Six New Diarylbutane Lignans from *Justicia procumbens*

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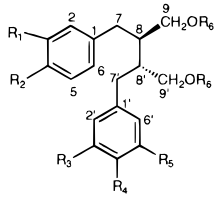
Six new diarylbutane lignans, namely, justin A (**1**), (−)-dihydroclusin diacetate (**2**), secoisolariciresinol dimethyl ether diacetate (**3**), 5-methoxy-4,4′-di-*O*-methylsecolariciresinol diacetate (**4**), justin B (**5**), and justin C (**6**), together with three known diarylbutane lignans [2,3-demethoxysecisolintetralin acetate (**7**), secoisolariciresinol dimethyl ether (**8**), and 5-methoxy-4,4′-di-*O*-methylsecolariciresinol (**9**)], were isolated from the whole plant of *Justicia procumbens*. Their structures were established by spectral analysis.

In a previous report,[1] bioassay-directed fractionation of the EtOH extract of *Justicia procumbens* L. (Acanthaceae) led to the isolation of 10 arylnaphthalide lignans. In our continuing research on this plant, we found that many spots were first observed on TLC as quenching spots under UV light (254 nm) and were then visualized as blue to dark blue spots after spraying with 10% H_2SO_4 and heating for 3 min. These phenomena encouraged us to reinvestigate the constituents of this plant, and six new diarylbutane lignans, justin A (**1**), (−)-dihydroclusin diacetate (**2**), secoisolariciresinol dimethyl ether diacetate (**3**), 5-methoxy-4,4′-di-*O*-methylsecolariciresinol diacetate (**4**), justin B (**5**), and justin C (**6**), along with three known diarylbutane lignans, 2,3-demethoxysecisolintetralin acetate (**7**),[2] secoisolariciresinol dimethyl ether (**8**),[3] and 5-methoxy-4,4′-di-*O*-methylsecolariciresinol (**9**)[4] were obtained. The present paper describes the structural elucidation of compounds **1**−**6**.



1 R_1-R_2 = O-CH_2-O, R_3 = OCH_3, R_4 = OH, R_5 = H, R_6 = Ac
2 R_1-R_2 = O-CH_2-O, R_3 = R_4 = R_5 = OCH_3, R_6 = Ac
3 R_1 = R_2 = R_3 = R_4 = OCH_3 , R_5 = H, R_6 = Ac
4 R_1 = R_2 = R_3 = R_4 = R_5 = OCH_3, R_6 = Ac
5 R_1-R_2 = O-CH_2-O, R_3 = R_5 = OCH_3, R_4 = OH, R_6 = Ac
6 R_1 = R_2 = R_3 = R_5 = OCH_3, R_4 = OH, R_6 = Ac
7 R_1-R_2 = O-CH_2-O, R_3 = R_4 = OCH_3, R_5 = H, R_6 = Ac
8 R_1 = R_2 = R_3 = R_4 = OCH_3 , R_5 = H, R_6 = H
9 R_1 = R_2 = R_3 = R_4 = R_5 = OCH_3, R_6 = H
10 R_1-R_2 = O-CH_2-O, R_3 = R_4 = R_5 = OCH_3, R_6 = H

Results and Discussion

Compounds **1**−**7** exhibited UV maxima around 235 and 285 nm and IR absorptions at ca. 1730 cm^{-1}. The ^{1}H-NMR signals at ca. δ 2.04 as one singlet (6H) or two singlets (each 3H) indicated the presence of two acetyl groups, and the downfield four-proton multiplet at ca. δ 4.00−4.16 was consistent with two acetylated CH_2-OH groups. These observations were supported by the signals at ca. δ 171.0, 64.3, and 21.0 in their ^{13}C-NMR spectra. From analyses of ^{1}H-NMR spectra, all of these compounds possessed four benzylic protons and two methine protons.

Compound **1** exhibited a molecular formula of $C_{24}H_{28}O_8$ on the basis of its HREIMS, 14 amu less than that of **7**. In the aromatic part of the ^{1}H-NMR spectrum, two pairs of ABX protons [δ 6.47−6.56 (4H, m), 6.67 (1H, d, J = 8.0 Hz), and 6.79 (1H, d, J = 8.0 Hz)] were observed. The ^{1}H-NMR spectrum also indicated a methoxy group (δ 3.80) and a methylenedioxy group (δ 5.90). Irradiation of the OCH_3 at δ 3.80 enhanced the signal at δ 6.51 (J = 1.5 Hz). The ^{1}H- and ^{13}C-NMR spectra gave clear evidence for the presence of both 3,4-methylenedioxyphenyl and 4-hydroxy-3-methoxyphenyl moieties. The MS fragments (*m*/*z* 135 and *m*/*z* 137) of **1** confirmed the presence of the methylenedioxybenzyl and hydroxymethoxybenzyl units, respectively. Thus, justin A was concluded to be represented by structure **1**.

Compound **2** had the molecular formula $C_{26}H_{32}O_9$ as deduced by HREIMS, 30 amu more than that of **7**. This additional mass corresponds to a methoxy group, evidenced in the ^{1}H-NMR spectrum containing three methoxy groups [δ 3.78 (6H) and 3.79 (3H)]. A singlet for two protons at δ 6.24 in the aromatic region was assigned to the symmetrical protons on the ring bearing the three contiguous methoxy groups, a conclusion that was also supported by the mass fragment (*m*/*z* 181). The other protons on the aromatic ring bearing a methylenedioxy group appeared as an ABX system at δ 6.48 (1H, dd, J = 1.8, 8.0 Hz), 6.49 (1H, d, J = 1.8 Hz), and 6.68 (1H, d, J = 8.0 Hz), the assignments of which were also supported by the mass fragment *m*/*z* 135. Thus, from the above experimental results and the ^{13}C-NMR spectrum, **2** was determined to be (−)-dihydroclusin diacetate. Compound **2** was previously prepared by the acetylation of (−)-dihydroclusin (**10**),[5,6] but this is the first reported isolation of **2** as a natural product.

Compound **3** gave a molecular ion peak at 474.2259 in its HREIMS, corresponding to the formula $C_{26}H_{34}O_8$. The ^{1}H-NMR spectrum exhibited four methoxy groups at δ 3.79 (6H) and 3.84 (6H). In the ^{13}C-NMR spectrum, only 13 signals, including those from the acetyl and 3,4-dimethoxyphenyl groups, were observed, suggesting that lignan **3** had a magnetically symmetrical structure.

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Two pairs of ABX protons [δ 6.53 (2H, d, J = 1.8 Hz), 6.55 (2H, dd, J = 1.8, 8.0 Hz), and 6.74 (2H, d, J = 8.0 Hz)] were observed in the aromatic part of the spectrum, and there was a significant mass fragment at *m/z* 151. On the basis of the above results, **3** was determined to be secoisolariciresinol dimethyl ether diacetate. Compound **3** was also obtained previously by the acetylation of secoisolariciresinol dimethyl ether (**8**);[3] and **8** was also obtained by our saponification of **3**. This is the first reported isolation of **3** as a natural product.

Compound **4** had a molecular formula of $C_{27}H_{36}O_9$, as deduced from its HREIMS, 30 amu more than that of **3**. The ^{1}H-NMR spectrum showed five methoxy groups at δ 3.75 (6H), 3.78, 3.79, and 3.81. A singlet for two protons at δ 6.22 in the aromatic region was assigned to the symmetrical protons on a ring bearing three contiguous methoxy groups. The other protons on the aromatic ring appeared as an ABX system at δ 6.53 (1H, d, J = 1.8 Hz), 6.56 (1H, dd, J = 1.8, 8.0 Hz), and 6.73 (1H, d, J = 8.0 Hz). Irradiation of the protons at δ 6.22 and 6.73 enhanced the methoxy signals at δ 3.75 and 3.81, respectively. On the basis of the above data, structure **4** was assigned, and the compound was named 5-methoxy-4,4′-di-*O*-methylsecolariciresinol diacetate. This structure was supported by its ^{13}C-NMR spectrum and MS fragmentation pattern (*m/z* 151 and 181). Saponification of **4** gave 5-methoxy-4,4′-di-*O*-methylsecolariciresinol (**9**).[4]

Compound **5** was assigned a molecular formula of $C_{25}H_{30}O_9$ by HREIMS, 14 amu more than that of **2**. Comparison of the ^{1}H-NMR spectra of **2** and **5** indicated the presence of one hydroxy group in **5** instead of a methoxy group in **2**. A singlet for two protons in the aromatic region at δ 6.24 was assigned to the symmetrical protons on a ring bearing one hydroxy and two methoxy groups, a conclusion that was supported by a mass fragment at *m/z* 167. Thus, compound **5** was determined to be the 4′-demethylated analogue of **2**.

Compound **6** exhibited a molecular formula of $C_{26}H_{34}O_9$ on the basis of its HREIMS, 14 amu less than that of **4**. The ^{1}H- and ^{13}C-NMR signals of **6** were similar to that of **4** except for the absence of one MeO signal and the presence of an additional phenolic proton signal at δ 5.39. Thus, compound **6** (justin C) was identified as the 4′-demethylated analogue of **4**. Compounds **1**−**6** showed negative specific rotations and are therefore assumed to have the same absolute configuration as compound **7**.[2]

Experimental Section

General Experimental Procedures. The physical data of the isolated compounds were obtained on the same instruments as those used in a previous paper.[1] Optical rotations were measured on a JASCO DIP-370 digital polarimenter.

Plant Material. Plant material, *J. procumbens*, was described previously.[1]

Extraction and Isolation. The aerial parts of the plant (3 kg) were extracted with EtOH. The extract was concentrated *in vacuo* to yield a thick, viscous, dark brown mass. This material was absorbed on Si gel (500 g), was stirred constantly until dried, and was then subjected to a Si gel column (70−230 mesh) eluted with a gradient solvent of *n*-hexane−$CHCl_3$ (5:1; 1:1, and 0:1), $CHCl_3$−Me_2CO (1:1), Me_2CO, and Me_2CO−MeOH (1:1). The fraction eluted with $CHCl_3$ was subjected to Si gel column chromatography using a *n*-hexane−Me_2CO (10:1 to 0:1) gradient system. After TLC analysis, the eluates of similar profiles were combined to give seven fractions. Among these fractions, several gave characteristic quenching spots on the TLC plate under UV light at 254 nm. After spraying the plate with 10% H_2SO_4 and heating for 3 min, these were visualized as blue to dark-blue spots. Each fraction was separately subjected to a Sephadex LH-20 column (MeOH), followed by a Si gel (230−400 mesh) column eluting with CH_2Cl_2−Me_2CO (50:1) and a C-18 column (2.5 × 35 cm) eluting with MeOH−H_2O (9:1). The following pure compounds were obtained: compounds **1** (7.6 mg), **2** (15.2 mg), **3** (12.7 mg), **4** (15.8 mg), **5** (13.6 mg), **6** (8.6 mg), **7** (10.7 mg), **8** (5.4 mg), and **9** (3.7 mg).

Justin A (1): colorless oil; $[\alpha]^{28}_D$ −20.0° (*c* 0.12, $CHCl_3$); UV λ_{max} (MeOH) 235, 285 nm; IR ν_{max} (film) 3465, 1733, 1590, 1506, 1243, 1127, 1037, 928 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.04 (6H, s, OAc), 2.07 (2H, m, H-8 and H-8′), 2.59 (4H, m, H-7 and H-7′), 3.80 (3H, s, 3′-OCH_3), 3.92−4.20 (4H, m, H-9 and H-9′), 5.47 (1H, s, 4′-OH), 5.90 (2H, s, O−CH_2−O), 6.47−6.56 (4H, m, H-2, H-2′, H-6, and H-6′), 6.67 (1H, d, J = 8.0 Hz, H-5), 6.79 (1H, d, J = 8.0 Hz, H-5); ^{13}C NMR ($CDCl_3$) δ 131.4 (s, C-1), 110.8 (d, C-2), 143.9 (s, C-3), 145.8 (s, C-4), 111.1 (d, C-5), 121.6 (d, C-6), 34.9 (t, C-7), 39.8 (d, C-8), 64.3 (t, C-9), 133.4 (s, C-1′), 109.1 (d, C-2′), 146.4 (s, C-3′), 147.6 (s, C-4′), 114.2 (d, C-5′), 121.8 (d, C-6′), 34.9 (t, C-7′), 39.8 (d, C-8′), 64.2 (t, C-9′), 21.0 (q, OAc), 100.8 (t, O−CH_2−O), 171.0 (s, C=O), 55.8 (q, Ar−OCH_3); EIMS *m/z* $[M]^+$ 444 (80), 189 (26), 137 (80), 135 (100); HREIMS *m/z* 444.1787, calcd for $C_{24}H_{28}O_8$ 444.1784.

(−)-Dihydroclusin diacetate (2): colorless oil; $[\alpha]^{28}_D$ −17.4° (*c* 1.37, $CHCl_3$); UV λ_{max} (MeOH) 236, 287 nm; IR ν_{max} (film) 1733, 1589, 1506, 1367, 1242, 1127, 1037, 927 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.04 (3H, s, OAc), 2.05 (3H, s, OAc), 2.10 (2H, m, H-8 and H-8′), 2.59 (4H, m, H-7 and H-7′), 3.78 (6H, s, 3′- and 5′-OCH_3), 3.79 (3H, s, 4′-OCH_3), 3.92−4.22 (4H, m, H-9 and H-9′), 5.90 (2H, s, O−CH_2−O), 6.24 (2H, s, H-2′ and H-6′), 6.48 (1H, dd, J = 1.8, 8.0 Hz, H-6), 6.49 (1H, d, J = 1.8 Hz, H-2), 6.68 (1H, d, J = 8.0 Hz, H-5); ^{13}C NMR ($CDCl_3$) δ 133.3 (s, C-1), 109.1 (d, C-2), 145.9 (s, C-3), 147.7 (s, C-4), 108.1 (d, C-5), 121.8 (d, C-6), 34.9 (t, C-7), 39.8 (d, C-8), 64.3 (t, C-9), 135.3 (s, C-1′), 105.7 (d, C-2′), 153.1 (s, C-3′), 135.4 (s, C-4′), 153.1 (s, C-5′), 105.7 (d, C-6′), 35.7 (t, C-7′), 39.5 (d, C-8′), 64.3 (t, C-9′), 21.0 (q, OAc), 100.9 (t, O−CH_2−O), 170.9 (s, C=O), 56.0 (q, Ar−OCH_3); EIMS *m/z* $[M]^+$ 488 (100), 181 (82), 135 (62); HREIMS *m/z* 488.2039, calcd for $C_{26}H_{32}O_9$ 488.2046.

Secoisolariciresinol dimethyl ether diacetate (3): colorless oil; $[\alpha]^{28}_D$ −16.6° (*c* 6.66, $CHCl_3$); UV λ_{max} (MeOH) 228, 280 nm; IR ν_{max} (film) 1732, 1589, 1518, 1233, 1029 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.05 (6H, s, OAc), 2.10 (2H, m, H-8 and H-8′), 2.64 (4H, m, H-7 and H-7′), 3.79 (6H, s, 3- and 3′-OCH_3), 3.84 (6H, s, 4- and 4′-OCH_3), 3.95−4.25 (4H, m, H-9 and H-9′), 6.53 (2H, d, J = 1.8 Hz, H-2 and H-2′), 6.55 (2H, dd, J = 1.8, 8.0 Hz, H-6 and H-6′), 6.74 (2H, d, J = 8.0 Hz, H-5 and H-5′); ^{13}C NMR ($CDCl_3$) δ 132.1 (s, C-1), 111.9 (d, C-2), 147.3 (s, C-3), 148.8 (s, C-4), 111.0 (d, C-5), 120.8 (d, C-6), 34.8 (t, C-7), 39.6 (d, C-8), 64.2 (t, C-9), 132.1 (s, C-1′), 111.9

(d, C-2′), 147.3 (s, C-3′), 148.8 (s, C-4′), 111.0 (d, C-5′), 120.8 (d, C-6′), 34.8 (t, C-7′), 39.6 (d, C-8′), 64.2 (t, C-9′), 20.9 (q, OAc), 170.9 (s, C═O), 55.6, 55.8 (each q, 2 × Ar−OCH_3); EIMS *m/z* $[M]^+$ 474 (35), 203 (15), 151 (100); HREIMS *m/z* 474.2259, calcd for $C_{26}H_{34}O_8$ 474.2254.

5-Methoxy-4,4′-di-*O*-methylsecolariciresinol diacetate (4): colorless oil; $[\alpha]^{28}_D$ −23.4° (*c* 8.52, $CHCl_3$); UV λ_{max} (MeOH) 235, 287 nm; IR ν_{max} (film) 1731, 1589, 1515, 1463, 1247, 1126, 1031 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.04 (6H, s, OAc), 2.08 (2H, m, H-8 and H-8′), 2.62 (4H, m, H-7 and H-7′), 3.75 (6H, s, 3′- and 5′- OCH_3), 3.78 (3H, s, 3- or 4′-OCH_3), 3.79 (3H, s, 3- or 4′-OCH_3), 3.81 (3H, s, 4- OCH_3), 3.92−4.22 (4H, m, H-9 and H-9′), 6.22 (2H, s, H-2′ and H-6′), 6.53 (1H, d, J = 1.8 Hz, H-2), 6.56 (1H, dd, J = 1.8, 8.0 Hz, H-6), 6.73 (1H, d, J = 8.0 Hz, H-5); ^{13}C NMR ($CDCl_3$) δ 132.0 (s, C-1), 112.0 (d, C-2), 147.3 (s, C-3), 148.8 (s, C-4), 111.1 (d, C-5), 120.8 (d, C-6), 35.7 (t, C-7), 39.6 (d, C-8), 64.2 (t, C-9), 135.3 (s, C-1′), 105.7 (d, C-2′), 153.0 (s, C-3′), 136.2 (s, C-4′), 153.0 (s, C-5′), 105.7 (d, C-6′), 34.8 (t, C-7′), 39.5 (d, C-8′), 64.2 (t, C-9′), 20.9 (q, OAc), 170.9 (s, C═O), 55.8, 56.9, 60.7 (each q, 3 Ar−OCH_3); EIMS *m/z* $[M]^+$ 504 (100), 181 (90), 151 (90); HREIMS *m/z* 504.2352, calcd for $C_{27}H_{36}O_9$ 504.2359.

Justin B (5): colorless oil; $[\alpha]^{28}_D$ −23.9° (*c* 1.58, $CHCl_3$); UV λ_{max} (MeOH) 235, 287 nm; IR ν_{max} (film) 3501, 1732, 1613, 1519, 1456, 1232, 1029 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.03 (3H, s, OAc), 2.04 (3H, s, OAc), 2.06 (2H, m, H-8 and H-8′), 2.59 (4H, m, H-7 and H-7′), 3.82 (6H, s, 3′- and 5′-OCH_3), 3.91−4.24 (4H, m, H-9 and H-9′), 5.40 (1H, s, 4-OH), 5.89 (2H, s, O−CH_2−O), 6.24 (2H, s, H-2′ and H-6′), 6.46 (1H, dd, J = 1.8, 8.0 Hz, H-6), 6.48 (1H, d, J = 1.8 Hz, H-2), 6.66 (1H, d, J = 8.0 Hz, H-5); ^{13}C NMR ($CDCl_3$) δ 133.0 (s, C-1), 109.1 (d, C-2), 145.8 (s, C-3), 147.6 (s, C-4), 108.0 (d, C-5), 121.8 (d, C-6), 35.4 (t, C-7), 39.7 (d, C-8), 64.3 (t, C-9), 130.5 (s, C-1′), 105.4 (d, C-2′), 146.9 (s, C-3′), 133.3 (s, C-4′), 146.9 (s, C-5′), 105.4 (d, C-6′), 35.0 (t, C-7′), 39.6 (d, C-8′), 64.1 (t, C-9′), 20.9 (q, OAc), 100.8 (t, O−CH_2−O), 170.9 (s, C═O), 56.1, 56.2 (each q, 2 × Ar−OCH_3); EIMS *m/z* $[M]^+$ 474 (100), 167 (60), 135 (55); HREIMS *m/z* 474.1878, calcd for $C_{25}H_{30}O_9$ 474.1890.

Justin C (6): colorless oil; $[\alpha]^{28}_D$ −37.6° (*c* 1.09, $CHCl_3$); UV λ_{max} (MeOH) 235, 285 nm; IR ν_{max} (film) 3500, 1732, 1615, 1520, 1456, 1233, 1116, 1030 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.04 (3H, s, OAc), 2.05 (3H, s, OAc), 2.06 (2H, m, H-8 and H-8′), 2.63 (4H, m, H-7 and H-7′), 3.76 (3H, s, 3′- OCH_3), 3.77 (6H, s, 3- and 5-OCH_3), 4′-OCH_3), 3.91−4.28 (4H, m, H-9 and H-9′), 5.39 (1H, s, 4-OH), 6.24 (2H, s, H-2 and H-6), 6.48 (1H, d, J = 1.8 Hz, H-2′), 6.53 (1H, dd, J = 1.8, 8.0 Hz, H-6′), 6.75 (1H, d, J = 8.0 Hz, H-5′); ^{13}C NMR ($CDCl_3$) δ 132.1 (s, C-1), 111.9 (d, C-2), 147.3 (s, C-3), 148.8 (s, C-4), 111.0 (d, C-5), 120.9 (d, C-6), 34.9 (t, C-7), 39.5 (d, C-8), 64.3 (t, C-9), 130.6 (s, C-1′), 105.3 (d, C-2′), 146.8 (s, C-3′), 132.9 (s, C-4′), 146.8 (s, C-5′), 105.3 (d, C-6′), 35.5 (t, C-7′), 39.4 (d, C-8′), 64.3 (t, C-9′), 21.0 (q, OAc), 170.9 (s, C═O), 55.7, 56.1 (each q, 2 × Ar−OCH_3); EIMS *m/z* $[M]^+$ 490 (67), 167 (75), 135 (100); HREIMS *m/z* 490.2209, calcd for $C_{26}H_{34}O_9$ 490.2203.

2,3-Demethoxysecoisolintetralin acetate (7): colorless prisms; mp 52−54 °C; $[\alpha]^{28}_D$ −9.65° (*c* 2.20, $CHCl_3$), [lit.[2] $[\alpha]^{28}_D$ −1.3° (*c* 1.6, $CHCl_3$)]; physical and spectroscopic data comparable to these reported in the literature.[2]

Saponification of 2−4. Each pure compound (10 mg) was separately saponified by treatment with 5 mL of 1% MeOH solution of NaOH. The reaction was stirred at room temperature overnight, and then 10 mL of H_2O was added. The mixture was neutralized with diluted HCl, and MeOH was removed under reduced pressure. The aqueous solution was extracted with 3 × 20 mL of ethyl ether, and the organic layer was evaporated to dryness. The residue was subjected to Si gel column chromatography eluting with CH_2Cl_2−Me_2CO (10:1) to yield **10**,[5,6] **8**,[3] and **9**,[4] respectively. The ^1H NMR of **8**, **9**, and **10** were identical with those reported.

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