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Lignans of *Rosa multiflora* Roots

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Five known lignans, (+)-pinoresinol (**1**), (+)-8-hydroxypinoresinol (**2**), (-)-dehydrodiconiferyl alcohol (**3**), (+)-*trans*-dehydrodiconiferyl alcohol (**4**), and (-)-olivil (**5**), were isolated from the roots of *Rosa multiflora* for the first time. Their structures were determined using spectroscopic data.

Key words: *Rosa multiflora,* Lignan, (+)-Pinoresinol, (+)-8-Hydroxypinoresinol, (-)-Dehydrodiconiferyl alcohol, (+)-*trans*-Dehydrodiconiferyl alcohol, (-)-Olivil

INTRODUCTION

Rosa multiflora Thunberg (Rosaceae) is a deciduous tree that is widespread in Korea and has been used in folk medicine for the treatments of diabetes, arthritis and dysentery. Previous phytochemical investigations on the roots of this species afforded pentacyclic triterpenes and their glucosides (Takahashi *et al.*, 1969; Yeo *et al.*, 1998). In this paper, we describe the isolation and structural elucidation of five lignans that were not identified in *R. multiflora* roots previously.

MATERIALS AND METHODS

General experimental procedures

Optical rotation was measured with a JASCO DIP-1000 digital polarimeter (Tokyo, Japan) and the CD spectra were recorded on a JASCO J-715 spectrometer. The melting point was determined by using a Büchi 535. EI-MS spectra were obtained on a VG-Trio 2 spectrometer. UV and IR spectra were recorded on a Shimadzu UV-2101 and Perkin Elmer 1710 spectrometer, respectively. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL JNM-LA 300 spectrometer at 300 MHz and at 75 MHz, respectively. Column chromatography was performed using Sephadex LH-20 (Pharmacia) and Kiesegel 60 (Art. 7734; Merck, Darmstadt, Germany). TLC was conducted on pre-coated Kiesegel 60 F_{254} plates (Art. 5715; Merck, Darmstadt, Germany). Spots on TLC were detected under UV radiation.

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Plant material

The roots of *R. multiflora* were collected at Mt. Chunma (Korea) in July 1995, and identified by Dr. Dae Suk Han, an emeritus professor at the College of Pharmacy at Seoul National University. A voucher specimen (SNUPH-0026) was deposited in the herbarium of our institute.

Extraction and isolation

The air-dried roots of *R. multiflora* (2.3 kg) were cut into pieces and applied with 80% MeOH. The methanolic extract was evaporated *in vacuo* to give a crude extract (300 g), which was successively distilled using $CHCl_3$ and *n*-BuOH. The $CHCl_3$ extract (26 g) was chromatographed over silica gel using *n*-hexane-EtOAc and $CHCl_3$-MeOH gradient to give thirty-six fractions. The twenty-first fraction (300 mg) was applied to the silica gel column chromatography (*n*-hexane-EtOAc = 10:1) followed by Sephadex LH-20 (MeOH) to afford **1** (16.0 mg). The twenty-fourth fraction (300 mg) was subjected to SiO_2 column chromatography (*n*-hexane-acetone=8:1) and then purified by Sephadex LH-20 (MeOH) to obtain **2** (8.0 mg). The twenty-sixth fraction (320 mg) was subjected to SiO_2 column chromatography (*n*-hexane-acetone=10:1) to afford 6 sub-fractions. The fifth sub-fraction was subjected to SiO_2 column chromatography (*n*-hexane-acetone=8:1) and then purified by Sephadex LH-20 (MeOH) to yield **3** (1.6 mg), **4** (8.8 mg), and **5** (8.1 mg).

Compound **1**: colorless oil, $[\alpha]_D^{20}$ +27.2° (*c* 0.01, $CHCl_3$); UV (EtOH) λ_{max} nm (log ε) : 240 (3.82), 280 (3.75); EI-MS (70 eV, *m/z*, rel. int.): 358 $[M]^+$ (99), 327 (26), 206 (36), 151 (99), 137 (100); IR (KBr) ν_{max} (cm^{-1}): 3368 (OH), 3020 (aromatic C-H stretching), 1605, 1517, 1480 (aromatic C=C), 1273, 1034 (C-O); ^{1}H-NMR (300 MHz, acetone-d_6) δ 3.09 (2H, m, H-8, 8'), 3.79 (2H, dd, *J* = 9.0, 3.7 Hz, H-9ax, H-9'ax), 3.83 (6H, s, 3-OMe, 3'-OMe), 4.19 (2H, dd, *J* = 9.0, 6.8 Hz, H-9eq, H-9'eq), 4.66 (2H, d, *J* = 4.4 Hz, H-7, H-7'), 6.78 (2H, d, *J* = 8.0 Hz, H-5, H-5'), 6.83 (2H, dd, *J* = 8.0, 1.7 Hz, H-6, H-6'), 6.98 (2H, d, *J* =1.7 Hz, H-2, H-2'); ^{13}C-NMR see Table I.

Compound **2**: colorless oil, $[\alpha]_D^{20}$ +113.7° (*c* 0.06, EtOH); UV (EtOH) λ_{max} nm (log ε): 232 (4.06), 280 (3.88); EI-MS (70 eV, *m/z*, rel. int.): 374 $[M]^+$ (3), 222 (2), 151 (100), 137 (22); IR (KBr) ν_{max} (cm^{-1}): 3392 (OH), 3020 (aromatic C-H stretching), 1606, 1519, 1480 (aromatic C=C), 1272, 1035 (C-O); ^{1}H-NMR (300 MHz, acetone-d_6) δ 3.01 (1H, ddd, *J* = 8.0, 6.2, 5.1 Hz, H-8'), 3.72 (1H, dd, *J* = 9.2, 6.2 Hz, H-9'ax), 3.82 (6H, s, OMe×2), 3.85(1H, d, *J* = 9.2 Hz, H-9ax), 4.03 (1H, d, *J* = 9.2 Hz, H-9eq), 4.44 (1H, dd, *J* = 9.2, 8.0 Hz, H-9'eq), 4.66 (1H, s, H-7), 4.81 (1H, d, *J* = 5.1 Hz, H-7'), 6.76 , 6.78 (each 1H, d, *J* =8.0 Hz, H-5, H-5'), 6.87, 6.89 (each 1H, dd, *J* = 8.0, 1.7 Hz, H-6, H-6'), 7.05, 7.07 (each 1H, d, *J* = 1.7 Hz, H-2, H-2'); ^{13}C-NMR see Table I.

Compound **3**: colorless oil, $[\alpha]_D^{20}$ -27.9° (*c* 0.01, acetone); UV (EtOH) λ_{max} nm (log ε): 220 (4.39), 278 (4.25); CD (MeOH) nm (Δε): 268 (+4.66), 294 (-4.86); EI-MS (70 eV, *m/z*, rel. int.): 358 $[M]^+$ (61), 340 (64), 151 (45), 137 (100); IR (KBr) ν_{max} (cm^{-1}): 3368 (OH), 3020 (aromatic C-H stretching), 1653 (vinylic C=C), 1605, 1520, 1508 (aromatic C=C), 1216, 1144, 1033 (C-O); ^{1}H-NMR (300 MHz, acetone-d_6) δ 3.52 (1H, ddd, *J* = 6.4, 5.6, 5.6 Hz, H-8), 3.81 (3H, s, 3'-OMe), 3.83 (1H, dd, *J* = 10.8, 5.6 Hz, H-9a), 3.84 (3H, s, 3-OMe), 3.87 (1H, dd, *J* = 10.8, 5.6 Hz, H-9b), 4.19 (2H, dd, *J* = 6.0, 1.6 Hz, H-9'), 5.55 (1H, d, *J* = 6.4 Hz, H-7), 6.23 (1H, dt, *J* = 15.6, 6.0 Hz, H-8'), 6.51 (1H, d, *J* = 15.6 Hz, H-7'), 6.79 (1H, d, *J* = 8.4 Hz, H-5), 6.87 (1H, dd, *J* = 8.4, 2.0 Hz, H-6), 6.94 (1H, d, *J* = 2.0 Hz, H-6'), 6.97 (1H, d, *J* = 2.0 Hz, H-2), 7.02 (1H, d, *J* = 2.0 Hz, H-2'); ^{13}C-NMR see Table I.

Compound **4**: colorless oil, $[\alpha]_D^{20}$ +9.4° (*c* 0.05, MeOH); UV (MeOH) λ_{max} nm (log ε): 230 (4.06), 281 (3.69); CD (MeOH) nm (Δε): 275 (-2.57), 281 (-5.68), 287 (+2.72); EI-MS (70 eV, *m/z*, rel. int.): 360 $[M]^+$ (61), 342 (100), 151 (28), 137 (90); IR ($CHCl_3$) ν_{max} (cm^{-1}): 3369 (OH), 3020 (aromatic C-H stretching), 1606, 1519, 1498 (aromatic C=C), 1212, 1034 (C-O); ^{1}H-NMR (300 MHz, CD_3OD) δ 1.81 (2H, tt, *J* = 7.7, 6.3 Hz, H-8'), 2.62 (2H, t, *J* = 7.7, H-7'), 3.46 (1H, m, H-8), 3.56 (2H, t, *J* = 6.3 Hz, H-9'), 3.71-3.77 (2H, m, H-9), 3.80 (3H, s, 3'-OMe), 3.84 (3H, s, 3-OMe), 5.48 (1H, d, *J* = 6.3 Hz, H-7), 6.72 (2H, brs, H-2', H-6'), 6.76 (1H, d, *J* = 8.0 Hz, H-5), 6.82 (1H, dd, *J* = 8.0, 2.0, H-6), 6.94 (1H, d, *J* = 2.0 Hz, H-2); ^{13}C-NMR see Table I.

Table I. ^{13}C-NMR chemical shifts of compounds **1-5** (75 MHz)

NO.	1†	2†	3†	4‡	5‡
1	134.62	129.28	134.88	135.61	136.16
2	111.05	112.25	110.97	111.32	112.37
3	148.78	147.89	148.87	149.87	149.82
4	147.31	146.94	147.79	148.32	147.99
5	116.00	115.16	116.16	116.93	116.61
6	120.07	121.10	120.07	120.50	121.57
7	87.08	88.70	89.01	89.78	86.61
8	55.68	92.66	55.27	56.25	62.71
9	72.66	75.86	65.10	65.78	61.58
1'	134.62	134.02	130.90	130.67	131.22
2'	111.05	111.00	112.19	114.87	116.05
3'	148.78	148.29	145.66	137.71	149.37
4'	147.31	147.07	149.45	146.00	146.96
5'	116.00	115.52	132.42	130.67	116.49
6'	120.07	120.00	116.56	118.71	124.68
7'	87.08	86.75	130.99	36.61	41.42
8'	55.68	62.20	128.87	33.70	83.39
9'	72.66	71.81	63.89	63.03	78.74
3-OMe	56.70	56.20	56.85	57.54	57.17
3'-OMe	56.70	56.20	56.74	57.15	57.17

† acetone-d_6
‡ CD_3OD

Compound **5**: white powder, m.p. 106-108°C (uncorrected); $[\alpha]_D^{20}$ -110° (*c* 0.05, MeOH); UV (MeOH) λ_{max} nm (log ε): 229 (3.93), 281 (3.51); EI-MS (70 eV, *m/z*, rel. int.): 376 $[M]^+$ (1), 151 (30), 137 (99); IR ($CHCl_3$) ν_{max} (cm^{-1}): 3369 (OH), 3020 (aromatic C-H stretching), 1604, 1516 (aromatic C=C), 1275, 1035 (C-O); ^{1}H-NMR (300 MHz, CD_3OD) δ 2.25 (1H, m, H-8), 2.86 (1H, d, *J* = 13.9 Hz, H-7'a), 2.94 (1H, d, *J* = 13.9 Hz, H-7'b), 3.56 (1H, d, *J* = 9.0 Hz, H-9'a), 3.69 (1H, dd, *J* = 11.2, 5.6 Hz, H-9a), 3.77 (1H, d, *J* = 9.0 Hz, H-9'b), 3.80 (6H, s, 3-OMe, 3'-OMe), 3.78-3.80 (1H, m, H-9b), 4.68 (1H, d, *J* = 7.3 Hz, H-7), 6.67-6.69 (3H, brd, *J* = 8.0 Hz, H-5, H-5', H-6'), 6.83 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 6.86 (1H, d, *J* = 2.0 Hz, H-2'), 7.10 (1H, d, *J* = 2.0 Hz, H-2); ^{13}C-NMR see Table I.

RESULTS AND DISCUSSION

The methanolic extract of the roots of *R. multiflora* were

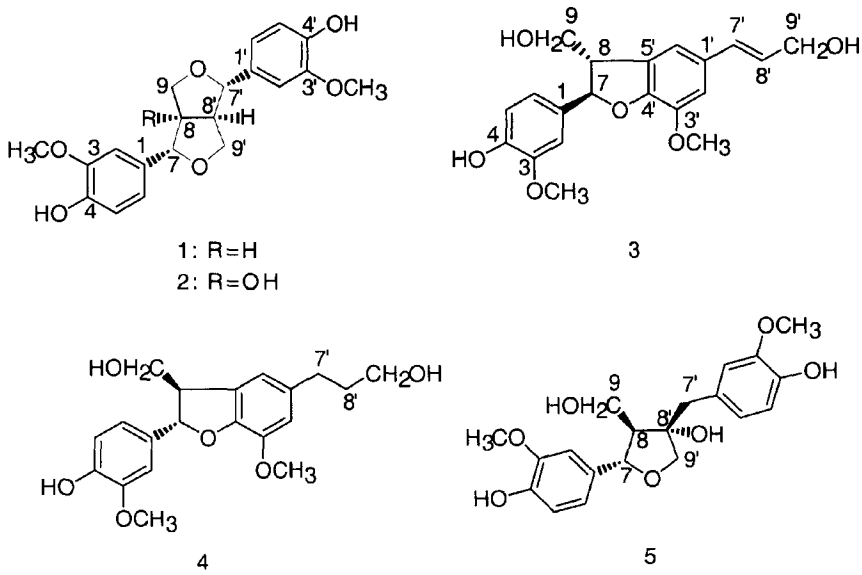

Fig. 1. Structures of compounds **1-5**

suspended with water and then partitioned using chloroform and *n*-BuOH. The chloroform soluble fraction was subjected to a series of column chromatography using SiO_2 and Sephadex LH-20, and finally five lignans (**1-5**) were obtained.

The mass spectrum of **1** exhibited an ion at *m/z* 358 $[M]^+$, two ions at *m/z* 151 $[ArCO]^+$, and 137 $[ArCH_2]^+$ assignable to guaiacyl group (Ar) ions, respectively (Katayama *et al.*, 1992). There were only ten carbon peaks in the ^{13}C-NMR spectrum. This suggests that **1** was symmetrical in structure. The ^1H-NMR spectrum of **1** indicated the typical pattern of two 1,3,4-trisubstituted benzene rings at δ_H 6.78 (2H, d, *J* = 8.0 Hz, H-5, H-5'), 6.83 (2H, dd, *J* = 8.0, 1.7 Hz, H-6, H-6'), and 6.98 (2H, d, *J* =1.7 Hz, H-2, H-2'), two methoxy groups at δ_H 3.83 and five signals at δ_H δ 3.09 (2H, m, H-8, H-8'), 3.79 (2H, dd, *J* = 9.0, 3.7 Hz, H-9ax, H-9'ax), 4.19 (2H, dd, *J* = 9.0, 6.8 Hz, H-9eq, H-9'eq), and 4.66 (2H, d, *J* = 4.4 Hz, H-7, H-7'), assigned to a *cis*-diequatorial substituted 7,7'-diaryldioxabicyclo[3,3,0]octane [Pérez *et al.*, 1995]. In conclusion, the structure of **1** was elucidated as (+)-pinoresinol, in agreement with the literature (Nishibe *et al.*, 1984).

Compound **2** showed a molecular ion peak at *m/z* 374 in the mass spectrum and an absorption maxima at 232 and 280 nm in the UV spectrum. The ^1H-NMR spectrum of **2** revealed aliphatic peaks at δ_H 3.85 (1H, d, *J* = 9.2 Hz, H-9ax), 4.03 (1H, d, *J* = 9.2 Hz, H-9eq), and 4.66 (1H, s, H-7) that are distinguishable from those of **1**. These results suggested that a hydroxylation occurred at C-8 of the tetrahydrofurofuran ring. In comparison with the literature, **2** proved to be (+)-8-hydroxypinoresinol (Tsukamoto *et al.*, 1984a; Tsukamoto *et al.*, 1984b).

Compound **3,** a colorless oil, displayed a molecular ion peak at *m/z* 358 corresponding to $C_{20}H_{22}O_6$. Absorption maxima appeared at 220 and 278 nm in the UV spectrum, as characteristic of lignan moiety. Its IR spectrum exhibited an absorption band at 1653 cm^{-1} ascribable to a conjugated vinylic group. In the ^1H-NMR spectrum, peaks at δ_H 6.79 (1H, d, *J* = 8.4 Hz, H-5), 6.87 (1H, dd, *J* = 8.4, 2.0 Hz, H-6), and 6.97 (1H, d, *J* = 2.0 Hz, H-2) assigned to a 1,3,4-trisubstituted benzene ring were connected to a C_3 unit belonging to the signals at δ_H 3.52 (1H, ddd, *J* = 6.4, 5.6, 5.6 Hz, H-8), 3.83 (1H, dd, *J* = 10.8, 5.6 Hz, H-9a), 3.87 (1H, dd, *J* = 10.8, 5.6 Hz, H-9b), and 5.55 (1H, d, *J* = 6.4 Hz, H-7). The signals at δ_H 6.94 (1H, d, *J* = 2.0 Hz, H-6') and 7.02 (1H, d, *J* = 2.0 Hz, H-2') showed the presence of a 1,3,4,5-tetrasubstituted benzene ring, and were linked to a C_3 unit that consisted of an olefinic functionality at δ_H 6.23 (dt, *J* = 15.6, 6.0 Hz) and 6.51 (d, *J* = 15.6 Hz), and a secondary alcohol at δ_H 4.19. All the above data suggested that **3** was composed of two phenylpropanoids. The link between two phenylpropanoid units was inferred from the signals of H-7 (δ_H 5.55) and H-8 (δ_H 3.52) that appeared in the downfield region. These implied that C-7 and C-8 were linked to aromatic ring of another phenylpropanoid unit through an *O*-linkage and *C*-linkage, respectively. The relative configuration of C-7 and C-8 was assumed to be *trans* on the basis of the chemical shifts of H-7 and H-8 (Tan *et al.*, 1990). The absolute configurations of C-7 and C-8 were confirmed as *R* and *S*, respectively, by observing the positive cotton effect at 268 nm and the negative cotton effect at 294 nm (Nabeta *et al.*, 1994; Hirai *et al.*, 1994). As a result, **3** turned out to be (-)-dehydrodiconiferyl alcohol.

Compound **4** was an optically active colorless oil with a molecular ion peak at *m/z* 360 $[M]^+$. Its ^1H- and ^{13}C-NMR

spectra were very similar to those of **3** except that two methylene signals appeared at δ_H 1.81 (2H, tt, J = 7.7, 6.3 Hz, H-8') and 2.62 (2H, t, J = 7.7, H-7') instead of the olefinic peaks in **3**. Therefore, **4** was (+)-*trans*-dehydrodiconiferyl alcohol. In the CD spectrum, the cotton effects opposite to those of **3** made it possible to determine the absolute configurations of C-7 and C-8 in **4** as *S* and *R*, respectively (Hirai *et al.*, 1994).

The ^{1}H-and ^{13}C-NMR spectra of **5** suggested that this compound was analogous to **2**. In the ^{1}H-NMR spectrum, a C_3 unit, which was comprised of a methine, an oxygenated methine, and a secondary alcohol, was ascertained by observing that the peak at δ_H 2.25 (H-8) was coupled with δ_H 4.68 (H-7), 3.69 (H-9a), and 3.78-80 (H-9b), respectively. The remaining C_3 unit was considered to have a methylene (δ_H 2.86 and 2.94) next to a benzene ring, oxygenated methylene (δ_H 3.56 and 3.77), and a tertiary alcohol at δ_c 83.39. The position of the tertiary alcohol was confirmed as C-8' because of the distinct geminal couplings between the signals at δ_H 2.86 and 2.94 (d, J = 13.9 Hz, H-7'), and between δ_H 3.56 and 3.77 (d, J = 9.0 Hz, H-9') without coupling to other protons in the ^{1}H-^{1}H COSY spectra. On the basis of the spectroscopic data, **5** was identified as (-)-olivil (Ghogomu-Tih *et al.*, 1985; Wang *et al.*, 1989).

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