Nitric Oxide Production Inhibitory Activity of Flavonoids Contained in Trunk Exudates of *Dalbergia sissoo*

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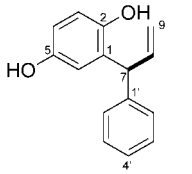
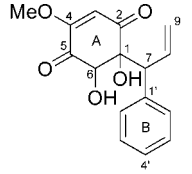
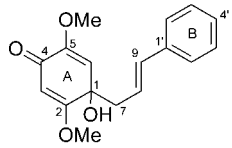
Methanolic extracts of trunk exudates of *Dalbergia sissoo* yielded two new open-chain neoflavonoids (**1**, **2**), a new flavonoid (**3**), a new flavanone (**4**), and 26 known compounds. Their structures were elucidated by detailed spectroscopic analyses. The ability of the isolated compounds to prevent nitric oxide (NO) production by LPS-stimulated J774.1 cells was also studied. All of the isolated compounds except **4**, formononetin, and zenognosin B exhibited significant activity in a concentration-dependent manner. Compounds **2** and **3** were among the most potent NO production inhibitors, with IC_{50} values of 3.19 and 6.22 μM, respectively, and compound **1** had an IC_{50} of 31.6 μM.

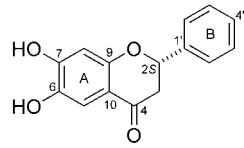
Dalbergia sissoo (Roxb.), belonging to legume family (Fabaceae), is a perennial tree found in the lowland region (300 to about 1000 m) of Nepal. Its distribution range extends across the sub-Himalayan region in India, Pakistan, Bangladesh, and Afghanistan. In addition to its use as a timber or firewood, it also is used by different ethnic groups to treat a variety of ailments.[1,2] *D. sissoo* has also been reported to possess various biological activities.[1,3]

In the course of our search for the source of Nepalese (Chitwan) propolis, the TLC and HPLC profiles of exudates of *D. sissoo* were found to be similar to those of Nepalese propolis.[4] Moreover, the trunk exudates were also found to inhibit the production of nitric oxide (NO) by LPS-induced J774.1 cells (IC_{50} 22 μg/mL) in our initial assay. To the best of our knowledge, studies of constituents of trunk exudates of *D. sissoo* have not been reported to date. Thus, in an attempt to find novel compounds with potent NO production inhibitory activity, we carried out a bioassay-guided fractionation of the exudates that resulted in the isolation of 30 flavonoids including four new ones (**1**–**4**). We report herein the details of isolation, structure elucidation, and NO production inhibitory activity of the isolated compounds.









Results and Discussion

The MeOH extract of trunk exudates of *D. sissoo* was subjected to a succession of chromatographic procedures to yield four new (**1**–**4**) and 26 known compounds. Compound **1** was assigned the molecular formula $C_{15}H_{14}O_2$ on the basis of the ion peak at *m*/*z* 226.0971 (calcd for $C_{15}H_{14}O_2$ $[M]^+$, 226.0994) observed in its HRFABMS. The general features of ^1H and ^{13}C NMR spectra (Tables 1 and 2) of compound **1** closely resembled those of 4-[(1*S*)-1-phenyl-2-propenyl]-1,3-benzenediol,[5] except for slight differences in the chemical shifts of signals due to H-7 and the ABX spin system of the trisubstituted benzene ring (ring A) in the ^1H NMR spectrum. After extensive analysis of the HMBC spectrum of **1**, the signals due to an ABX spin system observed in the ^1H NMR spectrum at δ_H 6.57 (1H, d, J = 8.5 Hz), 6.49 (1H, dd, J = 8.5, 2.5 Hz), and 6.53 (1H, d, J = 2.5 Hz) for ring A were assigned H-3, H-4, and H-6, respectively, on the basis of long-range correlations from H-6 to C-1, C-2, C-4, C-5, and C-7; from H-7 to C-1, C-2, and C-6, and from H-8 to C-1, which revealed **1** to have a 1,2,5-trisubstituted ring A instead of a 1,2,4-trisubstituted ring A. The positive optical rotation values and negative CD minimum observed at 286 nm in the LC-CD spectrum indicated that **1** has the same absolute configuration as 4-[(1*S*)-1-phenyl-2-propenyl]-1,3-benzenediol, i.e., 7*S*.[5–7]

Compound **2** had the molecular formula $C_{16}H_{16}O_5$ as determined by HRFABMS. Inspection of the ^1H and ^{13}C NMR spectra (Tables 1 and 2) revealed **2** to have two conjugated ketone groups, a monosubstituted benzene ring, a trisubstituted double bond, an oxymethine, an oxygenated quaternary sp^2-carbon, an oxygen-bearing quaternary carbon, a vinyl group, and a methoxyl group. The splitting patterns and the coupling constants of the signals due to the aliphatic methine at δ_H 4.54 (1H, d, J = 7.0 Hz, H-7) and the vinyl group at δ_H 5.94 (1H, ddd, J = 17.0, 8.5, 7.0 Hz, H-8), 5.27 (1H, dt, J = 8.5, 1.4 Hz, H-9a), and 5.04 (1H, dt, J = 17.0, 1.4 Hz, H-9b) corresponded closely to the allylic system C(7)H−C(8)H=C(9)H_2 of known open-chain neoflavonoids such as *S*-4′-hydroxy-4-methoxydalbergione. Thus, assuming **2** also to be an open-chain neoflavonoid, the monosubstituted benzene (ring B) was connected to C-7. This was also supported by long-range HMBC correlations from H-7 and H-8 to C-1′ and from H-2′,6′ to C-7. Similarly, HMBC correlations from H-7 to C-1, C-2, and C-6; from H-3 and H-6 to C-1, C-2, C-4, and C-5; and from the methoxyl protons to C-4 connected C-1 of ring A to C-7 and also showed that the two OH groups were located at C-1, C-6; the methoxyl group was connected to C-4; and the ketone groups were located at positions 2 and 5, respectively. The small amount of material that remained limited further studies of its absolute configuration.

Compound **3** was isolated as a colorless oil with the molecular formula $C_{17}H_{18}O_4$. The ^1H NMR spectrum showed signals due to a monosubstituted benzene ring, a methylene, two isolated olefinic and two *trans*-olefinic protons, and two methoxyl groups. The ^{13}C NMR spectrum displayed 17 signals including those of a conjugated ketone (δ_C 184.7), two oxygen-bearing olefinic carbons (δ_C 178.8, 151.0), and an oxygenated quaternary carbon (δ_C 73.5). The partial structure C(7)H_2−C(8)H=C(9)H was obtained from COSY and

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Table 1. ^{1}H NMR Spectroscopic Data for Compounds **1**–**4**

proton	**1**[a]	**2**[a]	**3**[b]	**4**[b]
2				5.43 dd 13.0, 3.0)
3	6.57 d (8.5)	5.74 s	5.54 s	2.98 dd (17.0, 13.0)
3				2.72 dd (17.0, 3.0)
4	6.49 dd (8.5, 2.5)			
5				7.19 s
6	6.53 d (2.5)	3.76 s	5.69 s	
7	4.90 d (7.0)	4.54 d (7.0)	2.82 dd (13.5, 8.0)	
			2.72 dd (13.5, 7.0)	
8	6.19 ddd (17.0, 9.5, 7.0)	5.94 ddd (17.0, 8.5, 7.0)	5.94 ddd (16.0, 8.0, 7.0)	6.41 s
9	5.18 dt (9.5, 1.5)	5.27 dt (8.5, 1.4)	6.42 d (16.0)	
	4.92 dt (17.0, 1.5)	5.04 dt (17.0, 1.4)		
2′	7.15 dt (8.0, 1.5)	7.22 dd (7.0, 1.5)	7.23 dd (6.5, 2.0)	7.48 d (7.0)
3′	7.23 dt (8.0, 1.5)	7.30 tt (7.0, 1.5)	7.26 dt (6.5, 2.0)	7.40 t (7.0)
4′	7.16 dt (8.0, 1.5)	7.26 tt (7.0, 1.5)	7.16 tt (6.5, 2.0)	7.35 t (7.0)
5′	7.23 dt (8.0, 1.5)	7.30 tt (7.0, 1.5)	7.26 dt (6.5, 2.0)	7.40 t (7.0)
6′	7.15 dt (8.0, 1.5)	7.22 dd (7.0, 1.5)	7.23 dd (6.5, 2.0)	7.48 d (7.0)
2 OMe			3.67 s	
4 OMe		3.75 s		
5 OMe			3.84 s	

[a] Recorded at 500 MHz in $CDCl_3$. [b] Recorded at 500 MHz in CD_3OD. δ_H in ppm, J in Hz.

Table 2. ^{13}C NMR Spectroscopic Data for Compounds **1**–**4**

position	**1**[a]	**2**[a]	**3**[b]	**4**[b]
1	130.8	64.0	73.5	
2	147.0	190.2	178.8	81.1
3	117.2	108.7	101.4	45.2
4	114.4	158.8	184.7	193.2
5	149.3	187.7	151.0	111.4
6	116.5	55.7	115.9	142.4
7	48.7	44.9	44.7	159.2
8	139.3	134.0	123.9	104.3
9	116.9	119.8	135.5	156.6
10	118.2			113.9
1′	141.7	137.6	138.6	140.9
2′	128.5	128.8	127.2	127.3
3′	128.6	128.6	129.6	129.6
4′	126.6	127.4	128.5	129.4
5′	128.6	128.6	129.6	129.6
6′	128.6	128.8	127.2	127.3
2 OMe			57.1	
4 OMe		56.4		
5 OMe			55.7	

[a] Recorded at 125 MHz in $CDCl_3$. [b] Recorded at 125 MHz in CD_3OD.

HMQC spectra. The HMBC cross-peak observed for H-9 to C-1′, C-2′, and C-6′ connected the phenyl ring to C-9, while the complete planar structure was deduced on the basis of HMBC correlations from H_2-7 to C-1, C-2, and C-6; from H-3 to C-1, C-4, and C-5; from H-6 to C-1, C-2, C-4, and C-5; from OCH_3-2 to C-2; and from OCH_3-5 to C-5. The absolute configuration at C-1 could not be determined due to insufficient amount of sample.

Compound **4** had the molecular formula $C_{15}H_{12}O_4$ as determined by HRFABMS. The ^{1}H NMR spectrum of **4** in CD_3OD revealed the presence of nonequivalent methylene protons at δ_H 2.98 (1H, dd, J = 17.0, 13.0 Hz, H-3ax) and 2.72 (1H, dd, J = 17.0, 3.0 Hz, H-3eq), a signal at δ_H 5.43 (1H, dd, J = 13.0, 3.0 Hz, H-2), and signals due to a monosubstituted benzene ring at δ_H 7.48 (2H, d, J = 7.0, Hz, H-2′,6′), 7.40 (1H, t, J = 7.0, Hz, H-3′,5′), and 7.35 (1H, t, J = 7.0, Hz, H-4′). Fifteen carbon signals including signals due to two aromatic rings (with one monosubstituted), a conjugated ketone at δ_C 193.2 (C-4), a methine at δ_C 81.1 (C-2), and a methylene at δ_C 45.2 (C-3) were observed in its ^{13}C NMR spectrum. These data collectively revealed **4** to be a flavanone with unsubstituted ring B.[8,9] Thus, the remaining two ^{1}H NMR signals, which showed their *para*-disposition by appearing as singlet signals at δ_H 6.41 and δ_H 7.19, were attributed to H-5 and H-8, respectively, while the presence of OH groups at positions 6 and 7 could be inferred from the molecular formula $C_{15}H_{12}O_4$. The negative optical rotation values and negative Cotton effect observed at 282 nm in the LC-CD spectrum revealed that the absolute configuration at C-2 was *S*.[10] From this evidence, **4** was concluded to be (2*S*)-6,7-dihydroxyflavanone. The long-range correlations observed in the HMBC spectrum were in agreement with the above structural assignment of **4**. Also, the OH group bearing carbons at δ_C 142.4 and 159.2 in the ^{13}C NMR spectrum were unambiguously assigned to positions 6 and 7, respectively, by comparison with literature values of similar compounds.[11]

In addition to the four new compounds described above, 26 known compounds were also isolated. By comparing their physical and spectroscopic data with those reported in the literature, they were characterized as *S*-4′-hydroxy-4-methoxydalbergione,[12] *S*-4-methoxydalbergone,[13] *S*-dalbergione,[14] *S*-4-methoxydalbergiquinol,[13] 4-[(1*S*)-1-phenyl-2-propenyl]-1,3-benzenediol,[5] (4*S*,6*S*)-4-hydroxy-3-methoxy-6-(1-phenyl-2-propenyl)-2-cyclohexen-1-one,[5] (4*R*,6*S*)-4-hydroxy-6-(1-phenyl-2-propenyl)-2-cyclohexen-1-one,[5] (2*S*,4*R*,5*S*)-4-hydroxy-5-methoxy-2-[(1*S*)-1-phenyl-2-propenyl)]cyclohexanone,[5] (2*S*,4*R*,6*S*)-4-hydroxy-2-methoxy-6-(1-phenyl-2-propenyl)cyclohexanone,[5] (1*S*,2*S*,4*S*,5*S*)-2-methoxy-5-[(1*R*)-1-phenyl-2-propenyl)]-1,4-cyclohexanediol,[5] cearoin,[7] 4-hydroxy-3-methoxy-4-(3-phenyl-2-propenyl)-2-cyclohexen-1-one,[5] isoliquiritigenin,[15] butein,[16] 2′-hydroxy-4′-methoxychalcone,[17] hydroxyobtustyrene,[18] (2*S*)-7-hydroxyflavanone,[19] (+)-pinocembrin,[20] plathymenin,[21] (±)-vestitol,[22] dihydrosepiol,[23] formononetin,[24] zenognosin B,[25] 4-hydroxymedicarpin,[26] (+)-medicarpin,[27] and (+)-vesticarpan.[28] To the best of our knowledge, this is the first report on the chemical analysis of exudates of *D. sissoo* and also the first report of the isolation of seven of the known compounds from a plant source.

Selected compounds were then evaluated for their ability to inhibit nitric oxide (NO) production by LPS-induced macrophage-like J774.1 cells. The inorganic free radical nitric oxide (NO), synthesized by a family of enzymes termed NO-synthase (NOS), acts as host defense by damaging pathogenic DNA and as a regulatory molecule with homeostatic activities.[29] However, excessive production has detrimental effects on many organ systems of the body, leading to tissue damage, even leading to a fatal development (septic shock).[30] Therefore, inhibition of NO production may be of therapeutic benefit in various diseases induced by pathological conditions of NO. In this assay, N^G-monomethyl-L-arginine (L-NMMA), a nonselective nitric oxide synthase (NOS) inhibitor,[31] was used as a positive control, and most of the tested compounds showed significant concentration-dependent inhibition. Compounds **2**, **3**, and 2′-hydroxy-4′-methoxychalcone were the most potent NO production inhibitors, with IC_{50} values 3.19, 6.22, and 5.4 μM, respectively, which is much less than that of the positive

control L-NMMA (IC_{50} 32.0 μM). Furthermore, *S*-4′-hydroxy-4-methoxydalbergione (IC_{50} 11.5 μM) showed stronger activity than *S*-4-methoxydalbergone (IC_{50} 38.8 μM) and *S*-dalbergione (IC_{50} 39.7 μM), indicating that the presence of an OH group at 4′ increases the activity, and the presence or absence of a methoxyl group at position 4 does not have much impact on NO production inhibitory activity of dalbergiones. Although the structure–activity relationship of dalbergiones bearing an OH group in ring B has not been investigated thoroughly, our results suggest an OH group in ring B may enhance the NO production inhibitory activity of dalbergiones remarkably. To find out whether inhibition of NO production was due to the cytotoxicity of the tested compounds, cell viability was also determined by the MTT method. In this test, compound **2** and *S*-4′-hydroxy-4-methoxydalbergione exhibited cytotoxicity above 100 μM, while 2′-hydroxy-4′-methoxychalcone exhibited cytotoxicity above 25 μM. Elsewhere,[5] certain of these compounds have been reported to inhibit NO production by LPS-activated macrophage-like J774.1 cells with IC_{50} values less than or comparable to that of positive control L-NMMA (IC_{50} 27.1 μM), and only (4*R*,6*S*)-4-hydroxy-6-(1-phenyl-2-propenyl)-2-cyclohexen-1-one has been reported to be cytotoxic in higher concentrations. However, the IC_{50} values of the compounds that showed cytotoxicity also lie within the nontoxic concentration range. Thus, we conclude that the inhibitory activity exhibited by the compounds from trunk exudates of *D. sissoo* is not due to their cytotoxic properties but is due to their ability to effect the mechanism of NO production.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL A-500 FT-NMR spectrometer, and the chemical shifts were expressed in the δ (ppm) scale with TMS as an internal standard. FABMS, HRFABMS, EIMS, and HREIMS were recorded on a JEOL JMS-700 spectrometer. Analytical and preparative TLC were performed on precoated silica gel 60 F_{254} or RP-18 F_{254}S plates (Merck, 0.25 or 0.50 mm thickness), and detection was carried out by spraying the gel with a EtOH−H_2SO_4 reagent followed by heating. Column chromatography was carried out on silica gel (silica gel 60, Merk), Lobar LiChroprep RP-18 (Merck), Diaion HP-20 (Mitsubishi Chemical Corporation), and MCI gel CHP-20P (Mitsubishi Chemical Corporation). HPLC was performed on a Shimadzu LC-10ADVP instrument with a Capcell Pak C_{18} column (5 μm, 15 mm i.d. × 250 mm, Shiseido Fine Chemicals) at 40 °C. HPLC-CD spectra were measured in stopped-flow mode using a Capcell Pak C_{18} column (5 μm, 4.6 mm i.d. × 250 mm, Shiseido Fine Chemicals) at 40 °C, eluted with MeOH−H_2O (2:3) at a flow rate of 0.5 mL min^{-1} on a Jasco CD-2095$_{Plus}$ chiral detector equipped with two Shimadzu LC-10ADVP pumps, CTO-10AVP column oven, and SCL-10AVP system controller.

Biological Material. The trunk exudates of *Dalbergia sissoo* (Roxb.) were collected in Chitwan, Nepal, in 2006 and verified by lecturer Mr. Khem Raj Neupane of Nepal University, Institute of Agriculture and Animal Sciences, Rampur campus, Chitwan, Nepal. A voucher specimen (DNM2005NepP-1) has been deposited at the Division of Natural Medicines, Kyoritsu University of Pharmacy.

Extraction and Isolation. Trunk exudates of *D. sissoo* (180 g) were exhaustively extracted with refluxing MeOH to give the methanol extract (175 g). Part of the MeOH extract (70.7 g) was passed through a porous polymer gel column (Diaion HP-20) eluted successively with 50% MeOH, MeOH, and $CHCl_3$ to obtain 50% MeOH (2.1 g), MeOH (58.3 g), and $CHCl_3$ (7.1 g) eluates, respectively. The MeOH eluate was further chromatographed on an MCI gel CHP-20P column eluted with a stepwise gradient of CH_3CN−MeOH−H_2O (1:1:4 → 1:1:0) to afford five fractions [1: CH_3CN−MeOH-H_2O (1:1:4) eluate, 5.35 g; 2: CH_3CN−MeOH−H_2O (1:1:3) eluate, 13.2 g; 3: CH_3CN−MeOH−H_2O (1:1:2) eluate, 26.3 g; 4: CH_3CN−MeOH−H_2O (1:1:1) eluate, 9.09 g; and 5: CH_3CN−MeOH−H_2O (1:1:0) eluate, 1.73 g].

Repeated chromatography of fraction 1 using a MCI gel CHP-20P column eluted with CH_3CN−H_2O (1:4 → 1:0), a Lobar RP-8 column with CH_3CN−MeOH−H_2O (1:1:4 → 1:1:0), normal-phase preparative TLC with CH_3CN−C_6H_6 (1:8), and ODS gel preparative HPLC (CH_3CN−H_2O, 1:3 → 4:1) gave **2** (4.12 mg), butein (95.2 mg), plathymenin (34.2 mg), formononetin (134.1 mg), zenognosin B (69.3 mg), (+)-medicarpin (517 mg), and (+)-vesticarpan (105 mg). Fraction 2 was chromatographed using similar materials and methods to those for fraction 1 to obtain **4** (9.6 mg), cearoin (180.3 mg), isoliquiritigenin (72.6 mg), (2*S*)-7-hydroxyflavanone (46.6 mg), (+)-pinocembrin (23.4 mg), and (+)-vesticarpan (31.4 mg). Fraction 3 was subjected to successive chromatographic separation using a MCI gel CHP-20P column eluted with CH_3CN−H_2O (1:4 → 1:0), normal-phase preparative TLC with CH_3CN−C_6H_6 (1:6), and ODS gel preparative HPLC (CH_3CN−MeOH−H_2O, 1:1:3 → 1:1:0) to isolate **1** (48.1 mg), **3** (6.21 mg), *S*-4-methoxydalbergiquinol (32.1 mg), (2*S*,4*R*,5*S*)-4-hydroxy-5-methoxy-2-[(1*S*)-1-phenyl-2-propenyl)]cyclohexanone (3.4 g), (2*S*,4*R*,6*S*)-4-hydroxy-2-methoxy-6-(1-phenyl-2-propenyl)cyclohexanone (410 mg), (1*S*,2*S*,4*S*,5*S*)-2-methoxy-5-[(1*R*)-1-phenyl-2-propenyl)]-1,4-cyclohexanediol (29.2 mg), 4-hydroxy-3-methoxy-4-(3-phenyl-2-propenyl)-2-cyclohexen-1-one (400 mg), 2′-hydroxy-4′-methoxychalcone (20.0 mg), hydroxyobtustyrene (40.5 mg), dihydrosepiol (45.7 mg), and 4-hydroxymedicarpin (18.6 mg). Similarly, repeated chromatography of fractions 4 and 5 using a silica gel column (4 × 30 cm) eluting with CH_3Cl followed by increasing concentration of MeOH (0% → 30%), normal-phase preparative TLC with CH_3CN−C_6H_6 (1:4), and recrystallization gave *S*-4-methoxydalbergone (812 mg), 4-[(1*S*)-1-phenyl-2-propenyl]-1,3-benzenediol (122 mg), (4*S*,6*S*)-4-hydroxy-3-methoxy-6-(1-phenyl-2-propenyl)-2-cyclohexen-1-one (57.5 mg), (4*R*,6*S*)-4-hydroxy-6-(1-phenyl-2-propenyl)-2-cyclohexen-1-one (11.7 mg), (−)-vestitol (59.2 mg), *S*-4′-hydroxy-4-methoxydalbergione (20.2 mg), *S*-4-methoxydalbergiquinol (307 mg), and *S*-dalbergione (12.0 mg).

Compound 1: dark brown oil; $[\alpha]^{25}_D$ +30.9 (*c* 0.02, $CHCl_3$); UV (MeOH) λ_{max} 296, 308 nm; CD (60% MeOH−H_2O) λ_{max} ($\Delta\epsilon$) 284 (−3.398), 236 (−2.997); IR ν_{max} 3365, 1629, 1596, 1375, 1280, 1006 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRFABMS *m*/*z* 226.0971 [M]$^+$, calcd for $C_{15}H_{14}O_2$, 226.0994.

Compound 2: yellow, amorphous powder; $[\alpha]^{25}_D$ +24.7 (*c* 0.02, $CHCl_3$); UV (MeOH) λ_{max} 256, 347 nm; IR ν_{max} 3417, 1710, 1670, 1610, 1230, 1007 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m*/*z*: 289.1053 [M + H]$^+$, calcd for $C_{16}H_{16}O_5$, 289.1076.

Compound 3: colorless oil; $[\alpha]^{26}_D$ –2.26 (*c* 0.016, CH_3OH); UV (MeOH) λ_{max} 246, 292 nm; IR ν_{max} 3369, 1629, 1596, 1446, 1375, 1155 cm^{-1}; H and ^{13}C NMR, see Tables 1 and 2; HREIMS *m*/*z* 286.1186 [M]$^+$, calcd for $C_{17}H_{18}O_4$, 286.1205.

Compound 4: yellow solid; $[\alpha]^{26}_D$ −39.2 (*c* 0.097, $CHCl_3$); UV (MeOH) λ_{max} 241, 281, 345 nm; CD (60% MeOH−H_2O) λ_{max} ($\Delta\epsilon$ 282 (−6.382); IR ν_{max} 3297, 3081, 3027, 1598, 1492, 1446, 1186 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRFABMS *m*/*z* 257.0835 [M + H]$^+$, calcd for $C_{15}H_{12}O_4$, 257.0814.

Cell Culture. The J774.1 cells were propagated in 25 cm^2 plastic culture flasks (Falcone, Becton Dickinson, NJ), containing DMEM supplemented with penicillin G (100 units/mL), streptomycin (100 μg/mL), and 10% FCS.

Nitric Oxide Inhibitory Assay. After discarding the nonadherent cells, fresh medium was added in the flask, and the adherent cells were recovered. The cells were suspended in medium, plated on 96-well plates (1 × 10^5 cells/well), and allowed to adhere for 6 h at 37 °C in a humidified atmosphere containing 5% CO_2. Then, the medium was replaced with fresh medium, containing LPS (1 μg/mL) and test compounds at various concentrations, and again incubated for 24 h. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using Griess reagent.[32] Briefly, 50 μL of the supernatant form incubates was mixed with an equal volume of Griess reagent (1% sulfanilamide and 0.1% naphthylenediamide dihydrochloride in 5% H_3PO_4), and the mixture was allowed to stand for 10 min at room temperature. Absorbance at 550 nm was measured using a Bio-Rad model 550 microplate reader (Bio-Rad, Japan). The blank correction was carried out by subtracting the absorbance due to medium only from the absorbance reading of each well. The percentage inhibition was calculated as follows: % inhibition = $[(A_c - A_s)/A_c] \times 100$, where A_c and A_s are absorbance of control group treated with LPS alone and with LPs and the sample, respectively.

Cell Viability. Cell viability was determined using the mitochondrial respiration-dependent MTT reduction method.[33] After transferring the supernatant to another plate for Griess assay, the remaining supernatant was aspirated from the 96-well plates, and 100 μL of fresh medium containing 2 mg/mL of MTT was added to each well. The cells were then incubated at 37 °C in a humidified atmosphere containing 5% CO_2. After incubating for 3 h, the medium was removed and the violet

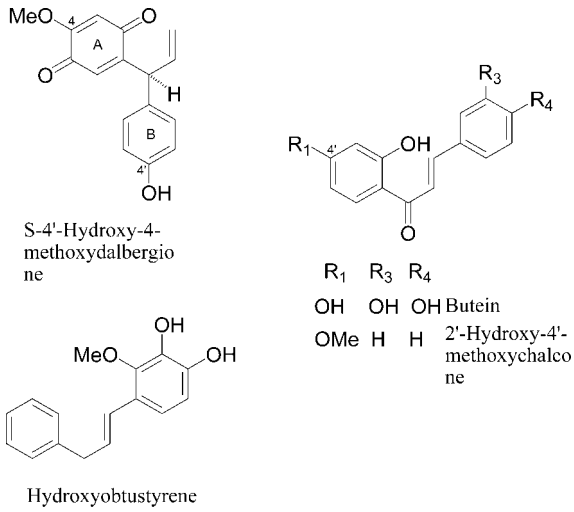

Figure 1. Structure of known compounds demonstrating greater activity than positive control L-NMMA.

crystals of formazan in viable cells were dissolved in dimethyl sulfoxide. Absorbance at 550 nm was measured using a Bio-Rad model 550 microplate reader (Bio-Rad, Japan).

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Supporting Information Available: Figure S-1 showing the structures of the known compounds isolated from trunk exudates of *Dalbergia sissoo* (Roxb.) is available free of charge via the Internet at http://pubs.acs.org.

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