Benzo[c]phenanthridine Alkaloids from *Corydalis incisa*

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Six benzo[c]phenanthridine alkaloids, corynoline (**1**), acetylcorynoline (**2**), corynoloxine (**3**), luguine (**4**), 6-oxocorynoline (**5**), and 12-hydroxycorynoloxine (**6**) were isolated from the aerial parts of *Corydalis incisa*, and **6** was isolated for the first time from nature. The structure was elucidated by NMR techniques.

Key words: *Corydalis incisa*, Benzo[c]phenanthridine alkaloids, 12-Hydroxycorynoloxine, Luguine, 6-Oxocorynoline

INTRODUCTION

Corydalis incisa Pers. (Fumariaceae) which is widely distributed in Korea has been used as a folk Medicine in China for its antipyretic, analgesic and diuretic properties, (Lee, 1989).

Earlier investigations on the chemical constituents of *C. incisa* afforded isoquinoline alkaloids such as corynoline, acetylcorynoline, corycavine, protopine (Nonaka *et al.*, 1973a), corydalic acid methyl ester (Nonaka *et al.*, 1973b), corydalispirone, corydalisol (Nonaka *et al.*, 1975a), 12-hydroxycorynoline, and 11-epicorynoline (Nonaka *et al.*, 1975b). For the isolation of isoquinoline alkaloids, MeOH extract of *C. incisa* was examined. Investigation on the extract afforded a new alkaloid, 12-hydroxycorynoloxine together with corynoline, acetylcorynoline, corynoloxine, luguine, and 6-oxocorynoline (Fig. 1). This paper reports their isolation and structure elucidation.

MATERIALS AND METHODS

^{1}H- and ^{13}C-NMR spectra were determined on a JEOL JMN-EX 400 spectrometer. EI/MS(70 eV) and HRMS were determined on a VG-VSEQ mass spectrometer (VG Analytical, UK). IR spectra were obtained on a JASCO FT/IR 410 spectrometer and UV spectra were recorded on Shimadzu UV-1601 UV-Visible spectrophotometer. TLC was carried out on Merck aluminium plates precoated with silica gel F_{254} and silica gel for column chromatography was Kiesel gel 60 (230-400 mesh, Merck). LPLC was carried out on Yamazen 540 pump with Merck Lichroprep Si 60 (Lobar A, 240-10 mm, 0.2 ml/min). All other chemicals and solvents were analyticlal grade and used without further purification.

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Plant materials

The aerial parts of *C. incisa* were collected in May 1998 at Moak mountain, Chonbuk, Korea. A voucher specimen (WSU-98-003) is deposited in the herbarium of College of Pharmacy, Woosuk University.

Extraction and isolation

The air-dried plant material (1.8 Kg) was finely ground and extracted at room temp. with MeOH. The resultant MeOH extract (350 g) was subjected to successive solvent

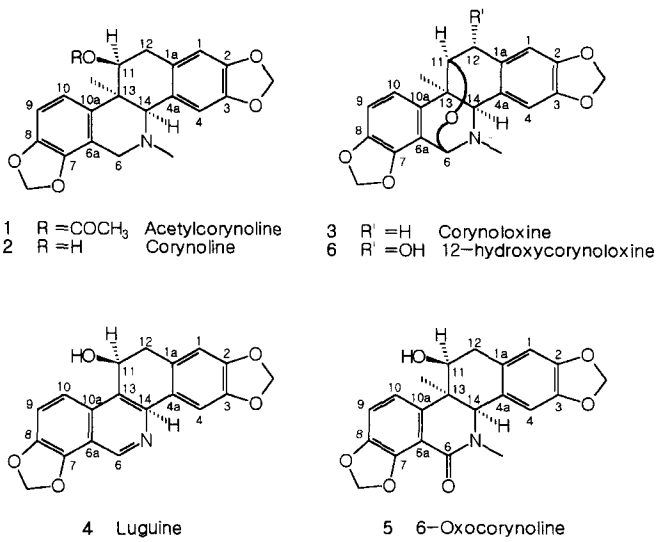

Fig. 1. Structures of compounds **1-6**

parition to give *n*-hexane (45 g), CH_2Cl_2 (20 g), *n*-BuOH (85 g), and H_2O soluble fractions. The CH_2Cl_2 soluble fraction was chromatographed over silica gel column using a solvent system of *n*-hexane-$CHCl_3$-MeOH (23:10:1) as an eluent to give five subfractions (MC I-MC V). Subfraction MC I (6.0 g) was rechromatographed on silica gel column ($CHCl_3$-EtOAc, 60:1) and purified by Sephadex LH-20 (Pharmacia, 25-100 μm, MeOH-$CHCl_3$, 8:2) to yield **1** (20 mg). Subfraction MC II (3.5 g) was rechromatographed on silica gel column with $CHCl_3$-EtOAc (50:1) to give two fractions (MC II a, MC II b). Fraction MC II a was recrystallized with MeOH to yield **2** (12 mg). Fraction MC II b was applied over silica gel column chromatography (*n*-hexane-$CHCl_3$-MeOH, 8:10:1) to yield **3** (10 mg). Subfraction MC III (2.0 g) was rechromatographed on silica gel column (CH_2Cl_2-acetone-EtOAc, 1:5:1) and purified by Lobar A column (*n*-hexane-$CHCl_3$-MeOH, 8:10:1) to give **4** (12 mg). Subfraction MC IV (2.8 g) was rechromatographed on silica gel column ($CHCl_3$-MeOH-H_2O, 40:10:1) and purified by Lobar A column (CH_2Cl_2-MeOH-H_2O, 10:10:1) to give **5** (10 mg). BuOH soluble fraction was applied over silica gel column using a solvent system of $CHCl_3$-MeOH-H_2O (5:5:1) as an eluent to give four subfractions (B-B), subfraction B III (2.5 g) was rechromatographed on silica gel column with $CHCl_3$-MeOH-H_2O (25:10:1) and purified by prep-TLC to yield **6** (9 mg).

Compound 1 (Acetylcorynoline): mp 159-160°C, EIMS *m/z* (rel. int.) 409 (M^+), 366, 349 (100), 334, 202, 190, ^{1}H-NMR (400MHz, $CDCl_3$) δ: 6.98 (1H, d, *J*=8.5, H-10), 6.87 (1H, s, H-4), 6.69 (1H, d, *J*=8.5Hz, H-9), 6.52 (1H, s, H-1), 5.93 (2H, s, -OCH_2O-), 5.92 (2H, s, -OCH_2O-), 5.21 (1H, dd, *J*=8.8, 6.4, H-11), 3.90 (1H, d, *J*=16.4, H-6), 3.53 (1H, d, *J*=16.4, H-6), 3.51 (1H, s, H-14), 2.96 (1H, dd, *J*=15.2, 8.4, H-12), 2.84 (1H, dd, *J*=15.2, 6.4, H-12), 2.48 (3H, s, *N*-CH_3), 1.87 (3H, s, CO$\underline{CH_3}$), 1.27 (3H, s, CH_3), ^{13}C-NMR (100MHz, $CDCl_3$) δ 170.4 ($\underline{C}OCH_3$), 146.6 (C-2)*, 145.9 (C-3)*, 144.5 (C-8)*, 142.8 (C-7), 132.9 (C-10a), 129.9 (C-4a), 127.4 (C-1a), 120.3 (C-10), 117.5 (C-6a), 109.4 (C-9), 108.3 (C-4), 106.2 (C-1), 100.9 (-OCH_2 O-), 100.7 (-OCH_2O-), 75.4 (C-11), 70.2 (C-14), 49.5 (C-6), 43.7 (*N*-CH_3), 42.5 (C-13), 32.8 (C-12), 27.8 (CH_3), 21.2 (CO$\underline{C}H_3$). *Assignments may be reversed.

Compound 2 (Corynoline): mp 217-218°C, EIMS *m/z* (rel. int.) 367 (M^+), 349 (100), 334, 202, 190, ^{1}H-NMR (400MHz, $CDCl_3$) δ: 6.93 (1H, d, *J*=8.5, H-10), 6.80 (1H, d, *J*=8.5Hz, H-9), 6.66 (1H, s, H-4), 6.64 (1H, s, H-1), 5.95 (2H, s, -OCH_2O-), 5.94 (2H, s, -OCH_2O-), 4.06 (1H, d, *J*=15.2, H-6), 3.96 (1H, m, H-11), 3.45 (1H, d, *J*=15.2, H-6), 3.31 (1H, s, H-14), 3.16 (1H, d, *J*=16.8, H-12), 3.08 (1H, dd, *J*=16.8, 4.4, H-12), 2.21 (3H, s, *N*-CH_3), 1.15 (3H, s, CO$\underline{CH_3}$), 1.27 (3H, s, CH_3), ^{13}C-NMR (100MHz, $CDCl_3$) δ 147.8 (C-2)*, 145.1 (C-3)*, 144.9 (C-8)*, 142.6 (C-7), 135.9 (C-10a), 127.7 (C-4a), 125.1 (C-1a), 118.5 (C-10), 116.7 (C-6a), 112.6 (C-4), 109.3 (C-9), 107.6 (C-1), 101.2 (-OCH_2O-), 100.9 (-OCH_2O-), 76.1 (C-11), 69.7 (C-14), 54.3 (C-6), 43.2 (*N*-CH_3), 40.8 (C-13), 36.7 (C-12), 23.4 (CH_3). *Assignments may be reversed.

Compound 3 (Corynoloxine): mp 205-207°C, EIMS *m/z* (rel. int.) 365 (M^+), 336, 306, 280, 189, 175, ^{1}H-NMR (400MHz, $CDCl_3$) δ: 6.82 (1H, d, *J*=8.5, H-10), 6.80 (1H, d, *J*=8.5Hz, H-9), 6.69 (1H, s, H-4), 6.61 (1H, s, H-1), 5.99 (2H, s, -OCH_2O-), 5.92 (2H, s, -OCH_2O-), 5.29 (1H, s, H-6), 3.64 (1H, m, H-11), 3.12 (1H, d, *J*=18.0, H-12), 2.94 (1H, dd, *J*=18.0, 3.2, H-12), 2.84 (1H, s, H-14), 2.15 (3H, s, *N*-CH_3), 1.28 (3H, s, CH_3), ^{13}C-NMR (100MHz, $CDCl_3$) δ: 147.1 (C-8)*, 146.4 (C-3)*, 145.8 (C-2)*, 141.2 (C-7), 134.2 (C-10a), 130.6 (C-4a), 124.0 (C-1a), 118.4 (C-6a), 114.3 (C-10), 109.6 (C-9), 108.9 (C-4), 106.7 (C-1), 101.3 (-OCH_2O-), 100.7 (-OCH_2O-), 77.5 (C-6), 72.1 (C-11), 64.1 (C-14), 39.6 (*N*-CH_3), 36.6 (C-13), 32.3 (C-12), 15.6 (CH_3). *Assignments may be reversed.

Compound 4 (Luguine): mp 280-282°C, EIMS (*m/z*): 335 (M^+), 317, 290, 275, 82, ^{1}H-NMR (400MHz, CD_3OD) δ: 9.62 (1H, s, H-6), 7.89 (1H, d, *J*=9.0Hz, H-10), 7.79 (1H, d, *J*=9.0Hz, H-9), 7.23 (1H, s, H-4), 6.92 (1H, s, H-1), 6.37 (2H, s, -OCH_2O-), 6.01 (2H, s, -OCH_2O-), 5.98 (1H, m, H-11), 3.06 (2H, m, H-12).

Compound 5 (6-Oxocorynoline): EIMS (*m/z*): 381 (M^+), 363, 348, 332, 190, ^{1}H-NMR (400MHz, DMSO-d_6) δ: 7.54 (1H, d, *J*=8.4Hz, H-9), 6.87 (1H, d, *J*=8.4Hz, H-10), 6.51 (1H, s, H-4), 6.49 (1H, s, H-1), 5.89 (2H, s, -OCH_2O-), 5.87 (2H, s, -OCH_2O-), 4.32 (1H, m, H-11), 4.00 (1H, s, H-14), 3.31 (3H, s, *N*-CH_3), 1.34 (3H, s, CH_3), ^{13}C-NMR (100MHz, DMSO-d_6) 160.6 (C-6), 146.7 (C-8)*, 146.5 (C-3)*, 146.1 (C-2)*, 145.8 (C-7)*, 135.8 (C-10a), 130.1 (C-4a), 127.1 (C-1a), 118.7 (C-10), 112.6 (C-6a), 110.3 (C-9), 108.0 (C-4), 105.3 (C-1), 101.3 (-OCH_2O-), 100.8 (-OCH_2O-), 71.9 (C-11), 67.1 (C-14), 43.1 (*N*-CH_3), 37.6 (C-13), 35.2 (C-12), 24.7 (CH_3). *Assignments may be reversed.

Compound 6 (12-Hydroxycorynoloxine): mp 216-218 °C, $[\alpha]_D^{20}$ 0.0° (c=0.3, MeOH), HRMS *m/z*: 381.3867 (M^+, calcd for $C_{21}H_{19}NO_6$, 381.3863), EIMS *m/z* (rel. int.) 381 (M^+, 35), 366 (25), 350 (23), 202 (100), 189 (38), IR ν_{max}^{KBr} cm^{-1} 3250, ^{1}H-NMR (400MHz, CD_3OD) δ: 6.90 (1H, s, H-4), 6.85 (1H, d, *J*=8.0Hz, H-10), 6.79 (1H, d, *J*=8.0Hz, H-9), 6.59 (1H, s, H-1), 5.91 (2H, s, -OCH_2O-), 5.86 (2H, s, -OCH_2O-), 5.14 (1H, s, H-6), 4.70 (1H, d, *J*=1.9, H-12), 3.37 (1H, d, *J*=1.9, H-11), 2.76 (1H, s, H-14), 1.94 (3H, s, *N*-CH_3), 1.30 (3H, s, CH_3), ^{13}C-NMR (100MHz, CD_3OD) δ 149.0 (C-8)*, 148.4 (C-3)*, 148.1 (C-2)*, 142.9 (C-7), 141.1 (C-10a), 135.4 (C-4a), 132.3 (C-1a), 128.5 (C-6a), 116.2 (C-10), 110.9 (C-9), 109.4

(C-4), 108.4 (C-1), 103.0 (-OCH_2O-), 102.3 (-OCH_2O-), 80.3 (C-6), 78.5 (C-12), 71.1 (C-11), 65.8 (C-14), 40.0 (*N*-CH_3), 36.9 (C-13), 16.9 (CH_3). *Assignments may be reversed.

RESULTS AND DISCUSSION

In the course of phytochemical study of the MeOH extract from *C. incisa*, six benzo[c]phenanthridine alkaloids, corynoline (**1**), acetylcorynoline (**2**), corynoloxine (**3**), luguine (**4**), 6-oxocorynoline (**5**), and 12-hydroxycorynoloxine (**6**) were isolated from the CH_2Cl_2 and *n*-BuOH soluble fractions.

Compounds **1-6** have similar patterns in their NMR spectra. Compounds **1-3** and **5** were readily elucidated as corynoline, acetylcorynoline, corynoloxine, 6-oxocorynoline, respectively, by comparison of reported spectroscopic data (Nonaka *et al.*, 1973a, Takao *et al.*, 1978, Kametani *et al.*, 1971, and Nonaka *et al.*, 1973b).

The EI-MS of **4** gave a molecular ion at *m/z* 335 [M^+]. In the NMR spectrum of **4**, the signals of a proton at oxygen-bearing carbon (1H, δ 5.98, m, H-11), the methylenedioxide group (δ 6.37, 6.01), and five aromatic protons at C-6, -10, -9, -4 and -1 (each 1H, δ 9.62, 7.89, 7,79, 7.23 and 6.92) were observed. A pair of doublets (J=9.0Hz) at δ 7.89 and 7.79 was assigned to the 10- and 9-protons, respectively. With the above evidences and by the direct comparison of its spectral data with those of the literature, the structure of **4** was identified as luguine, which has been previously isolated from *Glaucium flavum* var. *vestitum* (Castedo *et al.*, 1978).

Compound **6** was assigned the molecular formula $C_{21}H_{19}NO_6$ (*m/z*, 381.3867, [M^+]) by its EI- and HR-mass spectrometry. IR spectrum of **6** revealed the presence of hydroxyl group (3250 cm^{-1}). Its ^{1}H-NMR spectrum showed the presence of a methyl group (δ 1.30), an *N*-methyl group (δ 1.94), two methylenedioxy groups (δ 5.91, 5.86) and four aromatic protons (δ 6.90-6.59). These spectral data suggested that **6** had a benzo[c]phenanthridine skeleton (Nonaka *et al.*, 1975b). The ^{1}H- and ^{13}C-NMR data of **6** were very similar to that of **3**, except for the proton and carbon chemical shifts of C-12 position. The downfield shift of C-12 (δ 78.5) suggested that C-12 was carrying a hydroxyl group. In the ^{1}H-NMR spectrum of **6**, H-12 proton appeared more downfield at δ 4.70 (1H) while that of **3** showed at δ 3.11 and 2.94 (2H), indicating that the C-12 bears a hydroxyl group, and the proton signal at δ 4.70 (1H, d, J=1.9, H-12) showed correlation with the signal at 3.37 (1H, d, J=1.9, H-11). Therefore, the structure of **6** was characterized as 12-hydroxycorynoloxine. Finally, the structure and stereochemistry of compound **6** was identified by comparison with the spectral data of the synthesized compounds. (Nonaka *et al.*, 1975b).

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