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Cytotoxic Constituents from the Whole Plant of *Corydalis pallida*

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Here we report the cytotoxic activity of three known compounds isolated for the first time from *Corydalis pallida* (Papaveraceae). An isoquinoline alkaloid, berberine, exhibited cytototoxic activity against two human cancer cell lines, HT-1080 (human fibrosarcoma) and SNU-638 (human stomach adenocarcinoma), with IC_{50} values of 3.2 and 3.4 μg/mL, respectively. *N-trans*-feruloyltyramine and *N-trans*-feruloylmethoxytyramine were also isolated from this plant but were inactive.

Key words: *Corydalis pallida*, Papaveraceae, Berberine, *N-trans*-Feruloylmethoxytyramine, *N-trans*-Feruloyltyramine, Cytotoxicity

INTRODUCTION

Corydalis, one of the largest genus of the Papaveraceae family, contains various isoquinoline alkaloids (Ito *et al.*, 2001; Kametani *et al.*, 1976; Kaneko *et al.*, 1971; Polyakova *et al.*, 1970; Stekol'nikov *et al.*, 1970). The *Corydalis* species have shown various biological activities, including chemopreventive activity mainly by isoquinoline akaloids (Ito *et al.*, 2001). *C. pallida* has been used for fever, poison, and edema (Kim *et al.*, 1997) as a traditional medicine. Some isoquinoline alkaloids have been derived from mixtures of *C. bulosa* and *C. pallida* var. *sparstimamma* (Ito *et al.*, 2001). To the best of our knowledge, there is no previous report on the single species of *Corydalis pallida* (Thunb.) Pers.

In the present study, the EtOAc-soluble extract of *C. pallida* showed significant cytotoxic activity and was subjected to further laboratory phytochemical investigation. Three known compounds, berberine (**1**), *N-trans*-feruloyltyramine (**2**), and *N-trans*-feruloylmethoxytyramine (**3**) were isolated for the first time from this plant. Among them, compound **1** showed a significant cytotoxic activity. These isolates **1-3** were identified by spectroscopic data and by comparison with published values.

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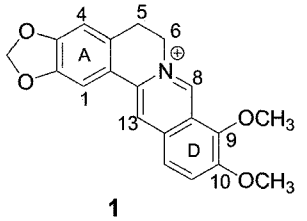

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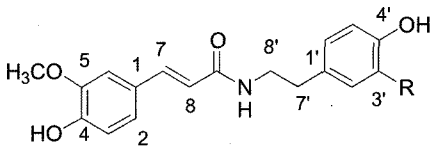

2 R = H
3 R = OCH_3

MATERIALS AND METHODS

Plant material

The whole plant of *C. pallida* was collected in Jeonbuk province, Korea, in April, 2003, and was identified by one of the authors, Prof. Nam Sook Lee, Ewha Womans University. A voucher specimen (No. EA229) has been deposited at the Natural Product Chemistry Laboratory, College of Pharmacy, Ewha Womans University.

General experimental procedures

The melting point was measured on a J-923 melting point apparatus (Jisico, Korea). Optical rotation was measured with a P-1010 polarimeter (Jasco, Japan) at

25°C. UV and IR spectra were recorded on a U-3000 spectrophotometer (Hitachi, Japan) and a FTS 135 FT-IR spectrometer (Bio-Rad, CA), respectively. 1D- and 2D-NMR experiments were performed on a UNITY INOVA 400 MHz FT-NMR instrument (Varian, CA). TMS was used as an internal standard. EIMS was obtained on a JMS 700 Mstation HRMS spectrometer (JEOL, Japan). TLC analysis was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness), with compounds visualized by dipping plates into 10% (v/v) H_2SO_4 reagent (Aldrich) followed by charring at 110°C for 5-10 min. Silica gel (Merck 60A, 200-400 mesh ASTM), YMC GEL ODS-A, and Sephadex LH-20 (Amersham Pharmacia Biotech) were used for column chromatography. All solvents used for chromatographic separations were distilled before use.

Extraction and isolation

The dried and milled plant material (2.5 kg) was extracted with MeOH (10 × 3 L) by maceration. The extracts were combined and concentrated *in vacuo* at 40°C. The concentrated extract was suspended in distilled water (1 L) and then partitioned with *n*-hexane (16 × 2 L) to produce an *n*-hexane-soluble syrup on drying. Next, the aqueous extract was partitioned again with EtOAc (9 × 2 L) to give an EtOAc-soluble extract and an aqueous residue. The EtOAc-soluble extract (25 g) was chromatographed over silica gel as a stationary phase, using a CH_2Cl_2-MeOH gradient (from 1:0 to 0:1 v/v) as the mobile phase, to afford 11 pooled fractions (F01-F11). Fraction F09 [eluted with CH_2Cl_2-MeOH (98:2 v/v); 3.5 g] was chromatographed over silica gel as a stationary phase, using a CH_2Cl_2-MeOH gradient (from 1:0 to 0:1 v/v) as mobile phase, to produce nine subfractions (F0901-F0921) and compound **1** (56 mg). Fraction F03 [eluted with CH_2Cl_2-MeOH (99.5:0.5 v/v); 5.15 g] was chromatographed over silica gel using a $CHCl_3$-MeOH gradient (from 99:1 to 0:1 v/v) to produce 18 pooled fractions (F0301-F0318). Fraction F0311 [eluted with MeOH; 255.2 mg] was purified on a reverse phase column using MeOH-H_2O (7:3) as eluent to yield **3** (28.6 mg). Fraction F0314 [eluted with MeOH; 173.1mg] was purified over a further Sephadex LH-20 column, with 100% MeOH as eluent, to give compound **2** (4.3 mg).

Assay methods

Cytotoxic potential was determined as described previously (Lee *et al.*, 1998). Briefly, cells (in log growth phase) were counted, diluted to 5×10^4 cells/mL with fresh medium, and added to 96-well microtiter plates (190 μL/well) containing test materials (10 μL in 10% aqueous DMSO). Test plates were incubated at 37°C for 3 days in a CO_2 incubator. For zero day controls, cells were incubated at 37°C for 30 min in CO_2 incubator. All treatments were performed in triplicate. After the incubation periods, cells were fixed by the addition of 50 μL of cold 50% aqueous trichloroacetic acid (4°C for 30 min), washed 4-5 times with tap water, and air-dried. The fixed cells were stained with sulforhodamin B (SRB) (0.4% w/v SRB in 1% aqueous acetic acid) for 30 min. Free SRB solution was then removed by rinsing with 1% acetic acid. The plates were then air dried, the bound dye was solubilized with 200 μL of 10 mM tris-base (pH 10.0), and absorbance was determined at 515 nm using an ELISA plate reader. Finally, the absorbance values obtained with each of the treatment procedures were averaged, and the averaged value obtained with the zero day control was subtracted. These results were expressed as a percentage, relative to solvent-treated control incubations, and IC_{50} values were calculated using non-liner regression analyses (percent survival versus concentration).

Berberine (1)

Yellow powder; m.p. 139-140°C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 9.88 (1H, s, H-8), 8.92 (1H, s, H-13), 8.20 (1H, d, J = 9.0 Hz, H-11), 7.99 (1H,d, J = 9.0 Hz, H-12), 7.78 (1H, s, H-1), 7.09 (1H, s, H-4), 6.17 (2H, s, 2,3-OCH_2O), 4.93 (1H, t, J = 6.2 Hz, H-6), 4.09 (3H, s, 10-OCH_3), 4.07 (3H, s, 9-OCH_3), 3.20 (2H, t, J = 6.4 Hz, H-5); ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C (ppm): 150.4 (C-10), 149.8 (C-3), 147.7 (C-2), 145.5 (C-8), 143.7 (C-9), 137.5 (C-13a), 133.0 (C-12a), 130.7 (C-4a), 126.8 (C-11), 123.5 (C-12), 121.4 (C-8a), 120.5 (C-13), 120.2 (C-13b), 108.4 (C-4), 105.4 (C-1), 102.1 (-OCH_2O-), 61.9 (9-OCH_3), 57.1 (10-OCH_3), 55.2 (C-6), 26.3 (C-5); LREIMS m/z (rel. int.): 336 $[M]^+$ (50), 335 (78), 320 (100), 276 (47).

N-trans-Feruloyltyramine (2)

Colorless crystals; m.p. 64-66°C; IR ν_{max} KBr 3430, 1640 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD) δ_H (ppm): 7.43 (1H, d, J = 15.2 Hz, H-8), 7.12 (1H, d, J = 2.0 Hz, H-6), 7.05 (2H, d, J = 8.8 Hz, H-3, H-5), 7.02 (1H, dd, J = 8.0, 2.0 Hz, H-2), 6.79 (1H, d, J = 8.4 Hz, H-3), 6.72 (2H, d, J = 8.0 Hz, H-2, H-6), 6.40 (1H, d, J = 15.2 Hz, H-7), 3.88 (3H, s, 5-OCH_3), 3.46 (2H, t, J = 7.6 Hz, H-8), 2.76 (2H, t, J = 7.6 Hz, H-7); ^{13}C-NMR (100 MHz, CD_3OD) δ_C (ppm): 169.3 (C-9), 157.0 (C-4), 149.9 (C-4), 149.3 (C-5), 142.2 (C-7), 131.4 (C-1), 131.0 (C-3, C-5), 128.4 (C-1), 123.3 (C-2), 118.9 (C-8), 116.6 (C-3), 116.4 (C-2, C-6), 111.7 (C-6), 56.9 (5-OCH_3), 42.9 (C-8), 36.2 (C-7); LREIMS *m/z* (rel. int.): 313 $[M]^+$ (18), 192 (8), 177 (100), 145 (19), 129 (10) 120 (26), 107 (17), 89 (10).

N-trans-Feruloylmethoxytyramine (3)

A white amorphous powder, m.p. 111-113°C; IR ν_{max} KBr 3430, 1640 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H (ppm):

7.44 (1H, d, *J* = 15.6 Hz, H-7), 7.11 (1H, d, *J* = 1.6 Hz, H-6), 7.01 (1H, dd, *J* = 8.6, 1.6 Hz, H-2), 6.81 (1H, d, *J* = 2.0 Hz, H-2), 6.79 (1H, d, *J* = 8.6 Hz, H-3), 6.72 (1H, d, *J* = 8.0 Hz, H-5), 6.66 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 6.42 (1H, d, *J* = 15.6 Hz, H-8), 3.87 (3H, s, 5-OCH_3), 3.81 (3H, s, 3-OCH_3), 3.48 (2H, t, *J* = 7.2 Hz, H-8), 2.76 (2H, t, *J* = 7.2 Hz, H-7); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ_C (ppm): 169.1 (C-9), 149.9 (C-5), 149.3 (C-4), 148.9 (C-3), 146.0 (C-4), 142.0 (C-7), 132.0 (C-1), 128.3 (C-1), 123.2 (C-2), 122.3 (C-6), 118.8 (C-8), 116.5 (C-3), 116.3 (C-5), 113.5 (C-2), 111.6 (C-6), 56.9 (5-OCH_3), 56.5 (3-OCH_3), 42.6 (C-8), 36.4 (C-7); LREIMS *m/z* (rel. int.): 343 $[M]^+$ (10), 207 (25), 194 (25), 177 (69), 150 (100).

RESULTS AND DISCUSSION

The bioassay-guided fractionation of the EtOAc-soluble extract from *C. pallida* led to the isolation of berberine (**1**), *N-trans*-feruloyltyramine (**2**), and *N-trans*-feruloylmethoxy-tyramine (**3**). Compound **1** was identified as berberine by comparison of its spectral data with previously published data (Dostal *et al.*, 2004; Blasko *et al.*, 1988; Furuya *et al.*, 1972). Berberine is a representative protoberberine, which is widely used, particularly for treating bacterial infections and stomach problems (Tan *et al.*, 2001; McDevitt *et al.*, 1998; Kedzia *et al.*, 1992; Kashiwabara *et al.*, 1977). In the present study, berberine (**1**) showed a significant cytotoxic activity against the HT-1080 (IC_{50} = 3.2 μg/mL) and SNU-638 (IC_{50} = 3.4 μg/mL) cell lines. Berberine was previously found in the *Corydalis* species, but this is the first report of isolation from *C. pallida* (Ito *et al.*, 2001).

The ^{1}H-NMR spectrum of **2** showed resonances for a set of ABX-type signals [δ 7.12 (1H, d, *J* = 2.0 Hz, H-6), 6.79 (1H, d, *J* = 8.4 Hz, H-3), 7.02 (1H, dd, *J* = 8.0, 2.0 Hz, H-2)]. A typical AABB signal was also observed at δ 7.05 (2H, d, *J* = 8.8 Hz) and 6.72 (2H, d, *J* = 8.0 Hz) for H-3,5 and H-2,6, respectively. In addition, two coupled triplets of methylene protons appeared at δ 2.76 and 3.46 (each 2H, t, *J* = 7.6 Hz) for H-7 and H-8, respectively. Olefinic protons appeared at δ 6.40 and 7.43 (each, 1H, d, *J* = 15.2 Hz), indicating *trans* couplings. Therefore, compound **2** was identified as *N-trans*-feruloyltyramine by NMR data and comparison with published values (Chen *et al.*, 1998).

Compound **3** showed similar NMR data with those of compound **2**, except for an additional methoxy signal. Compound **3** was identified as *N-trans*-feruloylmethoxy-tyramine by further analysis of 1D- and 2D-NMR data, and comparison with published values (Chen *et al.*, 1998).

Compounds **2** and **3** did not show any cytotoxic activity in the present study. Compound **2** was previously reported to exhibit the inhibitory activity on NO production (Yokozawa *et al.*, 2001) and antioxidative activity (Tomoike *et al.*, 2000; Cavin *et al.*, 1998). Compound **3** is used as an aroma and flavoring material for foods and oral hygiene products (Ley *et al.*, 2003).

In conclusion, we evaluated the cytotoxic activity of three known compounds isolated for the first time from *Corydalis pallida* (Papaveraceae). Berberine (**1**) showed cytotoxicity against cancer cells, whereas *N-trans*-feruloyltyramine (**2**) and *N-trans*-feruloylmethoxytyramine (**3**) did not.

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