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Spirobenzylisoquinoline Alkaloids from *Corydalis ochotensis*

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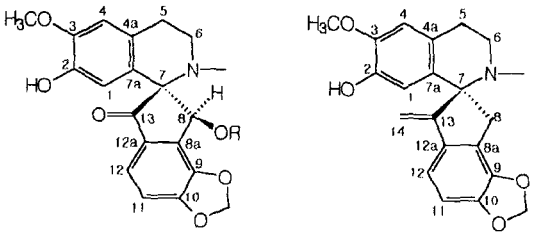
Separation of the alkaloids from the aerial parts of *Corydalis ochotensis* afforded a new spirobenzylisoquinoline alkaloid, 8-*O*-acetylcorysolidine along with two known spirobenzylisoquinoline alkaloids, isoochotensine and corysolidine.

Key words: *Corydalis ochotensis*, Spirobenzylisoquinoline alkaloids, Isoochotensine, Corysolidine, 8-*O*-acetylcorysolidine

INTRODUCTION

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

Earlier investigations on the chemical constituents of *C. ochotensis* mainly dealt with the isolation of isoquinoline alkaloids such as isoochotensine (Wu *et al.*, 1987), yenhusomine, corytenchine, corytenchirine, ochotensine, adlumidine, and protopine (Lu *et al.*, 1976). And raddeanamine, raddeanine, adlumidine, aobamine, protopine, and dihydrosanguinarine were isolated from *C. ochotensis* var. *raddeana* (Kametani *et al.*, 1977). For the isolation of isoquinoline alkaloids, BuOH and $CHCl_3$ soluble fractions were examined. Investigation on the two fractions afforded a new alkaloid, 8-O-acetylcorysolidine together with known corysolidine and isoochotensine (Fig. 1). This reports their isolation and structure elucidation.



Comp. I R = H
Comp. II R = $COCH_3$

Comp. III

Fig. 1. *Structures of compound* **I, II,** *and* **III.**

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MATERIALS AND METHODS

^1H- and ^{13}C-NMR spectra were determined on a JEOL JMN-EX 400 spectrometer. The EI/MS (70 eV) were determined on a VG-VSEQ mass spectrometer (VG Analytical, UK). The UV spectra were recorded on Shimadzu UV-1601 UV-Visible spectrophotometer. TLC was carried out on Merck precoated silica gel F_{254} plates and silica gel for column chromatography was Kiesel gel 60 (230-400 mesh, Merck). All other chemicals and solvents were analyticlal grade and used without further purification.

Plant materials

Corydalis ochotensis was collected in August 1998 at Jinankun, Chonbuk, Korea. A voucher specimen is deposited in the herbarium of college of pharmacy, Woosuk University (WSU-98-005).

Extraction and isolation

The air-dried plant materials (1.5 Kg) was finely ground and extracted at room temp. with MeOH. The resultant MeOH extract (290 g) followed by the successive solvent parition to give *n*-hexane (25 g), $CHCl_3$ (12 g), *n*-BuOH (60 g) and H_2O soluble fractions. The *n*-BuOH soluble fraction was chromatographed over silica gel column using a solvent system of CH_2Cl_2-EtOAc-MeOH (7:1:1) as an eluent to give eight subfractions, subfraction six (2.5 g) was rechromatographed on Lobar A column (Merck Lichroprep Si 60, 240-10 mm, CH_2Cl_2-EtOAc-MeOH, 10:1:1) to yield **I** (10 mg). The $CHCl_3$ soluble fraction was applied over silica gel column using a gradient solvent system of CH_2Cl_2-EtOAc-MeOH (6:1:0→40:1:1) as an eluent to give three subfractions, subfraction two (1.5 g) was rechromatographed on silica gel column with CH_2Cl_2-EtOAc (7:1) to give three fractions. Fractions two and three were applied

over Sephadex LH-20 column chromatography (Pharmacia, 25-100 μm, MeOH) to yield compounds **II** (8 mg) and **III** (15 mg), respectively.

Compound I (Corysolidine): EIMS (m/z): 369 [M^+], 359, 344, 324, 206, ^{1}H-NMR (400 MHz, $CDCl_3$) δ : 7.51 (1H, d, *J*=8.0 Hz, H-12), 7.01 (1H, d, *J*=8.0 Hz, H-11), 6.67 (1H, s, H-4), 6.20, 6.19 (each 1H, d, *J*=1.1Hz, O-CH_2-O), 6.09 (1H, s, H-1), 5.58 (1H, s, H-8), 3.86 (3H, s, OCH_3), 2.41 (3H, s, *N*-CH_3).

Compound II (8-*O*-Acetylcorysolidine): EIMS m/z (rel. int.) 411 (M^+, 92), 396 (15), 368 (100), 352 (63), 324 (60), 190 (47), IR ν_{max}^{KBr} cm^{-1} 1760, 1620, 1510, UV λ_{max}^{MeOH} 290, 227, ^{1}H-NMR (400 MHz, $CDCl_3$) δ : 7.54 (1H, d, *J*= 8.0 Hz, H-12), 7.04 (1H, d, *J*=8.0 Hz, H-11), 6.66 (1H, s, H-4), 6.56 (1H, s, H-8), 6.18, 6.17 (each 1H, d, *J*=1.2 Hz, O-CH_2-O), 6.14 (1H, s, H-1), 3.85 (3H, s, OCH_3), 2.40 (3H, s, *N*-CH_3), 1.71 (3H, s, $COCH_3$), ^{13}C-NMR (100 MHz, $CDCl_3$): 201.7 (C-13), 169.3 (C$\underline{O}$$CH_3$), 154.0 (C-10), 154.6 (C-9), 144.3 (C-3), 142.5 (C-2), 132.4 (C-12a), 129.1 (C-7a), 127.4 (C-8a), 125.0 (C-4a), 119.7 (C-12), 113.8 (C-4), 110.8 (C-1), 110.2 (C-11), 102.9 (O-CH_2-O), 77.1 (C-7), 69.9 (C-8), 55.7 (OCH_3), 48.5 (C-6), 39.3 (*N*-CH_3), 27.8 (C-5), 20.5 (CO$\underline{C}$$H_3$).

Compound III (Isoochotensine): EIMS (m/z): 351 [M^+], 334, 322, 190, 148, ^{1}H-NMR (400 MHz, $CDCl_3$) δ : 7.09 (1H, d, *J*=8.4 Hz, H-12), 6.78 (1H, d, *J*=8.4 Hz, H-11), 6.50 (1H, s, H-4), 6.36 (1H, s, H-1), 5.98, 5.97 (each 1H, s, O-CH_2-O), 5.61 (1H, s, H-14α), 4.91 (1H, s, H-14β), 3.85 (3H, s, OCH_3), 3.42 (1H, d, *J*=17.6 Hz, H-8α), 2.89 (1H, d, *J*=17.6 Hz, H-8β), 2.15 (3H, s, *N*-CH_3), ^{13}C-NMR (100 MHz, $CDCl_3$): 155.4 (C-13), 147.7 (C-10), 144.8 (C-9), 143.5 (C-3), 142.7 (C-2), 137.1 (C-7a), 136.8 (C-12a), 124.7 (C-4a), 123.5 (C-8a), 113.4 (C-12), 112.4 (C-4), 109.4 (C-1), 107.7 (C-11), 106.3 (C-14), 101.1 (O-CH_2-O), 71.6 (C-7), 55.7 (OCH_3), 48.2 (C-6), 38.7 (*N*-CH_3), 36.4 (C-8), 29.1 (C-5).

RESULTS AND DISCUSSION

In the course of phytochemical study of the MeOH extract from *C. ochotensis*, three spirobenzyl isoquinoline alkaloids, corysolidine (**I**), 8-*O*-acetylcorysolidine (**II**), and isoochotensine (**III**) were isolated from the *n*-BuOH and $CHCl_3$ soluble fractions.

Compounds **I-III** have similar patterns in their NMR spectra. The EI-MS of **I** gave a molecular ion at *m/z* 369 [M^+]. In the NMR spectrum of **I**, the signals of the *N*-methyl group (3H, δ2.41, *s*), methoxy group (3H, δ3.86, *s*), a proton at oxygen-bearing carbon (1H, δ5.58, *s*, H-8), the methylenedioxide group (δ6.20, 6.19, each 1H, *d*, *J*=1.1Hz), and the four aromatic protons at C-12, -11, -4, and -1 (each 1H, δ7.51, 7.01, 6.67, 6.09) were observed. A pair of doublets (*J*=8.0 Hz) at δ7.51 and 7.02 was assigned to the 12- and 11-protons, respectively, suggesting the existence of the carbonyl group at C-13, and the H-8 signal was observed at δ5.58 as a broad singlet, suggesting that the H-8 is oriented *syn* to the nitrogen atom (Lu *et al.*, 1976). Besides the above evidences, by the direct comparison of its spectral data with those of the reported literature, the structure of **I** was established to be corysolidine, which has been isolated from *C. solida* (Rahimizadeh *et al.*, 1986).

The EI-MS of **II** gave a molecular ion at *m/z* 411 [M^+]. IR spectrum showed the presence of an ester (1760 cm-1) group. The ^{13}C-NMR spectrum of **II** exhibited the two carbonyl carbons (δ201.7 and 169.3 ppm) and twelve aromatic carbon signals. The ^{1}H-NMR spectrum of **II** was very similar to that of **I**, suggesting it has the similar carbon skeleton. The main difference was H-8 chemical shift and the presence of an acetyl group. In the ^{1}H-NMR spectrum of **II**, H-8 proton, assigned by ^{13}C-^{1}H COSY, appeared more downfield at δ6.56 while that of **I** did at δ5.58, indicating that the C-8 bears acetyloxy group. In the HMBC spectrum, the methoxy proton signal (δ3.86) showed a ^{1}H-^{13}C long-range correlation with C-3, 4. Therefore, the structure of **II** was established as 8-*O*-acetylcorysolidine.

The EI-MS of **III** gave a molecular ion at *m/z* 351 [M^+]. The ^{1}H-NMR spectrum of **III** was very similar to that of **I**, suggesting the same skeleton. The main difference was the presence δ5.61 and δ4.91 ppm resonances in ^{1}H-NMR spectrum which can be assigned to methylene group (H-14). Compound **III** was identified as isoochotensine, which has been isolated and identified from this plant, by the comparison with ^{1}H- and ^{13}C-NMR data in the literature (Wu *et al.*, 1987).

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