5

(3.49), 253.5(3.41), 274(3.38), 292(3.41), 307(3.28), 368(3.21), 381(3.24), ^{1}H-NMR ($CDCl_3$, δppm), 4.80 (2H, s, CH_2O), 6.46(1H, d, J=3.3Hz, 3′-H), 7.18(1H, d, J=3.3Hz, 4′-H), 7.21～7.47 (1H, m, 7-H), 7.52～7.56(2H, m, 6,8-H×2), 7.83(1H, d, J=5.2Hz, 4-H), 8.08(1H, d, J=7.7Hz, 5-H), 8.41(1H, d, J=5.3Hz, 3-H), 9.44(1H, br. s., NH). Mass m/z (Rel. Int., %): 264(M^+, 100), 247(82.3), 246 (59.9), 235(12.3), 233(7.4), 218(16.8), 205 (26.5), 168(17), 167(39.3), 140(27.1), 114 (9.7).

Wonji-6: Fr. 3 (70mg) was dissolved in pyridine (2ml) and acetic anhydride (2ml) and standed overnight at room temperature. The reagents were removed with N_2 flash and purified by preparative TLC to give Dragendorff reaction positive crystalline mass. 18mg, pale yellow needles from benzene. mp 203～205°C, IRν_{max}^{KBr} cm^{-1}; 1660(C=O), UVλ_{max}^{MeOH} nm (log ε): 230.4 (4.20), 251(4.13), 264(5.96), 274(5.92), 282 (4.04), 289(5.81), 314(5.64), 324(5.72), 349 (5.10), ^{1}H-NMR($CDCl_3$, δppm): 2.93(3H, s, $COCH_3$), 7.42～7.72(2H, m, 6,7-H×2), 7.90 (1H, d, J=5Hz,4-H) 8.03～8.14 (1H, m, 8-H), 8.24(1H, d, J=8Hz, 5-H), 8.63(1H, J=5.2Hz, 3-H), 9.60 (1H, br. s, NH), Mass m/z (Rel. Int., %); 210(M^+, 18.6), 182(0.8), 168 (100), 140(15.6), 114(8.8), 113(8.1). Wonji-6 acetate (17mg) was dissolved in MeOH (2ml) and c-NH_4OH(2ml) and standed at room temperature for 2 hours and evaporated the solvent *in vicuo*. The residue was crystallized from acetone-water to yield needles. 12mg, mp 197°C, ^{1}H-NMR ($CDCl_3$, δppm): 7.18～7.35 (1H, m, 7H), 7.45～7.57 (2H, m, 6,8-H×2), 7.92(1H, d, J=5.4, 4-H), 8.13 (1H, d, J=8Hz, 5-H), 8.45 (1H, d, J=5.4Hz, 3-H), 8.91 (1H, s, 1-H), 9.12 (1H, br, NH), Mass m/z (Rel. Int.,%): 168(M^+, 100), 140(24.4), 114(14.4), 113(10.5).

Wonji-7; Fr. 4(70mg) was purified by preparative TLC in $CHCl_3$-MeOH (10 : 1). The solid obtained from the band of Rf 0.28 was crystallized from $CHCl_3$ to yield needles. 14mg, 1×10^{-4}%, mp 187～188°C, ^{1}H-NMR ($CDCl_3$, δppm): 2.82 (3H, s, CH_3), 7.2～7.37 (1H, m, 7-H), 7.51～7.56 (2H, m, 6,8-H×2), 7.82 (1H, d, J=5.4Hz, 4-H), 8.12 (1H, d, J=7.5 Hz, 5-H), 8.37 (1H, d, J=5.4Hz, 3-H), Mass m/z (Rel. Int., %): 182(M^+, 100), 167(1), 154(22.4), 140(5.7), 114(4.0), 113(4.5).

Synthesis of Alkaloids:

Perlolyrine acetate was synthesized from tryptophan and 5-acetoxymethyl-2-formylfuran by Jeffreys' method[9]. mp 160°C, Mass m/z: 306 (M^+). This was deacetylated with ammonia to give perlolyrine. mp 165°C, Mass m/z 264(M^+). In this synthetic procedure, N_9-formylharman was obtained as a by-product. mp 178°C, Mass m/z 210(M^+).

Harman, mp 188°C, Mass m/z 182(M^+) and 1-carbomethoxy-β-carboline, mp 166°C, Mass m/z 226(M^+) were prepared by Snyder's method[10]. 1-Carboethoxy-β-carboline was prepared by treatment of β-carboline-1-carboxylic acid[10] with dry hydrogen chloride in EtOH, mp 123°C, Mass m/z 240(M^+). 1-Carbobutoxy-β-carboline was prepared by trans esterification of 1-carbomethoxy-β-carboline in *n*-butanol with *p*-toluene sulfonic acid. mp 95°C, Mass m/z 268(M^+), ^{13}C-NMR (DMSO-d_6, δppm): 165.90(C=O), 141.62(C-1), 137.92(C-3), 136.35(C-8a), 130.96(C-8b), 130.17(C-4b), 128.97(C-6), 121.71(C-5), 120.34(C-4a), 120.07(C-7), 118.62(C-4), 113.01(CO-8), 64.66(OCH_2), 30.54(CH_2), 18.81(CH_2), 13.54(CH_3). Norharman was prepared by Kermank's method[13]. mp 197°C, Mass m/z 168(M^+).

RESULTS AND DISCUSSION

Wonji-1 was obtained as pale yellow needles with mp 178~179°C and the mass spectrum showed M^+, m/z 210($C_{13}H_{10}N_2O$). The IR spectrum showed a carbonyl group at 1670cm^{-1} and the UV spectrum showed characteristic spectrum of β-carboline[6]. ^{1}H-NMR spectrum showed the characteristic peaks of Harman with one additional singlet at δ10.25 which resisted to the exchange with D_2O. This additional proton was assigned as aldehyde proton. This suggestion was supported by mass fragment of m/z 182 (M^+-CO) and production of harman with alkaline hydrolysis of Wonji-1. From the above results, Wonji-1 was identified as N_9-formylharman, previously isolated from *Codonopsis lanceolata*[7], *Panax ginseng*[8] and it was also produced as by-product during the synthesis of perlolyrine[6].

Wonji-2 was obtained as plates with mp 95°C and showed M^+, m/z 268, $C_{16}H_{16}N_2O_2$, in its mass spectrum. Its IR spectrum showed a conjugated carbonyl group at 1,670cm^{-1} and the UV spectrum showed characteristic spectrum of β-carboline[6]. ^{1}H-NMR spectrum showed the same aromatic protons of unsubstituted β-carboline skeleton and a typical $CH_3CH_2CH_2CH_2O$ at δ0.99, 1.39, 1.74 and 4.54 with coupling constant (J=6.8Hz each). The above results suggested that Wonji-2 was 1-carbobutoxy-β-carboline and the structure was finally identified by direct comparisons of the physical properties and spectral data of 1-carbobutoxy-β-carboline synthesized by transesterification of 1-carbomethoxy-β-carboline in *n*-butanol with *p*-toluene sulfonic acid.

This alkaloid has not yet described in literature to our best knowledge.

Wonji-3 was obtained as needles with mp 123°C and showed M^+, m/z 240, $C_{14}H_{12}N_2O_2$ in its mass spectrum.

The IR spectrum showed a conjugated carbonyl group at 1,670cm^{-1} and its UV spectrum showed typical characteristics of β-carboline[6]. The ^{1}H-NMR spectrum showed a typical quartet and triplet pattern at δ4.45 and 1.37(J=7.2Hz) which was assignable to the ethyl group. It was also supported by its mass fragmentations, m/z 211, 195, 168, 167 produced by degradation of $COOC_2H_5$ from M^+, m/z 240. From the above results Wonji-3 was identified as 1-carboethoxy-β-carboline and was finally identified by direct comparison of spectral data with those of synthetic 1-carboethoxy-β-carboline by treatment of β-carboline-1-carboxylic acid[10] with dry hydrogen chloride in EtOH.

Wonji-4 was obtained as needles with mp 166°C and showed M^+, m/z 226 $C_{13}H_{10}N_2O_2$ in its mass spectrum. The IR spectrum showed a conjugated carbonyl group at 1,670cm^{-1}. Its UV, ^{1}H-NMR and mass spectra were compatible with those reported for 1-carbomethoxy-β-carboline[11] and the structure of Wonji-4 was finally identified as 1-carbomethoxy-β-carboline by direct comparison of physical properties and spectral data with synthetic 1-carbomethoxy-β-carboline[10].

Wonji-5 was obtained as brownish-yellow needles with mp 166°C showed M^+, m/z 264, $C_{16}H_{12}N_2O_2$ in its mass spectrum. Its ^{1}H-NMR spectrum suggested the presence of 5-hydroxymethyl furyl group at δ4.80 (2H, s.), 6.46 and 7.18 (each 1H, d, J=3.3Hz), in addition to protons assignable to unsubstituted β-carboline skeleton which was supported by the mass spectrum showing prominent ion peaks at m/z 247, 246, 233, 205 and 167 corresponding to the elimination of HO, H_2O, CH_3O, $C_2H_3O_2$ and C_5H_5

O_2 from M^+. Wonji-5 was identified as perlolyrine by the above results and by direct comparison of the spectral data with those of synthetic perlolyrine.[9]

Wonji-6 was obtained as its acetate mp 203~205°C after acetylation of Wonji-6 containing fraction and showed M^+, m/z 210, $C_{13}H_{10}N_2O$ in its mass spectrum. The IR spectrum showed a conjugated carbonyl group at 1,660cm^{-1} and its UV spectrum was compatible with those reported for β-carboline.[6,12] ^{1}H-NMR spectrum suggested the presence of an acetyl group at δ2.93 (3H, s.), which was also supported by the mass spectrum showing a prominent ion peak at m/z 167 corresponding to the elimination of an acetyl group from M^+(m/z 210). From the above results Wonji-6 acetate was identified as 1-acetyl-β-carboline, previously isolated from *Ailanthus malabarica*[6] and *Picrasma quassioides*[12], Wonji-6 acetate was easily deacetylated by 14% ammonia in 50% MeOH to yield norharman with mp 197°C. Norharman was identified by direct comparison with synthetic product prepared by Kermak's method[13].

Wonji-7 was obtained as needles with mp 187~188°C and showed M^+, m/z 182, $C_{12}H_{10}N_2$ in its mass spectrum. Its ^{1}H-NMR spectrum showed one methyl group at δ2.82(3H, s) and protons assignable to unsubstituted β-carboline. Wonji-7 was indentified as harman by direct comparison with synthetic harman[10].

LITERATURE CITED

1) Takiura, K. and Honda, S.: Sugar Components of the Root of *Polygala tenuifolia*. *Yakugaku Zasshi*, **84**, 1223 (1964).
2) Ito, H., Taniguchi, H., Kita, T., Matsukui, Y., Tachikawa, E. and Fujita, T.: Xanthones and a Cinnamic Acid Derivatives from *Polygala tenuifolia*. *Phytochemistry*, **16**, 1614 (1977).
3) Sakuma, S. and Shoji, J.: Studies on the Constituents of the Root of *Polygala tenuifolia* Willdenow (I), Isolation of Saponins and the Structures of Onjisaponins G. and F. *Chem. Pharm. Bull.*, **29**(9), 2431 (1981).
4) Sakuma, S. and Shoji, J.: Studies on the Constituents of the Root of *Polygala tenuifolia* Willenow (II), On the structures of Onjisaponins A,B and E. *Chem. Pharm. Bull.*, **30**, 810 (1982).
5) Kim, J.H.: Studies on the Alkaloid from *Polygala tenuifolia* Willdenow. *Yakhak Hoeji*, **8**, 59 (1964).
6) Joshi, B.S., Kamat, V.N. and Gawad, D.H.: Some β-Carboline Alakloids of *Ailanthus malabarica* DC. *Heterocycles*, **7**, 193 (1977).
7) Chang, Y.K.: Chemical Studies on the Alkaloid Constituents of *Codonopsis lanceolata*. Dissertation for Ph.D. Hyosung Women's University. (1985).
8) Han, B.H., Park, M.H. Han, Y.N. and Woo, L.K., in press elsewhere.
9) Jefferys, J. AD.: The Alkaloids of Perennial Rye-Grass (*Lolium pernne* L.) Part IV, Isolation of a New Base, Perlolyrine, The Crystal Structure of its Hydrobromide Dihydrate, and the Synthesis of the Base, *J. Chem. Soc.*(*C*), 1091 (1970).
10) Snyder, H.R., Walker, H.G. and Werber, F.X.: The Synthesis of β-Carboline V. *J. Am. Chem. Soc.*, **71**, 527 (1949).
11) Achenbach, H. and Biemann, K.: Isotuboflavine and Norisotuboflavine, Two New Alkaloid Isolated from *Pleiocarpa mutica* Benth. *J. Am. Chem. Soc.*, **87**, 4177 (1965).
12) Ohmoto, T. and Koike, K.: Studies on the Constituents, of *Picrasma quassioides* Bennet. I, On the alkaloidal Constituents. *Chem. Pharm. Bull.*, 30, 1204 (1982).
13) Kermank, W.O., Perkin, W.H. and Robinson, R.: Harmine and Harmaline Part V, The Synthesis of Norharman, *J. Chem. Soc.*, 119, 1602 (1921).