

Isoflavonoids from the Root Cortex of *Robinia pseudo-acacia*

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Abstract – From the CHCl_3 -fraction of the root cortex of *Robinia pseudo-acacia*, five compounds have been isolated. On the basis of spectral data, these compounds were identified as medicarpin, biochanin A, formononetin, 3'-methoxydaidzein

Key words – *Robinia pseudo-acacia*, Leguminosae, isoflavonoids, medicarpin, biochanin A, formononetin, 3'-methoxydaidzein

Introduction

Robinia pseudo-acacia (Leguminosae) has been used as traditional chinese medicine for the treatment of some breeding diseases (Kim *et al.*, 1997). The chemical constituents of the heart woods and the barks of *Robinia pseudo-acacia* have been well studied (Roux and Paulus, 1962; Drewes and Roux, 1963; Cui *et al.*, 1992; Cui *et al.*, 1993) but less is known of those in the root cortex.

We have isolated (–)-vestitol, isoliquiritigenin, adenosine, adenine and 7-*O*- β -D-glucopyranosyl-4',8-dimethoxyisoflavone (Kwon *et al.*, 2000) and now report four isoflavonoids.

This study deals with the isolation and structure elucidation of CHCl_3 soluble fractions of the root cortex of *Robinia pseudo-acacia*.

Experimental

Plant materials – The root cortex of *Robinia pseudo-acacia* were collected at Mt. Keumbyung, Kangwon province around mid-May, 1999 and then dried under shade. The voucher specimen has been deposited in the herbarium of the College of Pharmacy, Kangwon National University.

Reagents and Instruments – For column packing materials, Kieselgel 60 (70-230 mesh ASTM, Merck, Art 7734), Kieselgel 63 (230-400 mesh ASTM, Merck, Art 9385), and ODS (70-230 mesh, ODS-A, YMC-gel) were used. For TLC, Kieselgel 60F₂₅₄ (precoated, Merck, Art 5715) was employed.

The melting points were taken on a Fisher/Johns

melting point apparatus (uncorrected). UV absorption spectra were measured by Hitachi U-2000 UV-VIS spectrophotometer. FT-IR spectra were taken on Bio-Rad FTS-7 spectrophotometer using KBr disc. EIMS was taken with a JEOL JMS-AX505 WA. ¹H- and ¹³C-NMR were recorded with a Varian Gemini 200.

Extraction, fractionation and isolation – The air dried root cortex of *Robinia pseudo-acacia* (2.1 kg) was extracted repeatedly 3 times refluxing with MeOH for 4 hours. The total filtrate was concentrated to dryness *in vacuo* at 40°C to obtain MeOH extract (430 g) which was partitioned with *n*-hexane, CHCl_3 (21 g) and BuOH (22 g), successively.

The CHCl_3 soluble fraction (21 g) was subjected to stepwise silica gel column chromatography by eluting with CHCl_3 -MeOH (9:1) → CHCl_3 -MeOH (2:1) to obtain 5 subfractions. Subsequently, the subfraction 2 was further chromatographed on silica gel and ODS column eluting with CHCl_3 -MeOH (9:1) and MeOH-H₂O (50:50) to obtain compounds I (37 mg) and II (23 mg). The subfractions 3 and 4 were further chromatographed on silica gel and ODS eluting with CHCl_3 -MeOH (9:1) and MeOH-H₂O (50:50) to obtain compounds IV (12 mg) and III (8 mg), respectively.

Compound I – mp 127-128°C; IR, ν_{max} (KBr) 3400 (-OH), 1540, 1450 (C=C) cm^{-1} ; UV, λ_{max} (MeOH) 237.5, 271.5, 281, 312.5 nm; ¹H-NMR (200 MHz, Acetone-*d*₆) δ : 7.35 (1H, d, *J* = 8.6 Hz, H-1), 7.23 (1H, d, *J* = 8.2 Hz, H-7), 6.60 (1H, dd, *J* = 8.6, 2.4 Hz, H-2), 6.47 (1H, dd, *J* = 8.2, 2.0 Hz, H-8), 6.40 (2H, m, H-4 and H-10), 5.52 (1H, d, *J* = 5.8 Hz, H-11a), 4.28 (1H, m, H-6eq.), 3.76 (3H, s, OCH₃), 3.59 (2H, m, H-6ax. and H-6a); ¹³C-NMR (50 MHz,

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Acetone- d_6) δ : 160.45 (C-10a), 160.17 (C-3), 158.05 (C-9), 156.10 (C-4a), 131.49 (C-1), 124.21 (C-7), 118.80 (C-6b), 111.19 (C-11b), 108.88 (C-2), 105.24 (C-8), 102.32 (C-4), 95.59 (C-10), 77.76 (C-11a), 65.43 (C-6), 54.05 (-OCH₃), 38.69 (C-6a).

Compound II – mp 216-216°C; IR, ν_{\max} (KBr) 3400 (-OH), 1680 (C=O), 1550, 1480 (C=C)cm⁻¹; UV, λ_{\max} (MeOH) 262, 317 nm; ¹H-NMR (200 MHz, Acetone- d_6) δ : 8.23 (1H, s, H-2), 7.49 (2H, d, J = 8.6 Hz, H-2' and H-6'), 6.93 (2H, d, J = 8.6 Hz, H-3' and H-5'), 6.59 (1H, d, J = 2.6 Hz, H-8), 6.38 (1H, d, J = 2.6 Hz, H-6), 3.95 (3H, s, OCH₃).

Compound III – mp 264-267°C; IR, ν_{\max} (KBr) 3405 (-OH), 1634 (C=O), 1568, 1512 (aromatic C=C) cm⁻¹; UV, λ_{\max} (MeOH) 205, 248, 302 nm; λ_{\max} (MeOH+NaOAc) 208, 255, 335 nm; ¹H-NMR (200 MHz, DMSO- d_6) δ : 8.33 (1H, s, H-2), 7.96 (1H, d, J = 8.7 Hz, H-5), 7.50 (2H, d, J = 8.7 Hz, H-2' and H-6'), 6.98 (2H, d, J = 8.7 Hz, H-3' and H-5'), 6.95 (1H, d, J = 2.2 Hz, H-8), 6.89 (1H, dd, J = 8.7, 2.2 Hz, H-6), 3.77 (3H, s, -OCH₃); ¹³C-NMR (50 MHz, DMSO- d_6) δ : 174.59 (C-4), 162.54 (C-7), 158.93 (C-4'), 157.41 (C-9), 153.12 (C-2), 129.99 (C-2' and C-6'), 127.21 (C-5), 124.16 (C-3), 123.07 (C-1'), 116.53 (C-10), 115.09 (C-6), 113.50 (C-3' and C-5'), 102.01 (C-8), 54.96 (-OCH₃).

Compound IV – mp 258-259°C; IR, ν_{\max} (KBr) 3513 (-OH), 1730 (C=O), 1566, 1512 (aromatic C=C) cm⁻¹; UV, λ_{\max} (MeOH) 248, 290 nm; λ_{\max} (MeOH+NaOH) 254, 330 nm; λ_{\max} (MeOH+NaOAc) 257.5, 327.5 nm; ¹H-NMR (200 MHz, DMSO- d_6) δ : 8.32

(1H, s, H-2), 7.96 (1H, d, J = 8.8 Hz, H-5), 6.90-7.15 (5H, m, H-2', H-5', H-6, H-6', H-8), 3.78 (3H, s, -OCH₃); ¹³C-NMR (50 MHz, DMSO- d_6) see Table 1; EI-MS (m/z , rel. int.) 284 (M⁺, 100), 253 (M-OCH₃, 1.61), 148 [(C₉H₈O₂, B₁⁺), 10.13], 136 [(C₇H₄O₃, A₁⁺, 1.22], 108 (A₁-CO, 2.48).

Results and Discussion

Repeated column chromatography of the CHCl₃ soluble fractions of root cortex of *Robinia pseudo-acacia* led to the isolation of four compounds. Compounds I, II and III were identified as medicarpin (Mitscher *et al.*, 1988; Kwon *et al.*, 1998), biochanin A (Takeda *et al.*, 1977) and formononetin (Yahara *et al.*, 1988), respectively, by comparison of their physical and spectroscopic data in the literature (m.p., UV, IR, ¹H- and ¹³C-NMR).

Compound IV showed molecular ion peak at m/z 284 and displayed hydroxyl (3513 cm⁻¹), ester (1730 cm⁻¹) and aromatic (1566, 1512 cm⁻¹) absorption in its IR spectrum. Its UV spectrum, and ¹H-NMR spectrum [δ 6.90-7.15 (5H, m), 7.96 (1H, d, J = 8.8 Hz), 8.32 (1H, s) and 3.78 (3H, s, OCH₃)] suggested that compound IV was a 4',7-dihydroxylated isoflavone derivative (Mabry and Thomas, 1970). In the ¹³C-NMR spectrum (Table 1), fifteen carbon signals were observed due to five oxygenated carbons, three quaternary carbons, six carbons bearing an aromatic proton and one carbonyl carbon along with one methoxyl group. In a comparison of the ¹³C-NMR chemical shift with those of daidzein, a signal attributable to the C-3' of daidzein was shifted downfield from δ 114.9 to 147.06 in compound IV, and the signals of C-2', C-4' and C-6' located *ortho* and *para* to C-3' were shifted to higher field by 16.92, 10.74 and 8.6 ppm. Therefore, compound IV was identified as 3'-methoxydaidzein.

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Table 1. ¹³C-NMR chemical shifts for compound IV and daidzein (50 MHz, in DMSO- d_6)

Carbon No.	IV	daidzein ^{a)}
2	153.02	152.6
3	123.39	123.5
4	174.64	174.6
4a	116.05	116.6
5	127.21	127.2
6	115.05	115.1
7	162.53	162.4
8	101.96	102.0
8a	157.33	157.4
1'	122.89	122.5
2'	113.08	130.0
3'	147.06	114.9
4'	146.36	157.1
5'	116.48	114.9
6'	121.40	130.0
OMe	55.44	-

^{a)}Kinjo *et al.*, 1987.

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