

New Flavan 3,4-Diol Derivatives from the Heartwood of *Robinia Pseudoacacia**¹

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ABSTRACT

Two flavan 3,4-diol derivatives were isolated from the heartwood of *Robinia pseudoacacia* and characterized by spectroscopic methods including ¹H and ¹³C NMR and positive FAB-MS. The structures were identified as 2,3-*trans*-3,4-*cis*-3,4,7,3',4',5'-hexahydroxy flavan, one of isomeric leucorobinetinidins, and 4'-methoxy-2,3-*trans*-3,4-*cis*-3,4,7,3',5'-pentahydroxy flavan.

Keywords : *Robinia pseudoacacia*, heartwood, extractives, flavan 3,4-diol, derivatives, methoxylated

1. INTRODUCTION

Robinia pseudoacacia, the black locust, is one of most abundant hardwood tree species in Korea and has been artificially planted in the past because of its nitrogen-fixing and soil enhancement ability. However, most of forester people in this country avoid black locust today because this species rapidly propagates by vigorous sprouting and inhibits the growth of any other tree plants under its own canopy. Thus, its commercial utilization has been disregarded and limited only to bee forage for honey production. But black locust has already been the subject of a number of chemical investigation and its heartwood shows exceptionally high decay resistance and durability attributable to the high concentration of flavonoids such as robinetin and dihydro-robinetin (Rowe, 1989; Schultz *et al.*, 1995). The present work describes the isolation and struc-

ture identification of flavan 3,4-diol derivative which is methoxylated from the heartwood of black locust.

2. MATERIALS and METHODS

2.1 Plant material

An 18 years old of *Robinia pseudoacacia* was collected from the campus forest of Kangwon National University, Chunchon in April 1997 and dried for 2 weeks at room temperature after grinding.

2.2 General

¹H and ¹³C NMR spectra were obtained in methanol-d₄ with a Bruker DTX 400 instrument.

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FAB-MS were recorded using meta nitrobenzyl alcohol (MNBA) matrix in a positive mode with a Micromass Autospec M363 spectrometer. Sephadex LH-20 was used for column chromatography and 25 DC-Plastikfolien Cellulose F (Merck) plates for TLC, which developed in HOAc-H₂O(3:47, A) and *t*-BuOH-HOAc-H₂O(3:1:1, B). Substances were detected by UV light or by spraying with Vanillin-HCl-EtOH(60:0.15:6) followed by heating.

2.3 Extraction and isolation

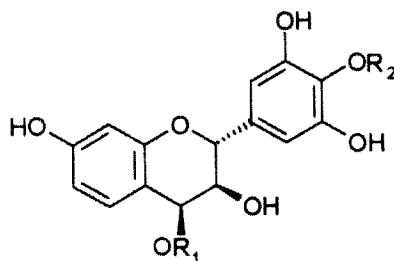
The air-dried ground heartwood of *Robinia pseudoacacia*(3.0 kg) was extracted with Me₂CO-H₂O(7:3). After filtration and concentration under reduced pressure, the aqueous residue was sequentially extracted with hexane, CH₂Cl₂ and EtOAc, then freeze dried. Part of the EtOAc extract(60 g) was chromatographed on a Sephadex LH-20 column eluted with MeOH-H₂O(1:1) affording 4 fractions. Fraction 2(26.5 g) was rechromatographed on Sephadex LH-20 column chromatography with MeOH-H₂O(1:2) to give 5 fractions. After sequentially washing with MeOH-H₂O(1:4 and 1:6) and EtOH-Hexane-EtOAc(3:1:1), fractions 2 and 3 gave 4 fractions. Fraction 1 was rinsed with EtOH-Hexane(1:1) to give 2,3-*trans*-3,4-*cis*-3,4,7,3',4',5'-hexahydroxy flavan(722 mg) and 4'-methoxy-2,3-*trans*-3,4-*cis*-3,4,7,3',5'-pentahydroxy flavan (41 mg).

2,3-*trans*-3,4-*cis*-3,4,7,3',4',5'-hexahydroxy flavan (1). R_f: 0.58(A), 0.56(B). FAB-MS: [M+H]⁺ at m/z 307. ¹H NMR: δ 3.87(1H, *dd*, *J* 3.42Hz, 9.2Hz, H-3), 4.56(1H, *d*, *J* 3.3Hz, H-4), 4.81(1H, *d*, *J* 9.2Hz, H-2), 6.26(1H, *d*, *J* 2.1Hz, H-8), 6.30(1H, *dd*, H-6), 6.49(2H, *s*, H-2', 6'), 7.12(1H, *d*, *J* 8.35Hz, H-5). ¹³C NMR: δ 67.87(C-3), 72.53(C-4), 78.71(C-2), 103.73(C-8), 108.24(C-2',6'), 110.16(C-6), 116.24(C-10), 131.40(C-5), 132.87(C-1'), 134.60(C-4'),

147.21(C-3',5'), 156.90(C-9), 160.26(C-7).

4'-methoxy-2,3-*trans*-3,4-*cis*-3,4,7,3',5'-pentahydroxy flavan (2). R_f: 0.63(A), 0.60(B). FAB-MS: [M+H]⁺ at m/z 321. ¹H NMR: δ 3.79(3H, *s*, H-4' OCH₃), 3.85(1H, *dd*, *J* 3.51Hz, 3.55Hz, H-3), 4.55(1H, *d*, *J* 3.46Hz, H-4), 4.82(1H, *d*, *J* 9.26Hz, H-2), 6.27(1H, *d*, *J* 9.26Hz, H-8), 6.40(1H, *dd*, *J* 2.4Hz, 8.39Hz, H-6), 6.48(2H, *s*, H-2', 6'), 7.12(1H, *d*, *J* 8.38Hz, H-5). ¹³C NMR: δ 61.05(C-4' OCH₃), 67.68(C-3), 72.40(C-4), 78.39(C-2), 103.60(C-8), 108.32(C-2',6'), 110.08(C-6), 116.10(C-10), 132.69(C-5), 136.50(C-1'), 136.89(C-4'), 151.83(C-3',5'), 156.66(C-9), 160.19(C-7).

3. RESULTS and DISCUSSION



- 1 R₁ = R₂ = H
2 R₁ = H ; R₂ = Me

The stereochemistry of the C-ring was confirmed by the presence of two doublets at 4.56(H-4) and 4.81 ppm(H-2), and one double doublet at 3.87 ppm(H-3) in the ¹H NMR spectrum of 1 (Table 1). The OH group attached to C-3 was assigned to the *trans* configuration in relation to the B-ring because H-2/H-3 system showed a high coupling constant(*J*=9.2Hz) and the other OH group attached to C-4 was assigned to the *cis* configuration in relation to the OH group of C-3 because H-3/H-4 system showed a low coupling constant(*J*=3.3Hz) (Drewes & Ilesley, 1969).

The one singlet of the B-ring at 6.49 ppm indicated one set of symmetrical protons that could arise from H-2' and H-6' of pyrogallol ring (Harborne *et al.*, 1975). The signals at 7.12(*d*, $J=8.35\text{Hz}$), 6.30(*dd*, $J=8.35$ and 2.1Hz) and 6.26 ppm(*d*, $J=2.1\text{Hz}$) were assigned to protons H-5, H-6 and H-8 of the resorcinol A-ring, respectively. The ^{13}C NMR spectrum of 1 exhibited typical signals of flavan 3,4-diol skeletons as shown in Table 1 : C-2 at 78.71, C-3 at 72.53 and C-4 at 67.87 ppm for the C-ring; C-5 at 131.40, C-6 at 110.16, C-7 bonding to an OH group at 160.26, C-8 at 103.73, C-9 at 156.90 and C-10 at 116.24 ppm for the A-ring; C-1' at 132.87, two sets of symmetrical carbons that could arise from C-2'/C-6' and C-3'/C-5' containing OH groups at 108.2 and 147.21 ppm, respectively and C-4' containing an OH group at 134.60 ppm. These carbon resonances were also assigned by comparison with literature values (Agrawal, 1989 and Czochanska *et al.*, 1980). A

positive FAB-MS spectrum of 1 showed molecular ion at m/z 307[(M+H)⁺] consistent with the molecular formula $\text{C}_{15}\text{H}_{14}\text{O}_7$. Thus, this compound was identified as 2,3-*trans*-3,4-*cis*-3,4,7,3',4',5'-hexahydroxy flavan, one of isomeric leucorobinetinidins.

As shown in Table 1, the ^1H NMR spectrum of 2 was very similar to that of 1, except for one singlet indicative of a OCH_3 group attached to C-4' at 3.79 ppm. However, comparison of the ^{13}C NMR spectra of 1 and 2 showed that for 2 the signal of C-1' caused downfield shift by 3.6 ppm when compared to the same one for 1(132.9 ppm) due to the paramagnetic effect of the OCH_3 (61.1 ppm) group attached to C-4' (136.9 ppm) (Günther, 1980) and one set of symmetrical carbons C-3' and C-5' also caused downfield shift by 4.6ppm due to the electronegative effect from the same OCH_3 group. The signal of C-4' shifted downfield by 2.3 ppm due to the electronegativity of its own OCH_3 group when

Table 1. ^1H and ^{13}C NMR chemical shift assignments of compounds 1 and 2

Position	1			2		
	^1H	$J(\text{Hz})$	^{13}C	^1H	$J(\text{Hz})$	^{13}C
2	4.81 <i>d</i>	9.2	78.7	4.82 <i>d</i>	9.26	78.4
3	3.87 <i>dd</i>	3.42, 9.2	67.9	3.85 <i>dd</i>	3.51, 9.2	67.7
4	4.56 <i>d</i>	3.46	72.5	4.55 <i>d</i>	3.46	72.4
5	7.12 <i>d</i>	8.35	131.4	7.12 <i>d</i>	8.38	132.7
6	6.30 <i>dd</i>	2.16, 8.3	110.2	6.40 <i>dd</i>	2.4, 8.49	110.1
7			160.3			160.2
8	6.26 <i>d</i>	2.13	103.7	6.27 <i>d</i>	2.28	103.6
9			156.9			156.7
10			116.2			116.1
1'			132.9			136.5
2'	6.49 <i>s</i>		108.2	6.48 <i>s</i>		108.3
3'			147.2			151.8
4'			134.6			136.9
5'			147.2			151.8
6'	6.49 <i>s</i>		108.2	6.48 <i>s</i>		108.3
OCH_3				3.79 <i>s</i>		61.1

compared to the same one for 1(134.6 ppm). The position of the B-ring resonances are more or less independent of A-ring oxidation pattern or C-ring stereochemistry so that the patterns are similar regardless of the type of flavanoid and these carbon resonance values of the B-ring were very close to the literature data (Agrawal, 1989). A positive FAB-MS spectrum of 2 showed molecular ion at m/z 321[(M+H)⁺] consistent with the molecular weight. This compound was identified as 4'-methoxy-2,3-*trans*-3,4-*cis*-3,4,7,3',5'-penta-hydroxy flavan.

4. CONCLUSION

Two flavan 3,4-diol derivatives were isolated by Sephadex LH-20 column chromatography on the ethylacetate extract of the heartwood of *Robinia pseudoacacia* and analyzed by spectroscopic methods using ¹H and ¹³C NMR and positive FAB-MS. The structures were identified as 2,3-*trans*-3,4-*cis*-3,4,7,3',4',5'-hexahydroxy flavan, one of isomeric leucorobinetinidins, and 4'-methoxy-2,3-*trans*-3,4-*cis*-3,4,7,3',5'-penta-hydroxy flavan. These compounds are new and novel structures that have not been previously reported in the literature.

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