Insecticidal Alkaloids on Aphids from *Corydalis turtschaninovii* Tubers

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Tubers of *Corydalis turtschaninovii* were extracted with 80% aqueous methanol (MeOH), and the concentrated extract was successively partitioned with *n*-hexane, ethyl acetate (EtOAc), butanol (*n*-BuOH), and H$_2$O. An activity-guided fractionation to search for insecticidal compounds against aphids led to the isolation of six alkaloids from the *n*-hexane and EtOAc fractions using a repeated silica gel, an octadecyl silica gel (ODS), and Sephadex LH-20 column chromatographic separations. Based on the spectroscopic data of NMR, mass spectroscopy (MS), and IR, the chemical structures of the compounds were determined to be: (+)-stylopine (1), (+)-corydaline (2), demethylcorydalmine (3), isocorypalmine (4), glaucine (5), and pseudoprotopine (6). These compounds showed significant insecticidal effects against *Aphis gossypii,* 69.7±7.2, 46.9±2.4, 68.5±8.6, 75.5±4.2, 80.2±9.7, and 78.9±11.3% at 1,000 ppm, respectively.

Key words: aphid, (+)-corydaline, *Corydalis turtschaninovii*, demethylcorydalmine, glaucine, insecticide, isocorypalmine, pseudoprotopine, (+)-stylopine

Aphids (Aphididae), also known as plant lice, have long, small bodies and suck the sap out of plants [Paik, 1972]. *Aphis gossypii,* the cotton aphid, is indigenous to tropical, subtropical, and temperate regions. The damage caused by sap-sucking leads to leaf shrinkage, indirect sooty mold, and the transmission of plant viruses [Shim *et al.*, 1979]. To prevent damage, synthetic insecticides are currently used in agricultural practices; however, this procedure has many negative side effects. Synthetic insecticides are predominantly composed of organic bases, organic phosphorus, and carbamates. Because these are highly toxic and not easily decomposed in nature, they accumulate at all stages of the food chain [Lee, 1997]. As a result, side effects such as environmental pollution, resistance to chemicals, and the poisoning of both humans and animals occur [Yamamoto, 1999].

Recently, there has been an increasing interest in the production of environment-friendly agricultural products.

The latest development of insecticides utilizes the microorganism *Bacillus thuringiensis,* an insect growth regulator (IGR), which inhibits the acceptance of neurotransmitters [Hsu, 1991; Takeda *et al.*, 1991; Jang *et al.*, 1998]. In line of this approach, neonicotinoid-type compounds, nitenpyram and acetamprid, were developed by modifying the structure of nicotine. However, pesticide resistance has become problematic [Jang *et al.*, 1998]. As a result, alkaloid compounds have been considered as replacements for insecticide. Thus, the present study focused on the Papaveraceae plants, because they contain many bioactive alkaloids. Specifically, *Corydalis turtschaninovii* alcohol extract was selected to test significant activity against *A. gossypii.*

The plant *C. turtschaninovii,* belonging to the Papaveraceae family, grows to a height of approximately 20 cm, with wide oval leaves 3~4 cm in length. Its pale red-purple flowers bloom in April and are roughly 10 mm long [Lee *et al.*, 2009]. As a traditional herb medicine, the *C. turtschaninovii* tuber has been used for the treatment of cardiac arrhythmia disease, dysmenorrheal, rheumatism, gastric issues, and duodenal ulcers [Lin *et al.*, 1996; Bae, 2002]. The major alkaloids found in this plant include

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berberine, protopine, palmatine, corydaline, and corynoline [Sagara *et al*., 1985; Matsuda *et al.,* 1988; Kim *et al*., 1999; Saito *et al*., 2004]. Therefore, this research aimed to determine which of these alkaloids show insecticidal effects against *A. gossypii*. The *C. turtschaninovii* tubers were extracted with 80% MeOH and partitioned using different polarities of solvents. The solvent fractions were evaluated for their insecticidal effects against *A. gossypii*. The repeated column chromatographies of the active fractions afforded six purified alkaloids which were then identified using spectroscopic methods of NMR, MS, and IR. The alkaloids were evaluated in terms of their insecticidal effects against *A. gossypii*.

Materials and Methods

Plant materials. The *C. turtschaninovii* tubers were collected at the Kyungdong herb market, Seoul, Korea in 2009 and were identified by Professor Dae-Keun Kim (Woosuk University, Jeonju, Korea). A voucher specimen (KHU-09-810) was reserved at the Laboratory of Natural Products Chemistry, Kyung Hee University, Yongin, Korea.

General experimental procedure. Silica gel (SiO_2), octadecyl silicagel (ODS), and Sephadex LH-20 resins used for column chromatography were Kieselgel 60 (Merck, Darmstadt, Germany), RP-18 F254$_S$ (Merck), and Sephadex LH-20 (Amersham Pharmacia Biotech Co., Uppsala, Sweden), respectively. Thin Layer Chromatography (TLC) analysis was carried out using Kiesel gel 60 F_{254} and RP-18 F_{254S} (Merck), and detected using a UV lamp, Spectroline Model ENF-240 C/F (Spectronics Corporation, New York, NY) and Dragendorff's reagent. The melting point was determined on a Fisher-John's apparatus and was not corrected. The optical rotation was measured on a JASCO P-1010 digital polarimeter (Tokyo, Japan). The IR spectrum was obtained from a Perkin Elmer Spectrum One Fourier Transform (FT)-IR spectrometer (Buckinghamshire, UK). The EI (Electron Ionization-Mass Spectrometer)-MS was recorded on a JEOL JMSAX-700 (Tokyo, Japan). The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded using a Varian Unity Inova AS-400 FT-NMR spectrometer (Palo Alto, CA).

Isolation of alkaloids from the *C. turtschaninovii* tuber. The dried and powdered *C. turtschaninovii* tubers (3 kg) were extracted with 80% aqueous MeOH (20 L× 2) at room temperature for 12 h and was subsequently filtered through a filter paper. The filtrate was evaporated *in vacuo,* leaving a dark-brown residue. The MeOH extract was suspended in water (3 L) and then successively partitioned with *n*-hexane (3 L×3), EtOAc (3 L×5) and

n-BuOH (3 L×2) to afford 23, 6, and 26 g extracts, respectively. The *n*-hexane fraction (23 g) was applied to Flash column chromatography (c.c.) (SNAP Cartridge KP-Sil, 100 g, Biotage@, Uppsala, Sweden) using *n*-hexane-EtOAc (1:1, 1 L) and $CHCl_3$-MeOH (30:1, 1.4 L →15:1, 4.3 L→8:1, 2 L) as eluents, which were monitored via TLC to produce 13 fractions (CTH-1 to CTH-13). Fraction CTH-2 [Ve/Vt (elution volume/total volume) 0.011-0.022, 1.8 g] was subjected to a SiO_2 column (Φ 4×15 cm) eluted with $CHCl_3$-MeOH (200:1, 1.6 L→ 100:1, 4 L) to produce 13 fractions (CTH-2-1 to CTH-2-13). Fraction 2-8 [Ve/Vt 0.155-0.200, 369 mg] was subjected to the SiO_2 column (Φ 3×13 cm) eluted with *n*-hexane-EtOAc (10:1, 2.5 L) to produce 9 fractions (CTH-2-8-1 to CTH-2-8-9), including a purified compound **1** [CTH-2-8-5, Ve/Vt 0.623-0.697, 21 mg, TLC (Kieselgel 60 F_{254}) R_f 0.60, *n*-hexane-EtOAc (1:1)] and a purified compound **2** [CTH-2-8-7, Ve/Vt 0.759-0.882, 170 mg, TLC (Kieselgel 60 F_{254}) R_f 0.56, *n*-hexane-EtOAc (1:1)]. The EtOAc fraction (6 g) was chromatographed over a Flash c.c. (SNAP Cartridge KP-Sil, 100 g, Biotage@) using *n*-hexane-EtOAc (1:1, 2 L) and $CHCl_3$-MeOH (30:1, 5 L→15:1, 4 L→8:1, 2 L) as eluents and was monitored via TLC, resulting in the production of 16 fractions (CTE-1 to CTE-16). Fraction CTE-4 [Ve/Vt 0.086-0.129, 287.0 mg] was subjected to the SiO_2 column (Φ 3.5×12 cm) and eluted with $CHCl_3$-MeOH (40:1, 2 L) to give 10 fractions (CTE-4-1 to CTE-4-10). Fraction CTE-4-5 [Ve/Vt 0.063-0.174, 19.0 mg] was subjected to a Sephadex LH-20 column (Φ 2×26 cm) eluted with MeOH (200 mL) to produce three fractions (CTE-4-5-1 to CTE-4-5-3), yielding a purified compound **3** [Ve/Vt 0.775-0.999, CTE-4-2, 8.6 mg, TLC (Kieselgel 60 F_{254}) R_f 0.56, $CHCl_3$-MeOH (7:1)]. Fraction CTE-3 [Ve/Vt 0.031-0.085, 1.5 g] was subjected to the SiO_2 column (Φ 4.5×13 cm) eluted with $CHCl_3$-MeOH (70:1, 0.5 L→ 15:1, 1.2 L) to give 11 fractions (CTE-3-1 to CTE-3-11). Fraction CTE-3-3 [Ve/Vt 0.090-0.200, 586.0 mg] was subjected to the SiO_2 column (Φ 4×12 cm) eluted with *n*-hexane-$CHCl_3$-MeOH (1:80:1, 1 L), to give 5 fractions (CTE-3-3-1 to CTE-3-3-5). Fraction CTE-3-3-3 [Ve/Vt 0.145-0.403, 517.0 mg] was subjected to the Sephadex LH-20 column (Φ 2.5×53 cm) eluted with acetone-H_2O (5:1, 0.25 L) to produce 9 fractions (CTE-3-3-3-1 to CTE-3-3-3-9), yielding a purified compound **4** [CTE-3-3-3-6, Ve/Vt 0.224-0.285, 19.0 mg, TLC (Kieselgel 60 F_{254}) R_f 0.41, $CHCl_3$-MeOH (15:1)]. Fraction CTE-8 [Ve/Vt 0.327-0.365, 3.5 g] was subjected to the SiO_2 column (Φ 4×15 cm) eluted with $CHCl_3$-MeOH (50:1, 3 L) to give 9 fractions (CTE-8-1 to CTE-8-9). Fraction CTE-8-2 [Ve/ Vt 0.084-0.120, 670 mg] was subjected to the ODS column (Φ 3.5×8 cm) eluted with MeOH-H_2O (5:1,

1.2 L) to give 9 fractions (CTE-8-2-1 to CTE-8-2-9). Fraction CTE-8-2-5 [Ve/Vt 0.300-0.473, 42 mg] was subjected to the Sephadex LH-20 column (Φ 1×35 cm) eluted with MeOH-H_2O (6:1, 0.1 L) to produce 8 fractions (CTE-8-2-5-1 to CTE-8-2-5-8), yielding a purified compound **5** [Ve/Vt 0.465-0.551, CTE-8-2-5-3, 28.0 mg, TLC (Kieselgel 60 RP-18) R_f 0.25, MeOH-H_2O (10:1)]. Fraction CTE-10 [Ve/Vt 0.466-0.562, 650 mg] was subjected to the SiO_2 column (Φ 4×15 cm) eluted with $CHCl_3$-MeOH (15:1, 10 L), resulting in 17 fractions (CTE-10-1 to CTE-10-17). Fraction CTE-10-8 (Ve/Vt 0.143-0.207, 148 mg) was subjected to the ODS column (Φ 3×7 cm) eluted with MeOH-H_2O (1:1, 0.5 L) to give 13 fractions (CTE-10-8-1 to CTE-10-8-13), yielding compound **6** [CTE-10-8-12, Ve/Vt 0.937-0.999, 31.0 mg, TLC (Kieselgel 60 RP-18) R_f 0.50, MeOH-H_2O (10:1)].

Compound 1 ((+)-stylopine). yellow powder; m.p. 209-212°C; $[\alpha]_D^{16}$ +297.0° (*c*=0.18, $CHCl_3$); IR (KBr, ν) 2922, 2805 2750, 1590, 1487, 1466 cm⁻¹; EI-MS *m/z* 323 [M]⁺, 308, 174, 148; ¹H-NMR (400 MHz, $CDCl_3$, δ_H) 6.69 (1H, s, H-1), 6.64 (1H, d, J=8.0 Hz, H-12), 6.60 (1H, d, J=8.0 Hz, H-11), 6.54 (1H, s, H-4), 5.91 (1H, d, J=1.6 Hz, -O-CH_2-O-), 5.89 (1H, d J=1.6 Hz, -O-CH_2-O-), 5.86 (2H, s, -O-CH_2-O-), 4.02 (1H, d, J=15.2 Hz, H-8α), 3.54 (1H, dd, J=3.6, 11.6 Hz, H-14), 3.49 (1H, d, J=15.2 Hz, H-8β), 3.24 (1H, dd, J=3.6, 16.4 Hz, H-13α), 3.12 (1H, m, H-6α), 3.04 (1H, m, H-5α), 2.75 (1H, dd, J=11.6, 16.4 Hz, H-13β), 2.63 (1H, m, H-5β), 2.58 (1H, m, H-6β); ¹³C-NMR (100 MHz, $CDCl_3$, δ_C) 146.8 (C-9), 146.6 (C-3), 145.5 (C-2), 143.7 (C-10), 130.4 (C-14a), 128.3 (C-12a), 127.6 (C-4a), 121.5 (C-8a), 116.4 (C-12), 108.7 (C-11), 107.3 (C-4), 105.5 (C-1), 101.5 (-O-CH_2-O-), 101.2 (-O-CH_2-O-), 60.1 (C-14), 53.1 (C-8), 51.6 (C-6), 36.1 (C-13), 29.2 (C-5).

Compound 2 ((+)-corydaline). yellow powder; m.p. 138-140°C; $[\alpha]_D^{25}$ +317.2° (*c*=0.20, $CHCl_3$); IR (KBr, ν) 2785, 2735, 1620 cm⁻¹; EI-MS *m/z* 369 [M]⁺; ¹H-NMR (400 MHz, $CDCl_3$, δ_H) 6.88 (1H, d, J=8.4 Hz, H-12), 6.79 (1H, d, J=8.4 Hz, H-11), 6.66 (1H, s, H-1), 6.58 (1H, s, H-4), 4.17 (1H, d, J=15.6 Hz, H-8α), 3.88 (9-OMe), 3.85 (3-OMe), 3.85 (10-OMe), 3.83 (2-OMe), 3.66 (3H, br.s, H-14), 3.47 (1H, d, J=15.6 Hz, H-8β), 3.19 (1H, m, H-13), 3.06 (2H, m, H-6), 2.57 (2H, m, H-5), 0.92 (3H, d, J=6.8 Hz, 13-Me); ¹³C-NMR (100 MHz, $CDCl_3$, δ_C) 149.9 (C-10), 147.5 (C-2), 147.0 (C-3), 144.7 (C-9), 134.8 (C-12a), 128.3 (C-8a), 128.2 (C-4a), 128.2 (C-4a), 123.9 (C-12), 111.0 (C-11), 110.8 (C-4), 108.6 (C-1), 62.9 (C-14), 60.0 (9-OMe), 56.0 (2-OMe), 55.7 (3-OMe), 55.7 (10-OMe), 54.3 (C-8), 51.3 (C-6), 38.1 (C-13), 29.6 (C-5), 18.2 (C-13Me).

Compound 3 (demethylcorydalmine). yellow powder; m.p. 142-143°C; $[\alpha]_D^{16}$ 0° (*c*=0.20, CH_3OH); IR (KBr, ν) 3347, 2842, 1590 cm⁻¹; EI-MS *m/z* 327 [M]⁺; ¹H-NMR (400 MHz, $CDCl_3$, δ_H) 6.80 (1H, s, H-1), 6.71 (1H, d, J=8.4 Hz, H-11), 6.64 (1H, d, J=8.4 Hz, H-12), 6.57 (1H, s, H-4), 4.21 (1H, d, J=15.6 Hz, H-8α), 3.84 (6H, s, 2, 3-OMe), 3.49 (2H, m, H-14, 8β), 3.20 (2H, m, H-6α, 13α), 3.12 (1H, m, H-5α), 2.79 (1H, dd, J=15.6, 11.2 Hz, H-13β), 2.63 (2H, m, H-5β, 6β); ¹³C-NMR (100 MHz, $CDCl_3$, δ_C) 144.8 (C-3), 143.8 (C-2), 143.7 (C-10), 141.2 (C-9), 130.3 (C-14a), 127.9 (C-12a), 125.9 (C-4a), 121.0 (C-8a), 119.2 (C-12), 111.2 (C-1), 110.4 (C-4), 108.8 (C-11), 59.1 (C-14), 56.1 (2-OMe), 55.8 (3-OMe), 53.4 (C-8), 51.5 (C-6), 36.2 (C-13), 29.1 (C-5).

Compound 4 (isocorypalmine). yellow powder; m.p. 228-235°C; $[\alpha]_D^{25}$ -173.0° (*c*=2.44, $CHCl_3$); IR (KBr, ν) 3336, 3000, 2928, 1661, 1609, 1495, 1271 cm⁻¹; EI-MS *m/z* 341 [M]⁺; ¹H-NMR (400 MHz, $CDCl_3$, δ_H) 7.24 (1H, s, H-1), 6.90 (1H, d, J=8.4 Hz, H-12), 6.84 (1H, d, J=8.4 Hz, H-11), 6.75 (1H, s, H-4), 4.40 (1H, d, J=16.0 Hz, H-8α), 3.86 (3H, s, 9-OMe), 3.74 (3H, s, 3-OMe), 3.71 (3H, s, 10-OMe), 3.52 (1H, m, H-13α), 3.47 (1H, m, H-8β), 3.34 (1H, dd, J=15.6, 3.2 Hz, H-14), 3.18 (1H, m, H-5β), 3.11 (1H, m, H-6β), 2.96 (1H, dd, J=15.6, 11.2 Hz, H-13β), 2.58 (2H, m, H-5α, 6α); ¹³C-NMR (100 MHz, $CDCl_3$, δ_C) 150.6 (C-10), 147.1 (C-3), 146.4 (C-2), 145.5 (C-9), 131.1 (C-14a), 129.3 (C-12a), 128.6 (C-8a), 125.5 (C-4a), 124.2 (C-12), 113.8 (C-1), 112.2 (C-4), 111.5 (C-11), 60.0 (9-OMe), 59.7 (C-14), 55.9 (3, 10-OMe), 54.6 (C-8), 52.1 (C-6), 37.0 (C-13), 29.6 (C-5).

Compound 5 (glaucine). yellow powder; m.p. 117-118°C; $[\alpha]_D^{25}$ −99.6° (*c*=1.00, $CHCl_3$); IR (KBr, ν) 2800, 1610, 1450, 1320, 1200 cm⁻¹; EI-MS *m/z* 355 [M]⁺; ¹H-NMR (400 MHz, $CDCl_3$, δ_H) 7.94 (1H, s, H-11), 6.85 (1H, s, H-8), 6.64 (1H, s, H-3), 3.84 (3H, s, 1-OMe), 3.82 (3H, s, 2-OMe), 3.81 (3H, s, 9-OMe), 3.57 (3H, s, 10-OMe), 3.06 (1H, m, H-5α), 3.03 (1H, m, H-7α), 3.02 (1H, m, H-4α), 2.99 (1H, m, H-5β), 2.90 (1H, m, H-6α), 2.63 (1H, m, H-4β), 2.49 (3H, s, N-CH_3), 2.43 (1H, m, H-7β), 2.40 (1H, m, H-7β); ¹³C-NMR (100 MHz, $CDCl_3$, δ_C) 153.4 (C-2), 149.5 (C-9), 148.6 (C-10), 145.4 (C-1), 130.4 (C-7a), 129.7 (C-3a), 127.7 (C-1b), 127.4 (C-1a), 125.4 (C-11a), 113.1 (C-11), 112.3 (C-8), 111.6 (C-3), 63.7 (C-6a), 60.4 (1-OMe), 56.5 (2-OMe), 56.2 (9-OMe), 56.1 (10-OMe), 54.0 (C-5), 43.9 (6-Me), 34.0 (C-7), 29.5 (C-4).

Compound 6 (pseudoprotopine). yellow powder ($CHCl_3$-MeOH); m.p. 200-202°C; $[\alpha]_D$ 0° (*c*=1.25, $CHCl_3$); IR (KBr, ν) 1679, 1618, 1495 cm⁻¹; EI-MS *m/z* 353 [M]⁺; ¹H-NMR (400 MHz, $CDCl_3$, δ_H) 6.88 (1H, s, H-4), 6.65 (1H, s, H-1), 6.64 (1H, s, H-12), 6.62 (1H, s, H-9), 5.92 (2H, s, -O-CH_2-O-), 5.90 (2H, s, -O-CH_2-O-), 3.64 (2H, m, H-13), 3.50 (2H, m, H-8), 2.52 (2H, m, H-5), 2.45 (2H, m, H-6), 1.90 (3H, s, 7-Me); ¹³C-NMR (100
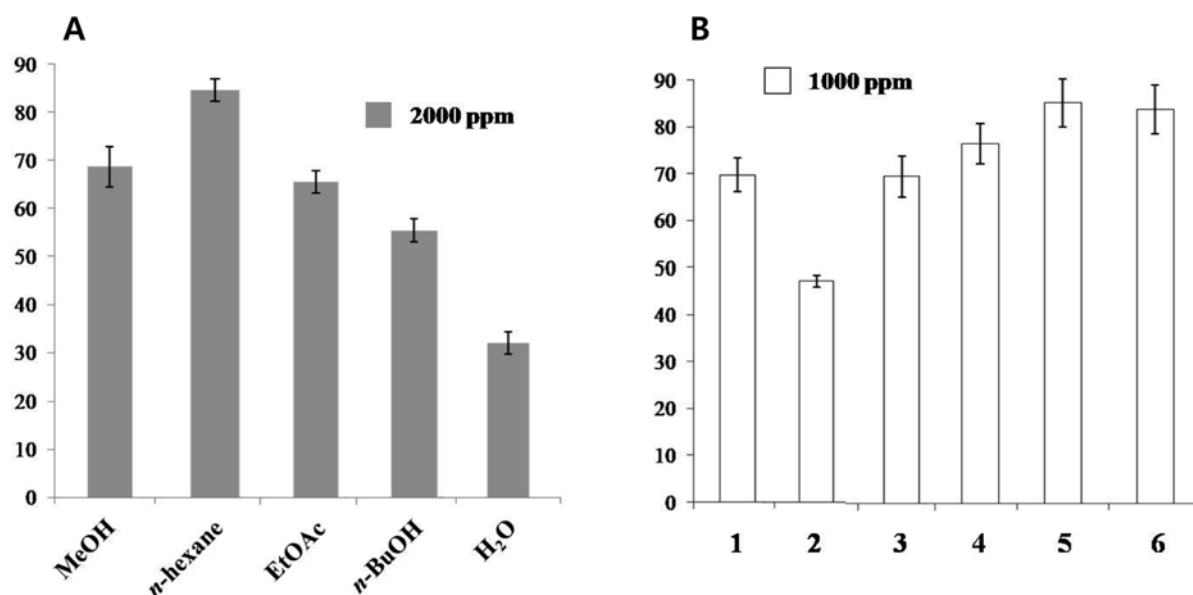
Fig. 1. Insecticidal activity of alcohol extract, solvent fractions, and alkaloids isolated from the tuber of *C. turtschaninovii* on *A. gossypii*. A, insecticidal activities of MeOH extract, *n*-hexane, EtOAc, *n*-BuOH, and H$_2$O fractions at 2000 ppm against *A. gossypii*; B, insecticidal activity of alkaloids, (+)-stylopine (**1**), (+)-corydaline (**2**), demethylcorydalmine (**3**), isocorypalmine (**4**), glaucine (**5**), and pseudoprotopine (**6**) against *A. gossypii* at 1,000 ppm. The 3-4th leaf stages of cucumber seedlings were used for the experiments. After *A.gossypii* were transferred to the plants, the infested plants were sprayed with the solutions and compounds dissolved in 5% methanol and suspended in distilled water containing Triton X-100 at 250 mg mL^{-1}. The data are presented as mean±SD of three replications.

MHz, CDCl$_3$, δ$_C$) 194.2 (C-14), 147.8 (C-3), 146.1 (C-10), 145.8 (C-2), 145.7 (C-11), 135.8 (C-4a), 132.5 (C-14a), 128.7 (C-12a), 124.9 (C-12), 117.6 (C-8a), 110.3 (C-9), 108.1 (C-4), 106.6 (C-1), 101.1 (-O-CH$_2$-O-), 100.7 (-O-CH$_2$-O-), 57.7 (C-6), 50.7 (C-8), 46.3 (C-13), 41.4 (N-CH$_3$), 31.9 (C-5).

Insecticidal activity assay against *A. gossypii*. Insecticidal activities of alcohol extracts and solvent fractions, and the purified alkaloids from the tubers of *C. turtschaninovii* extract were assessed on cucumber plants against *A. gossypii*. Cucumber seedlings at 3-4th leaf stages were used for the experiments. *A. gossypii* (50 to 100 per seedling) were transferred to cucumber seedlings. The number of *A. gossypii* on the plants was counted, and then the infested plants were sprayed with each solution. The extracts, fractions, and compounds were dissolved in 5% MeOH and were suspended in distilled water containing Triton X-100 at 250 μg mL^{-1}. The control values were determined using Abbott's formula: % control=[survival ratio (%) of aphids in untreated plants–survival ratio (%) of aphids in treated plants]×100/ [survival ratio (%) of aphids in untreated plants], and the total number of aphids surviving was counted [Abbott, 1925]. Insecticidal activity was evaluated three days after the applications in three replicates per treatment.

Results and Discussion

C. turtschaninovii tubers were extracted in aqueous methanol, and the obtained extracts were successively partitioned using *n*-hexane, EtOAc, *n*-BuOH, and H$_2$O. The *n*-hexane and EtOAc fractions showed high insecticidal activities against *A. gossypii* (Fig. 1). Developing both fractions on TLC with Dragendorff's regent indicated the presence of alkaloids as major components. Thus, column chromatographic separations of the fractions were carried out. Repeated silica gel, ODS, and Sephadex LH-20 c.c. were used to produce six purified compounds, **1-6**.

Compound **1**, a yellow powder, showed absorbance bands due to the double bond (1590 cm^{-1}) in the IR spectrum. The molecular weight was determined to be 323. In the ^{1}H-NMR (400 MHz, CDCl$_3$) spectrum, two singlet olefine methines (δ$_H$ 6.69, H-1; δ$_H$ 6.54, H-4), two doublet olefine methines showing vicinal coupling (δ$_H$ 6.64, d, H-12; δ$_H$ 6.60, d, H-11; *J*=8.0 Hz), and two dioxymethylene proton signals (δ$_H$ 5.91, d; δ$_H$ 5.89, d, *J*=1.6 Hz; δ$_H$ 5.86, s) were observed. Additionally, two nitrogenated methylenes (δ$_H$ 4.02, d, H-8a; δ$_H$ 3.49, d, *J*=15.2 Hz, H-8β; δ$_H$ 3.12, m, H-6α; δ$_H$ 2.58, m, H-6β) and two methylene proton signals (δ$_H$ 3.24, dd, *J*=3.6, 16.4 Hz, H-13α; δ$_H$ 2.75, dd, *J*=11.6, 16.4 Hz, H-13β; δ$_H$
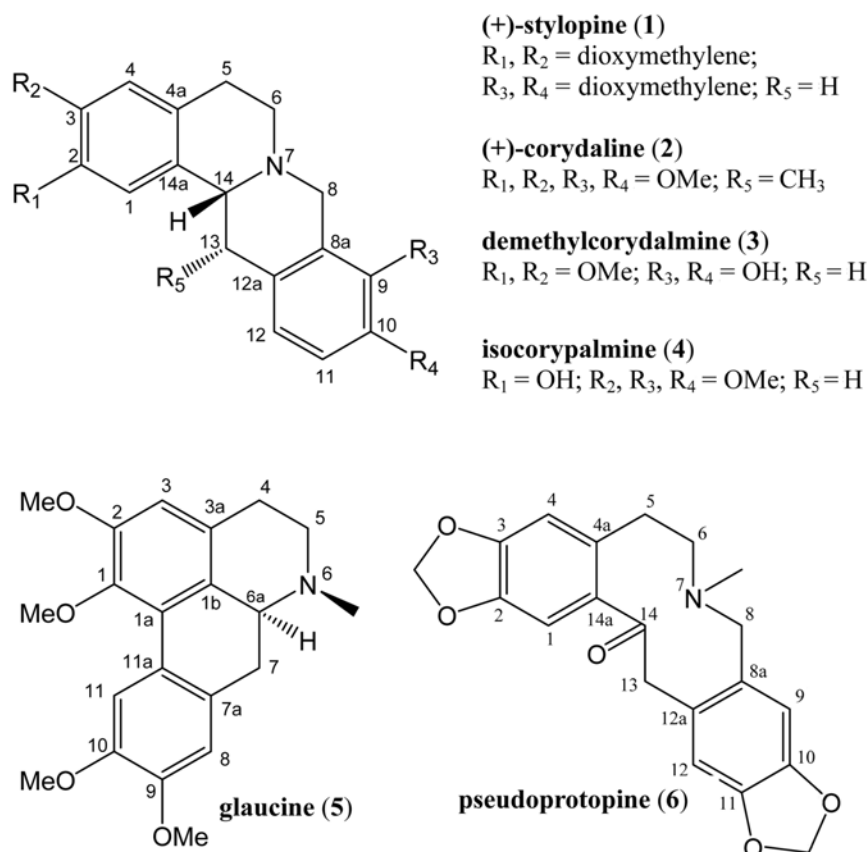
(+)-stylopine (1)
R₁, R₂ = dioxymethylene;
R₃, R₄ = dioxymethylene; R₅ = H

(+)-corydaline (2)
R₁, R₂, R₃, R₄ = OMe; R₅ = CH₃

demethylcorydalmine (3)
R₁, R₂ = OMe; R₃, R₄ = OH; R₅ = H

isocorypalmine (4)
R₁ = OH; R₂, R₃, R₄ = OMe; R₅ = H

glaucine (5)

pseudoprotopine (6)

Fig. 2. Chemical structures of alkaloids from the tubers of *C. turtschaninovii*.

3.04, m, H-5α; δ_H 2.63, m, H-5β) were observed. The ^{13}C-NMR (100 MHz, CDCl₃) spectrum showed 19 carbon signals: four oxygenated olefine quaternaries (δ_C 146.8, C-9; 146.6, C-3; 145.5, C-2; 143.7, C-10), four olefine quaternaries (δ_C 130.4, C-14a; 128.3, C-12a; 127.6, C-4a; 121.5, C-8a), four olefine methines (δ_C 116.4, C-12; 108.7, C-11; 107.3, C-4; 105.5, C-1), two dioxymethylenes (δ_C 101.5, -O-CH₂-O-; 101.2, -O-CH₂-O-), one nitrogenated methine (δ_C 60.1, C-14), two nitrogenated methylenes (δ_C 53.1, C-8; 51.6, C-6), and two methylenes (δ_C 36.1, C-13; 29.2, C-5), the multiplicities of which were determined using distortionless enhancement by polarization transfer (DEPT) experiments. The positions of the two dioxymethylene groups were determined to be between C-2 and C-3 and between C-9 and C-10, respectively, using hetero-nuclear multiple bonding connectivity (HMBC) experiments. The two dioxymethylene proton signals (δ_H 5.89, 5.86) showed correlations with four oxygenated olefine quaternary carbon signals (C-2, δ_C 145.5; C-3, δ_C 146.6; C-9, δ_C 146.8; C-10, δ_C 143.7). Thus, Compound **1** was identified as a protoberberine-type alkaloid, (+)-stylopine, and this was confirmed by comparison of the spectroscopic data with the published data. In addition, the stereostructure was determined to be

(+)-stylopine through comparison of specific rotation value of the compound, $[\alpha]_D^{16}$ +297.0°, with that reported in the literature [Lee *et al*., 2009].

Compound **2**, a yellow powder, showed absorbance bands due to the double bond (1620 cm^{-1}) in the IR spectrum. The molecular weight was determined to be 369. The ^{1}H-NMR (400 MHz, CDCl₃) and ^{13}C-NMR (100 MHz, CDCl₃) spectra of compound **2** were almost the same as those of compound **1**, except for four methoxies (δ_H 3.88, s , 9-OMe; δ_H 3.85, s , 3-OMe; δ_H 3.85, s , 10-OMe; δ_H 3.83, s , 2-OMe; δ_C 60.0, 9-OMe; 56.0, 2-OMe; 55.7, 3-OMe; 55.7, 10-OMe) and one doublet methyl (δ_H 0.92, d, J=6.8 Hz, 13-Me; δ_C 18.2, 13-Me). Thus, compound **2** was identified as a protoberberine-type alkaloid, corydaline. After comparing the specific rotation value with that reported in the literature [Qu *et al*., 2007], the stereostructure was determined to be (+)-corydaline.

Compound **3**, a yellow powder, showed absorbance bands due to the hydroxy group (3347 cm^{-1}) and the double bond (1590 cm^{-1}) in the IR spectrum. The molecular weight was determined to be 327. The ^{1}H-NMR (400 MHz, CDCl₃) and ^{13}C-NMR (100 MHz, CDCl₃) spectra of compound **3** were very similar to those

Table 1. ¹³C-NMR (100 MHz) chemical shifts of compounds 1-6 from the tuber of *C. turtschaninovii* (CDCl₃)

No of C	1	2	3	4	5	6
1	105.7	108.6	111.2	113.8	145.4	106.6
2	145.5	147.5	143.8	146.4	153.4	145.8
3	146.6	147.0	144.8	147.1	111.6	147.8
4	107.3	110.8	110.4	112.2	29.5	108.1
5	29.2	29.2	29.1	29.6	54.0	31.9
6	51.6	51.3	51.5	52.1	-	57.7
7	-	-	-	-	34.9	-
8	53.1	54.3	53.4	54.6	112.3	50.7
9	146.8	144.7	141.2	145.5	149.5	110.3
10	143.7	149.9	143.7	150.6	148.6	146.1
11	108.7	111.0	108.8	111.5	113.1	145.7
12	116.4	123.9	119.2	124.2	-	124.9
13	36.1	38.1	36.2	37.0	-	46.3
14	60.1	62.9	59.1	59.7	-	194.2
1a		-	-	-	127.4	-
1b	-	-	-	-	127.7	-
3a	-	-	-	-	129.7	-
4a	127.6	128.2	125.9	125.5	-	135.8
6a	-	-	-	-	63.7	-
7a	-	-	-	-	130.4	-
8a	121.5	128.3	121.0	128.6	-	117.6
11a	-	-	-	-	125.4	
12a	128.3	134.8	127.9	129.3	-	128.7
14a	130.4	128.2	130.3	131.1	-	132.5
N-Me	-	-	-	-	43.9	41.4
O-Me	-	60.0	56.1	60.0	60.4	-
O-Me	-	56.0	55.8	55.9	56.5	-
O-Me	-	55.7	-	55.9	56.2	-
O-Me	-	55.7	-	-	56.1	-
13-Me	-	18.2	-	-	-	-
O-CH₂-O	101.5	-	-	-	-	101.1
O-CH₂-O	101.2	-	-	-	-	100.7

of compound **1**, with the exception of two methoxies (δ_H 3.84, s, 2,3-O-Me; δ_C 56.1, 2-OMe; 55.8, 3-OMe). In the HMBC experiment, the two methoxy proton signals (δ_H 3.84, s, 2,3-OMe) had correlations with two oxygenated olefine quaternaries (C-3, δ_C 144.8; C-2, δ_C 143.8), enabling the position of the methoxy groups to be determined. Thus, compound **3** was identified as demethylcorydalmine [Rucker *et al.*, 1994; Lee *et al.*, 2009].

Compound **4**, a yellow powder, showed absorbance bands due to the hydroxyl bond (3336 cm⁻¹) and double bond (1609 cm⁻¹) in the IR spectrum. The molecular weight was determined to be 341 from the molecular ion [M]⁺ at *m/z* 341 in the EI-MS spectrum. The ¹H-NMR (400 MHz, CDCl₃) and ¹³C-NMR (100 MHz, CDCl₃) spectra of compound **4** were almost same as those of

compound **1**, except for three methoxies (δ_H 3.86, s, 9-OMe; δ_H 3.74, s, 3-OMe; δ_H 3.71, s, 10-OMe; δ_C 60.0, 9-OMe; 55.9, 3,10-OMe). The positions of the three methoxy groups were determined to be at C-3, C-9, and C-10, respectively using HMBC experiment. The three methoxy proton signals (δ_H 3.86, 3.74, 3.71) showed correlations with three oxygenated olefine quaternary carbon signals (C-3, δ_C 147.1; C-9, δ_C 145.5; C-10, δ_C 150.6). Thus, compound **4** was identified as a protoberberine-type alkaloid, isocorypalmine [Cutter *et al.*, 2002].

Compound **5**, a yellow powder, showed absorbance bands due to its double bond (1610 cm⁻¹) in the IR spectrum. The molecular weight was determined to be 355. In the ¹H-NMR (400 MHz, CDCl₃) spectrum, three singlet olefine methines (δ_H 7.94, H-11; δ_H 6.85, H-8; δ_H 6.64, H-3) and four methoxy proton signals (δ_H 3.84, s, 1-

OMe; δ_H 3.82, s, 2-OMe; δ_H 3.81, s, 9-OMe; δ_H 3.57, s, 10-OMe) were observed. In a high magnetic field, one nitrogenated methylene (δ_H 3.06, m, H-5α; δ_H 2.99, m, H-5β), two methylenes (δ_H 3.03, m, H-7α; δ_H 3.02, m, H-4α; δ_H 2.63, m, H-4β, δ_H 2.40, m, H-7β), one nitrogenated methine (δ_H 2.90, m, H-6α) and one nitrogenated methyl proton signals (δ_H 2.49) were observed. The ^{1}H-NMR data indicated that compound **3** is a benzylisoquinoline alkaloid. The ^{13}C-NMR (100 MHz, CDCl$_3$) spectrum showed 21 carbon signals: four oxygenated olefine quaternaries (δ_C 153.4, C-2; 149.5, C-9; 148.6, C-10; 145.4, C-1), five olefine quaternaries (δ_C 130.4, C-7a; 129.7, C-3a; 127.7, C-1b; 127.4, C-1a; 125.4, C-11a), three olefine methines (δ_C 113.1, C-11; 112.3, C-8; 111.6, C-3), four methoxies (δ_C 60.4, 1-OMe; 56.5, 2-OMe; 56.2, 9-OMe; 56.1, 10-OMe), one nitrogenated methine (δ_C 63.7, C-6a), one nitrogenated methylene (δ_C 54.0, C-5), one nitrogenated methyl (δ_C 43.9, 6-Me) and two methylenes (δ_C 34.0, C-7; δ_C 29.5, C-4), the multiplicities of which were determined through DEPT experiments. The positions of the four methoxy groups were determined to be between C-1 and C-2 and between C-9 and C-10 by using HMBC experiment. The four methoxy proton signals (δ_H 3.84, 1-OMe; 3.82, 2-OMe; 3.81, 9-OMe; 3.57, 10-OMe) correlated with four oxygenated olefine quaternary carbon signals (C-2, δ_C 153.4; C-9, δ_C 149.5; C-10, δ_C 148.6 and C-1, δ_C 145.4). Thus, Compound **5** was identified as a benzylisoquinoline alkaloid, glaucine [Goralski *et al.*, 1997].

Compound **6**, a yellow powder, showed absorbance bands due to the double bond (1679 cm^{-1}) in the IR spectrum. The molecular weight was determined to be 353 from the molecular ion [M]$^+$ at *m/z* 353. In the ^{1}H-NMR (400 MHz, CDCl$_3$) spectrum, four singlet olefine methines (δ_H 6.88, H-4; δ_H 6.65, H-1; δ_H 6.64, H-9; δ_H 6.62, H-12), and two dioxymethylene proton signals (δ_H 5.92, s, -O-CH$_2$-O-; δ_H 5.90, s, -O-CH$_2$-O-) were observed. In the high magnetic field, two nitrogenated methylenes (δ_H, 3.50, m, H-8α, H-8β; δ_H 2.45, m, H-6α, H-6β), two methylenes (δ_H 3.64, m, H-13α, H-13β; δ_H 2.52, m, H-5α, H-5β), and one nitrogenated methyl proton signals (δ_H 1.90, s, 7-Me) were observed. The ^{13}C-NMR (100 MHz, CDCl$_3$) spectrum showed 20 carbon signals: one ketone (δ_C 194.2, C-14), four oxygenated olefine quaternaries (δ_C 147.8, C-3; 146.1, C-10; 145.8, C-2; 145.7, C-11), four olefine quaternaries (δ_C 135.8, C-4a; 132.5, C-14a; 128.7, C-12a; 117.6, C-8a), four olefine methines (δ_C 124.9, C-12; 110.3, C-9; 108.1, C-4; 106.6, C-1), two dioxymethylenes (δ_C 101.1, -O-CH$_2$-O-; 100.7, -O-CH$_2$-O-), two nitrogenated methylenes (δ_C 57.7, C-6; 50.7, C-8), one nitrogenated methyl (δ_C 41.4, 7-Me), and two methylenes (δ_C 46.3, C-13; δ_C 31.9, C-5). The positions of

the two dioxymethylene groups were determined to be between C-2 and C-3, and C-10 and C-11. The two dioxymethylene proton signals (δ_H 5.92, 5.90) correlated with four oxygenated olefine quaternary carbon signals (C-2, δ_C 145.8; C-3, δ_C 147.8) and (C-10, δ_C 146.1; C-11, δ_C 145.7). Thus, compound **6** was identified as a berberine-type alkaloid, pseudoprotopine [Goralski *et al*, 1997].

The six alkaloids isolated from the *C. turtschaninovii* tubers were evaluated for insecticidal activity against *A. gossypii*. Compounds **1-6** showed insecticidal activities against *A. gossypii* of 69.7±7.2, 46.9±2.4, 68.5±8.6, 75.5±4.2, 80.2±9.7, and 78.9±11.3%, respectively, at 1,000 ppm (Fig. 1). Although the insecticidal potential of the alcohol extract obtained from the *C. turtschaninovii* tuber was almost equivalent to that of neem oil, a well-known insecticidal material against aphids [Boopathi *et al.*, 2010], the isolated alkaloids showed lower insecticidal activities than expected. We suggest other compounds in the extract also contributed to the insecticidal activity of the alcohol extract from the *C. turtschaninovii* tuber. However, different alkaloids such as macropodumines J and K, which are α-amino nitrile type, showed no insecticidal activity against *A. gossypii*. This indicates that compounds **1-6**, which are isoquinoline type alkaloids, are useful for the development of natural based-insecticide [Li *et al.*, 2009].

References

Abbott WS (1925) A method of computing the effectiveness of an insecticide. *J Econ Entomol* **18**, 265-267.

Bae KH (2002) In *The Medicinal Plants of Korea*, p. 180, Kyo-Hak Publishing Co., Seoul, Korea.

Boopathi T, Pathak KA, Ngachan SV, and Nabajyoti D (2010) Field efficacy of different neem products and insecticides against *Aphis gossypii* (GLOV.) on okra. *Pestology* **34**, 48-51.

Cutter PS, Miller RB, and Schore NE (2002) Synthesis of protoberberines using a silyl-directed pictet-spengler cyclization. *Tetrahedron* **58**, 1471-1478.

Goralski CT, Hasha DL, and Henton DR (1997) Isoquinoline alkaloids. 3. Synthesis of the 6-ethyl and 6-butyl analogs of *d,l*-6-methyl-5,6,6a,7-tetrahydro-1,2,9,10-tetramethoxy-4*H*-dibenzo [*de,g*]- quinoline 1.5 phosphate (*d,l*-glaucine 1.5 phosphate). *Org Process Res Dev* **1**, 280-286.

Hsu ACT (1991) 1,2-Diacyl-1-alkylhydrazines: A new class of insect growth regulator. In *Synthesis and Chemistry of*

Agrochemicals II, Baker DR, Fenyes JG, and Moderg WK (eds.), pp. 478-490, American Chemical Society, Washington, WA.

Jang C, Hwang IC, Yu YM, and Choe KR (1998) Action properties and insecticidal effects of thiamethoxam to the melon aphid, *Aphis gossypii*, and diamondback moth, *Plutella xylostella. Korean J Pestic Sci* **2**, 126-136.

Kim SR, Hwang SY, Jang YP, Park MJ, Markelonis GJ, Oh TH, and Kim YC (1999) Protopine from *Corydalis ternate* has anticholinesterase and antiamnesic activities. *Planta Med* **65**, 218-221.

Lee JK, Cho JG, Song MC, Yoo JS, Lee DY, Yang HJ, Han KM, Kim DH, Oh YJ, Jeong TS, and Baek NI (2009) Isolation of isoquinoline alkaloids from the tuber of *Corydalis turtschaninovii* and their inhibition activity on low density lipoprotein oxidation. *J Korean Soc Appl Biol Chem* **52**, 646-654.

Lee KS (1997) Evaluation on the effects of pesticide residues to agroecosystem in Korea. *Kor J Environ Agri* **16**, 80-93.

Li ZY, Gu YC, Irwin D, Sheridan J, Clough J, Chen P, Penga SY, Yang YM, and Guo YW (2009) Further daphniphyllum alkaloids with insecticidal activity from the bark of *Daphniphyllum macropodum* Miq. *Chem Biodivers* **6**, 1744-1750.

Lin MT, Chueh FY, Hsieh MT, and Chen CF (1996) Antihypertensive effects of DL-tetrahydropalmatine: An active principle isolated from Corydalis. *Clin Exp Pharmacol Physiol* **23**, 738-742.

Matsuda H, Shiomoto H, Naruto S, Namba K, and Kubo M (1988) Anti-thrombic action of methanol extract and alkaloidal components from Corydalis tuber. *Planta Med* **54**, 27-33.

Paik WH (1972) Illustrated encyclopedia of fauna and flora of Korea 13, In *Insecta V*, p. 751, Samwha Publishing Co., Seoul, Korea.

Qu Y, Liu M, Wu Z, Gao H, Sun B, Su Q, and Wu L (2007) 2,3,9,10-Tetraoxygenated protoberberine alkaloids from *Corydalis yanhusuo* W. T. Wang. *Asian J Trad Med* **2**, 61-65.

Rucker G, Breitmaier E, Zhang GL, and Mayer R (1994) Alkaloids from *Dactylicapnos torulosa. Phytochemistry* **36**, 519-523.

Sagara K, Ito Y, Ojima M, Oshima T, Suto K, Misaki T, and Itokawa H (1985) Quantitative analysis of tertiary and quaternary alkaloids in Corydalis tuber by ion-pair high-performance liquid chromatography and its application to an oriental pharmaceutical preparation. *Chem Pharm Bull* **33**, 5369-5374.

Saito SY, Tanaka SY, Tanaka M, Matsugana K, Li Y, and Ohizumi Y (2004) The combination of rat mast cell and rabbit aortic smooth muscle is the simple bioassay from the screening of antiallergic ingredient from methanolic extract of Cordalis tuber. *Biol Pharm Bull* **27**, 1270-1274.

Shim JY, Park JS, and Paik WH (1979) Studies on the life history of cotton aphid, *Aphid gossypii* Glover (Homoptera). *Korean J Plant Prot* **18**, 85-88.

Takeda S, Tateishi K, and Kiuchi M (1991) Effects of nonsteroidal ecdysone mimics RH5849 on the development of lepidopterous insects. *J Plant Prot* **45**, 21-24.

Yamamoto I (1999) Nicotine to nicotinoids: 1962 to 1997. In *Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor,* Yamamoto I and Casida JE (eds.), pp. 3-27, Springer-Verlag, Tokyo, Japan.