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DNA Strand-Nicking Principles of *Mucuna birdwoodiana*

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Abstract – During our research program to find DNA strand-scission agents from higher plants, the MeOH extracts of the stems of *Mucuna birdwoodiana* Tutcher. (Leguminosae) exhibited the most potent activity with an IC_{50} value of 4.9 μg/ml. Thus, detailed laboratory investigation was performed, and led to the isolation of known compounds, (±)-catechin (**1**) and (−)-epicatechin (**2**) as active principles. Compounds **1** and **2** showed significant activity of DNA strand-scission with IC_{50} values of 10.8 and 7.5 μg/ml, respectively (positive control, bleomycin: IC_{50} 3.3 μg/ml).
Keywords – DNA strand-scission, *Mucuna birdwoodiana*, Leguminosae, (±)-catechin, (−)-epicatechin.

Introduction

The dried stems of *Mucuna birdwoodiana* Tutcher. (Leguminosae) have been used as traditional medicine to treat the stagnant blood (Goda *et al.*, 1987). Several triterpenes (Ding *et al.*, 1991) and some phenolic compounds (Goda *et al.*, 1987; Kwon *et al.*, 1999) have been isolated previously as the constituents from *M. birdwoodiana*. There have been several reports on the biological activities of compounds from *M. birdwoodiana* such as inhibitions of prostaglandin biosynthesis and platelet aggregation (Goda *et al.*, 1987), and inhibitory activity on 3-hydroxysteroid dehydrogenase and anti-inflammatory effect (Kwon *et al.*, 1999).

In present study, the DNA strand-scission assay has been employed for the bioassay-guided fractionation. The DNA strand-scission assay was developed by Hecht (Sugiyama *et al.*, 1985), and used for bioassay-guided fractionation in the research program to find anti-neoplastic agents from plants by Wall and Wanis group (Chaudhuri *et al.*, 1995; Huang *et al.*, 1998; Seo *et al.*, 2000; Seo *et al.*, 1999). Several natural products such as biphenyl compounds (Seo *et al.*, 1999) and benzophenones (Seo *et al.*, 2000) isolated from the Guttiferae plants were reported previously as the DNA strand-nicking agents.

During our screening to find DNA-strand scission active plant extracts, the MeOH extracts of the dried stems of *M. birdwoodiana* exhibited a potent DNA strand-scission activity with an IC_{50} value of 4.9 μg/ml (Choi *et al.*, 2002). To the best of our knowledge, the DNA strand-scission activity of *M. birdwoodiana* has never been reported before. Therefore *M. birdwoodiana* was subjected to the detailed laboratory investigation.

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Experimental

General – Melting points were measured on a J-923 (Jisico, Korea) and is uncorrected. Optical rotations were measured on a P-1010 digital polarimeter (JASCO, Japan) at 25°C. UV spectrum was obtained using a U-3000 spectrophotometer (Hitachi, Japan) and IR spectrum was recorded on a FTS-135 FT-IR spectrometer (Bio-Rad, USA). NMR experiments were run on a Unity INOVA 400 MHz FT-NMR (Varian, CA), and TMS was used as an internal standard. Mass data were obtained using a JMS-700 Mstation HRMS spectrometer (JEOL, Japan). Flash column chromatography was carried out on Si gel 60 (70-230 mesh, Merck, Darmstadt, Germany). Column chromatography was monitored by TLC (Si gel 60 F_{254} plates, 0.25 mm thickness) with visualization under UV light (254 and 365 nm) and 1% sulfuric acid in EtOH.

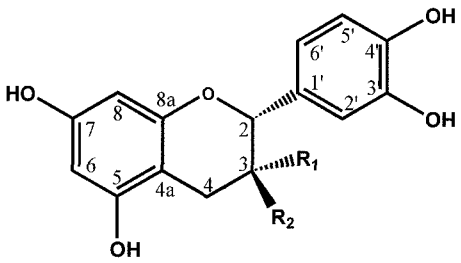
Plant materials – The stems of *Mucuna birdwoodiana* Tutcher. (Leguminosae) were purchased from an herb market (Han-Yag Yutong Co.) in Seoul, Korea in 2000. A voucher specimen (No. EAB009) has been deposited at the Herbarium of College of Pharmacy, Ewha Womans University, Seoul, Korea.

Extraction and isolation – The dried stems of *M. birdwoodiana* (2.7 kg) were ground and extracted with MeOH (5×0.5 L) for 24 h by percolation. The filtered

MeOH solutions were evaporated under vacuum, and then water (2.5 L) was added. The aqueous MeOH extract was partitioned with *n*-hexane (15×1 L), EtOAc (25×1 L), and BuOH (9×1 L), successively. A Si gel vacuum liquid column chromatography of the EtOAc extract (74.7 g) using a gradient solvent system of CH_2Cl_2-MeOH (100:0 → 0:100) afforded 10 fractions (IX). Fractions V and VI eluted with CH_2Cl_2-MeOH (19.9:0.1 → 18.6:1.4) from the first column chromatography were combined due to their overlapping TLC pattern. These combined fraction was subjected to a Si gel CC using another gradient mobile phase of CH_2Cl_2-MeOH (100:0 → 0:100), giving 22 subfractions (122). Fraction 11 eluted with CH_2Cl_2-MeOH (19.2:0.8) was further separated on Sephadex LH-20 using MeOH as eluent to afford 11 fractions. Fractions (68) from Sephadex LH-20 were purified by a revered phase (C18) preparative HPLC (YMC Jsphere ODS-H80, 2.0×25 cm, 4 μm, flow rate: 10 ml/min, UV detection: 220 nm) using a MeOH-H_2O (35:65) to provide pure compounds **1** (199.8 mg, t_R 10 min) and **2** (548.7 mg, t_R 14 min).

(±)-catechin (1): Amorphous solid; m.p.: 173175°C; $[\alpha]_{25}^{D}$: 0° (MeOH, c 0.101); IR ν_{max} (KBr) cm^{-1}: 3278, 1631, 1469, 1145, 1032; UV (MeOH) λ_{max} (log): 280 (3.5); ^{1}H-NMR (acetone-d_6, 400 MHz) : 6.89 (1H, d, J = 1.8 Hz, H-2'), 6.79 (1H, d, J = 8.0 Hz, H-5'), 6.75 (1H, dd, J = 8.0, 1.8 Hz, H-6'), 6.02 (1H, d, J = 2.4 Hz, H-8), 5.87 (^{1}H, d, J = 2.4 Hz, H-6), 4.55 (^{1}H, d, J = 8.0 Hz, H-2), 3.99 (1H, ddd, J = 8.0, 8.0, 5.6 Hz, H-3), 2.91 (1H, dd, J = 16.1, 5.4 Hz, H-4), 2.53 (1H, dd, J = 16.1, 8.4 Hz, H-4); ^{13}C-NMR (acetone-d_6, 100 MHz) : 157.8 (C-7), 157.3 (C-8a), 156.9 (C-5), 145.8 (C-3'), 145.7 (C-4'), 132.2 (C-1'), 120.1 (C-6'), 115.8 (C-5'), 115.3 (C-2'), 100.7 (C-4a), 96.2 (C-8), 95.5 (C-6), 82.8 (C-2), 68.4 (C-3), 28.9 (C-4); ^{1}H-^{13}C HMBC correlations (acetone-d_6, 400 MHz): H-4,/C-3, C-4a, C-2, C-8a; H-3/C-2, C-1'; H-2/C-3, C-1', C-8a, C-2', C-6'; H-6/ C-5, C-7, C-4a, C-8; H-8/C-8a, C-4a, C-6; H-6'/C-2, C-2', C-4'; H-5'/C-4', C-1', C-3'; H-2'/C-3', C-2, C-4', C-6'; FABMS *m/z*: 291 [M] $^+$ calcd. 290.27 for $C_{15}H_{14}O_6$.

(−)-epicatechin (2): Amorphous solid; m.p.: 232-234°C; $[\alpha]_{25}^{D}$: -36° (MeOH, c 0.11); IR ν_{max} (KBr) cm^{-1}: 3175, 2932, 1623, 1469, 1144, 1095; UV (MeOH) λ_{max} (log ε): 280 (3.7); ^{1}H-NMR (acetone-d_6, 400 MHz) δ: 7.04 (1H, d, J = 1.8 Hz, H-2'), 6.83 (1H, dd, J = 8.2, 1.8 Hz, H-6'), 6.78 (1H, d, J = 8.2 Hz, H-5'), 6.01 (1H, d, J = 2.2 Hz, H-6), 5.91 (1H, d, J = 2.2 Hz, H-8), 4.87 (1H, s, H-2), 4.02 (1H, t, J = 2.8 Hz, H-3), 2.85 (1H, dd, J = 16.8, 4.8 Hz, H-4β), 2.73 (1H, dd, J = 16.8, 3.2 Hz, H-4α); ^{13}C-NMR (acetone-d_6, 100 MHz) δ: 157.7 (C-7), 157.6 (C-5), 157.2 (C-8a), 145.5 (C-4'), 145.4 (C-3'), 132.3 (C-1'), 119.4 (C-6'), 115.5 (C-5'), 115.3 (C-2'), 99.8 (C-4a), 96.2 (C-6), 95.7 (C-8), 79.5 (C-2), 67.0 (C-3), 29.0 (C-4); ^{1}H-^{13}C HMBC correlations (acetone-d_6, 400 MHz): H-4α/C-3, C-4a, C-2, C-5, C-8a; H-4β/C-4a, C-8a; H-3/C-4a; H-2/C-1', C-4, C-8a, C-2', C-6'; H-8/C-8a, C-4a, C-6; H-6/C-5, C-7, C-4a, C-8; H-5'/C-4', C-1'; H-6'/ C-5', C-2, C-2', C-4'; H-2'/C-3', C-2, C-4', C-6'; FABMS *m/z*: 291 [M] $^+$ calcd. 290.27 for $C_{15}H_{14}O_6$.



1. R_1 = H, R_2 = OH
2. R_1 = H, R_2 = H

Chemicals – All chemicals and reagents used were of highest purity. Bleomycin sulfate, cacodylic acid, curric chloride, ferrous sulfate, ethylenediaminetetraacetic acid (EDTA), bromophenol blue, xylene cyanole FF, ficoll, boric acid, lauryl sulfate, glycerol, and Trizma base were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Electrophoresis grade agarose and pBR322 plasmid DNA were obtained from Gibco BRL (Life Technologies, Grand Island, NY, USA). SYBR Green I Nucleic Acid Gel Stain was obtained from Roche (Indianapolis, IN, USA).

DNA strand-scission assay – The DNA strand-scission assay modified the procedure described by Sugiyama *et al.* (1985) and Chaudhuri *et al.* (1995). In brief, the assay reaction mixtures (40 μl total volume) contained 25 mM cacodylate buffer pH 7.0, 0.3 mM $CuCl_2$, and 500 ng of supercoiled DNA pBR322 as a substrate, and various concentrations of the test compounds (initially dissolved in 0.5 μl of 100% DMSO, final 1.25% DMSO). The reaction mixture was incubated for 30 min at 25 °C while protected from light, then stopped the reaction by addition of 5 μl of stop solution (7 mM EDTA, 0.15% bromophenol blue, 75% glycerol). The reaction mixture was analyzed by electrophoresis at 80 volts for 7 h on a 1% agarose gel in 0.5×TBE buffer (45 mM Tris-borate, 1 mM EDTA), and then stained with SYBR Green I fluorescence, which was photographed using luminescence image analyzer, LAS-1000plus (Fuji film, Japan). The bands of the pBR322 were measured using Image Gauge software (Fuji film, Japan). Each experiment included DMSO and bleomycin sulfate as negative and positive controls, respectively. The results were calculated as the relative percentage of the DNA scission rations as compared to negative control group.

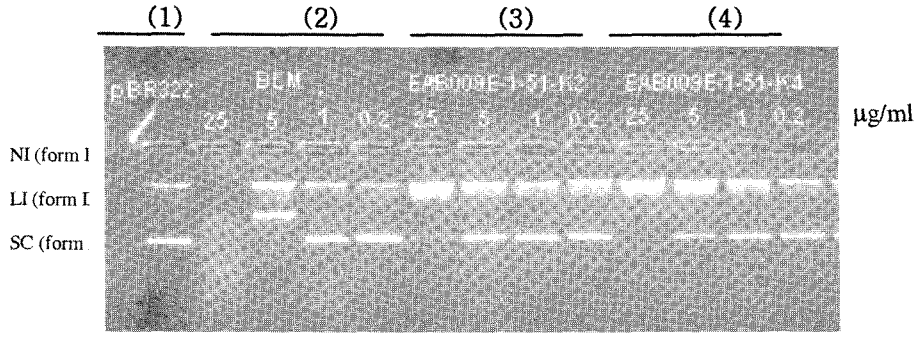


Fig. 1. The DNA strand-scission assay of supercoiled pBR322 plasmid DNA by (±)-catechin (1, EAB009E-1-51-K2) and (−)-epicatechin (2, EAB009E-1-51-K4) from *M. birdwoodiana*. (1) A control reaction was run in the absence of any Fe^{++}/bleomycin (BLM) congener. (2) Positive control reaction (bleomycin) (3) The reactions with (±)-catechin (4) The reactions with (−)-epicatechin. PBR322 form I DNA denotes a supercoiled (SC) and form II DNA is produced by nicking (NI) one strand of the supercoiled DNA, while nicking both strands at proximal sites produces a linear (LI) duplex (Form III DNA).

DNA scission ratio =
density of DNA scission (nicked DNA)/density of total DNA (supercolied DNA+ nicked DNA)×100

Results and Discussion

During the screening of DNA strand-scission activity, the MeOH extracts of the dried stems of *M. birdwoodiana* exhibited a potent DNA strand-scission activity with an IC_{50} value of 4.9 μg/ml (Choi *et al.*, 2002). The MeOH extracts of *M. birdwoodiana* was subjected to the bioassay-guided fractionation utilizing the DNA strand-scission assay system. The aqueous MeOH extracts were suspended in aqueous solution and partitioned by hexanes and ethyl acetate, successively. Each fraction was tested and showed the most potent DNA strand-scission activity with IC_{50} 8.4, 4.6, and 13.7 μg/ml, respectively.

The active ethyl acetate fraction was subjected to detailed phytochemical investigation, affording the two major active compounds **1** and **2** with IC_{50} values of 10.8 and 7.5 g/ml, respectively (positive control, bleomycin: IC_{50} 3.3 g/ml) as shown in Fig. 1. They were identified as (±)-catechin (**1**) and (−)-epicatechin (**2**), respectively, by analysis of 1D and 2D NMR data as well as by comparison of their spectral data with published values (Davis *et al.*, 1996). (−)-Epicatechin was previously found as the DNA strand-nicking principle of *Celastrus pringli* Rose (Chrisey *et al.*, 1988).

Several other compounds containing catechol groups have showed DNA strand-scission activity in previous studies (Huang *et al.*, 1998, Seo *et al.*, 1999). These results indicate that the catechol group plays an important role for the DNA strand-scission activity which can be related to anticancer activity.

In present study, the stems of *M. birdwoodiana* was found as one of the DNA strand-nicking plants for the first time. In addition, this is the first report on the isolation of the two known active compounds (±)-catechin (**1**) and (−)-epicatechin (**2**) from *M. birdwoodiana*.

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