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Flavan-3,4-diol Derivatives from the Heartwood of *Robinia pseudoacacia*[*1]

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ABSTRACT

Three flavan-3,4-diol derivatives were isolated from the heartwood of *Robinia pseudoacacia* and characterized by spectroscopic methods including ^{1}H and ^{13}C NMR and positive FAB-MS. The structures were identified as 4,4'-dimethoxy-, 4-ethoxy- and 4-ethoxy-4'-methoxy-2,3-*trans*-3,4-*cis*-(+)-leucorobinetinidin.

Keywords : *Robinia pseudoacacia,* heartwood, flavan-3,4-diols, methoxylated, ethoxylated

1. INTRODUCTION

Black locust(*Robinia pseudoacacia*) is one of abundant hardwood species in Korea and has been artificially planted in the past because of its nitrogen-fixing ability. However, many people avoid the tree because it rapidly propagates by vigorous sprouting and does not develop any other tree plants under its own canopy. Thus, its commercial utilization has been disregarded and limited only to bee forage for honey production. Black locust has already been the subject of a number of chemical investigation and the heartwood has been reported to have exceptionally high decay resistance and durability attributable to the high concentration of flavonoids such as robinetin and dihydrorobinetin (Hart, 1989; Schultz *et al.*, 1995) This report describes the isolation and structure identification of new flavan-3,4-diol derivatives which are methylated or ethylated or both 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

^{1}H and ^{13}C NMR spectra were obtained in methanol-d_4 with a Bruker DTX 400 instrument. FAB-MS were recorded using meta nitrobenzyl alcohol (MNBA) matrix in a positive mode with a Micromass Autospec M363 spectrometer.

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Sephadex LH-20 was used for column chromatography and 25 DC-Plastikfolien Cellulose F(Merck) plates for TLC, which developed in HOAc-H_2O (3 : 47, A) and *t*-BuOH-HOAc-H_2O (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 Me_2CO-H_2O (7 : 3). After filtration and concentration under reduced pressure, the aqueous residue was sequentially extracted with hexane, CH_2Cl_2 and EtOAc, then freeze dried. Part of the EtOAc extract (60 g) was chromatographed on a Sephadex LH-20 column eluted with MeOH-H_2O (1 : 1) affording 4 fractions. Fraction 2 (26.5 g) was rechromatographed on Sephadex LH-20 CC with MeOH-H_2O (1 : 2) to give 5 fractions. After sequentially washing with MeOH-H_2O (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 flavan-3,4-diol (722 mg) and 4'-methoxy flavan-3,4-diol (41 mg), fraction 2 gave 4,4'-dimethoxy flavan-3,4-diol (47 mg) after rewashing with EtOH-Hexane-EtOAc (3 : 1 : 1) and fraction 4 was purified with EtOH-H_2O (2 : 1) to isolate 4-ethoxy flavan-3,4-diol (107 mg) and 4-ethoxy-4'-methoxy flavan-3,4-diol (13 mg).

2.3.1 4,4'-*dimethoxy*-2,3-*trans*-3,4-cis-(+)-*leucorobinetinidin* (3)

R_f 0.67(A), 0.41(B).

FAB-MS: [M+H]+ at m/z 334.

^{1}H NMR: δ 3.44(3H, *s*, H-4 OCH_3), 3.83 (3H, *s*, H-4' OCH_3), 4.16(1H, *s*, H-4), 6.29(1H, *s*, H-8), 6.38(1H, *m*, H-6), 6.49(2H, *s*, H-2', 6'), 7.07(1H, *d*, *J* 8.26Hz, H-5).

^{13}C NMR: δ 57.24(C-4 OCH_3), 61.05(C-4' OCH_3), 71.63(C-3), 77.53(C-4), 78.72(C-2), 103.88(C-8), 109.22(C-2',6'), 110.48(C-6), 115.28 (C-10), 132.83(C-5), 136.91(C-1'), 137.05(C-4'), 151.83(C-3',5'), 157.20(C-9), 160.66(C-7)

2.3.2 4-*ethoxy*-2,3-*trans*-3,4-cis-(+)-*leucorobinetinidin* (4)

R_f 0.69(A), 0.74(B).

FAB-MS: [M+H]+ at m/z 334.

^{1}H NMR: δ 1.20(3H, *t*, *J* 6.94Hz, ethoxy CH_3), 3.66(2H, *dd*, *J* 3.08Hz, 6.99Hz, ethoxy CH_2), 3.94(1H, *dd*, *J* 3.29Hz, 9.14Hz H-3), 4.27(1H, *d*, *J* 3.25Hz, H-4), 4.90(1H, *d*, *J* 9.3 Hz, H-2), 6.27(1H, *d*, *J* 2.31Hz, H-8), 6.36(1H, *dd*, *J* 2.38Hz, 8.29Hz, H-6), 6.44(2H, *s*, H-2', 6'), 7.06(1H, *d*, *J* 8.32Hz, H-5).

^{13}C NMR: δ 16.07(ethoxy CH_3), 66.05 (ethoxy OCH_2), 71.45(C-3), 75.84(C-4), 79.20 (C-2), 103.92(C-8), 108.13(C-2',6'), 109.35(C-6), 114.22(C-10), 131.74(C-1'), 132.69(C-5), 134.60 (C-4'), 147.22(C-3',5'), 156.85(C-9), 160.53 (C-7).

2.3.3 4-*ethoxy*-4'-*methoxy*-2,3-*trans*-3,4-*cis*-(+)-*leucorobinetinidin* (5)

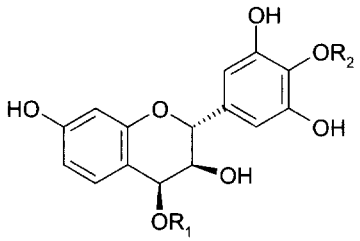
R_f 0.69(A), 0.72(B).

FAB-MS: [M+H]+ at m/z 349.

^{1}H NMR: δ 1.20(3H, *t*, *J* 7.0Hz, ethoxy CH_3), 3.66(2H, *dd*, *J* 3.57Hz, 7.0Hz ethoxy OCH_2), 3.79(3H, *s*, H-4' OCH_3), 3.92(1H, *d*, *J* 9.1Hz, H-3), 4.27(1H, *d*, *J* 3.2Hz, H-4), 4.91(1H, *d*, *J* 9.2Hz, H-2), 6.28(1H, *d*, *J* 2.47, H-8), 6.37(1H, *dd*, *J* 2.35Hz, 8.25Hz, H-6), 6.45(2H, *s*, H-2', 6'), 7.06(1H, *d*, *J* 8.27Hz, H-5).

^{13}C-NMR: δ 15.68(ethoxy CH_3), 60.78(C-4' OCH_3), 65.62(ethoxy OCH_2), 71.40(C-3), 75.45 (C-4), 78.62(C-2), 103.56(C-8), 107.99(C-2',6'), 109.02(C-6), 113.79(C-10), 132.32(C-5), 136.59 (C-4'), 136.64(C-1'), 151.62(C-3',5'), 156.39(C-9), 160.26(C-7).

3. RESULTS and DISCUSSION



1 $R_1 = R_2 = H$
2 $R_1 = H$; $R_2 = CH_3$
3 $R_1 = R_2 = CH_3$
4 $R_1 = CH_2CH_3$; $R_2 = H$
5 $R_1 = CH_2CH_3$; $R_2 = CH_3$

In the ^1H NMR spectrum of **1**, the stereochemical structure 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) as shown in 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 ($J = 9.2$ Hz) 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 ($J = 3.3$ Hz) (Drewes and Ilsley, 1969). The one singlet of the B-ring at 6.49ppm revealed one pair of symmetrical protons that could arise from H-2' and H-6' of pyrogallol ring (Markham and Mabry, 1975). The signals at 7.12(d, $J = 8.35$ Hz), 6.30(dd, $J = 8.35$ and 2.1 Hz) and 6.26 ppm(d, $J = 2.1$ 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 2.

Table 1. ^1H NMR chemical shift assignments of compounds 1-5.

Protons	1	2	3	4	5
H-2	4.81 *d* (9.2)	4.82 *d* (9.26)	4.91 *s*	4.90 *d* (9.3)	4.91 *d* (9.2)
H-3	3.87 *dd* (3.42, 9.2)	3.85 *dd* (3.51, 3.55)	3.92 *br s*	3.94 *dd* (3.29, 9.14)	3.92 *d* (9.1)
H-4	4.56 *d* (3.46)	4.55 *d* (3.46)	4.16 *br s*	4.27 *d* (3.25)	4.27 *d* (3.2)
H-5	7.12 *d* (8.35)	7.12 *d* (8.38)	7.07 *d* (8.26)	7.06 *d* (8.32)	7.06 *d* (8.27)
H-6	6.30 *m*	6.40 *dd* (2.4, 8.49)	6.38 *m*	6.36 *dd* (2.38, 8.29)	6.37 *dd* (2.35, 8.25)
H-8	6.26 *d* (2.1)	6.27 *d* (9.26)	6.29 *s*	6.27 *d* (2.31)	6.28 *d* (2.47)
H-2',6'	6.49 *s*	6.48 *s*	6.49 *s*	6.44 *s*	6.45 *s*
OCH_3(C-4')		3.79 *s*	3.83 *s*		3.79 *s*
OCH_3(C-4)			3.44 *s*		
$O\underline{CH_2}CH_3$(C-4)				3.66 *dd* (3.08, 6.99)	3.66 *dd* (3.57, 7.0)
$OCH_2\underline{CH_3}$(C-4)				1.20 *t* (6.94)	1.20 *t* (7.0)

Coupling constants J (in Hz) in parentheses.

Table 2. ^{13}C NMR chemical shift assignments of compounds **1-5**.

Carbons	1	2	3	4	5
2	78.7	78.4	78.7	79.2	78.6
3	67.9	67.7	71.6	71.5	71.4
4	72.5	72.4	77.5	75.8	75.5
5	131.4	132.7	132.8	132.7	132.3
6	110.2	110.1	110.5	109.4	109.0
C-7	160.3	160.2	160.7	160.5	160.3
C-8	103.7	103.6	103.9	103.9	103.6
C-9	156.9	156.7	157.2	156.9	156.4
C-10	116.2	116.1	115.3	114.2	113.8
C-1'	132.9	136.5	136.9	131.7	136.6
C-2'	108.2	108.3	109.2	108.1	108.0
C-3'	147.2	151.8	151.8	147.2	151.6
C-4'	134.6	136.9	137.1	134.6	136.6
C-5'	147.2	151.8	151.8	147.2	151.6
C-6'	108.2	108.3	109.2	108.1	108.0
OCH_3(C-4)			57.2		
OCH_3(C-4')		61.1	61.1		60.8
$O\underline{CH_2}CH_3$(C-4)				66.1	65.6
$OCH_2\underline{CH_3}$(C-4)				16.1	15.7

C-2 at 78.71, C-3 at 72.53 and C-4 at 67.87 ppm in 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 in 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' at 108.2 and 147.21 ppm, respectively and an OH group containing C-4' at 134.60 ppm. These carbon chemical shifts were assigned by comparison with literature values (Agrawal *et al.*, 1989; Bae, Ham and Kim, 2000; Czochanska *et al.*, 1980) and a positive FAB-MS spectrum showed molecular ion at m/z 307[(M+H)+] consistent with the molecular weight. Thus, this compound was 2,3-*trans*-3,4-*cis*-3,4,7,3',4',5'-hexahydroxy-flavan as already reported by Bae *et al.* (2000).

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 (Table 1). 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 4.6 ppm 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 compared to the same one for **1** (134.6 ppm). The B-ring carbons of **2** showed very similar values to those mentioned previously (Agrawal, 1989) and a positive FAB-MS spectrum showed molecular ion at m/z 321[(M+H)+] consistent with the molecular weight. This compound was identical to an authentic sample, as 4'-methoxy-2,3-*trans*-3,4-*cis*- leucorobinetinidin, isolated by Bae *et al.*(2000).

The ^1H NMR spectrum of **3** (Table 1) was similar to that of **2**, except for the C-ring signals indicative of a OCH_3 group attached to C-4. The H-4 signal caused upfield shift by 0.4 ppm when compared to the same one for 2 (4.56 ppm) due to the shield effect of the OCH_3 group. However, The signals of H-2 and H-3 shifted slightly downfield due to the electronegativity of the OCH_3 group. In the A-ring, H-5 signal caused slightly upfield shift because of the shield effect of the OCH_3 group. The OCH_3 groups attached to C-4 and C-4' showed two singlets at 3.83 and 3.44 ppm, respectively. Comparison of the ^{13}C NMR data of **2** and **3** also was similar to each other, except for the C-ring carbons (Table 2). Differences in chemical shifts between **2** and **3**: C-3 (-4 ppm) and C-4 (-5 ppm), are indicative of the electronegative effect of the OCH_3 group attached to C-4. The OCH_3 groups showed two singlets at 61.1 ppm and 57.2 ppm, respectively. A

positive FAB-MS spectrum **3** gave a molecular ion at m/z 334[(M+H)+] consistent with its molecular weight. This compound was identified as 4,4'-dimethoxy-2,3-*trans*-3,4-*cis*-(+)-leucorobinetinidin.

The ^{1}H NMR spectrum of **4** was similar to that of **1**, except for the C-ring proton signals as shown in Table 1. The OCH_2CH_3 group attached to C-4 showed one quartet at 3.66 ppm for methylene and one triplet at 1.20 ppm for methyl group. Proton signals for the C-ring were similar to those of **3** and this fact indicated that the OCH_2CH_3 group is attached to C-4. The ^{13}C NMR spectrum of **4** also was similar to that of **1**, except for the C-ring carbons that are similar to those of **3** (Table 2). However, the C-4 signal caused upfield shift by 2 ppm when compared to the same one for 3 (77.5 ppm) and this finding indicated that the electronegativity of OCH_2CH_3 group is less than that of OCH_3 group. The OCH_2CH_3 group showed two singlets at 66.1 ppm for methylene and at 16.1 ppm for methyl carbon. A positive FAB-MS spectrum of **4** showed molecular ion at m/z 334 [(M+H)+]. Thus, this compound was identified as 4-ethoxy-2,3-*trans*-3,4-*cis*-(+)-leucorobinetinidin.

The ^{1}H NMR spectrum of **5** was very similar to that of **4**, except for a signal indicative of a OCH_3 group attached to C-4' at 3.79 ppm (Table 1). As shown in Table 2, comparison of the ^{13}C NMR data of **4** and **5** showed that for **5** the signals were similar to those of **4**, except for the B-ring carbons that are similar to those of **2**. A positive FAB-MS spectrum **5** indicated molecular ion at m/z 349[(M+H)+] and this compound was identified as 4-ethoxy-4'-methoxy-2,3-*trans*-3,4-*cis*-(+)-leucorobinetinidin.

4. CONCLUSION

Three flavan 3,4-diol derivatives were isolated by Sephadex LH-20 column chromatography on the extractives of the heartwood of *Robinia pseudoacacia* and spectroscopic analytical methods such as ^{1}H, ^{13}C NMR and positive FAB-MS were used to characterize the isolated compounds.

The structures were identified as 4,4'-dimethoxy-2,3-*trans*-3,4-*cis*-(+)-leucorobinetinidin, 4-ethoxy-2,3-*trans*-3,4-*cis*-(+)-leucorobinetinidin, 4-ethoxy-4'-methoxy-2,3-*trans*-3,4-*cis*-(+)-leucorobinetinidin.

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