Low-Density Lipoprotein-Antioxidant Constituents of *Saururus chinensis*

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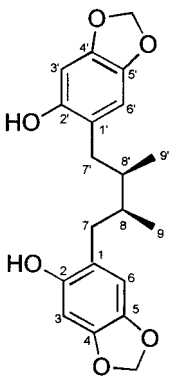
A new diarylbutane lignan, saururin A (**1**), and a known 8-*O*-4′-type neolignan, machilin D (**2**), were isolated from a total methanol extract of the underground parts of *Saururus chinensis*. The structures of **1** and **2** were elucidated by spectroscopic data analysis. Compounds **1**, **2**, and virolin (**3**) (the methyl ether of **2**) exhibited significant low-density lipoprotein (LDL)-antioxidant activity in the thiobarbituric acid-reactive substance (TBARS) assay with IC_{50} values of 8.5, 2.9, and 4.3 μM, respectively.

The oxidation of low-density lipoprotein (LDL) cholesterol has been proposed as an important step in the formation of atherosclerotic lesions.[1,2] Evidence to support this hypothesis is based in part on observational studies that show associations between oxidized LDL cholesterol and both the presence of atherosclerotic lesions[3] and the progression of carotid artery atherosclerosis.[4] The structurally different antioxidants probucol, butylated hydroxytoluene, and *N*,*N*′-diphenylphenylenediamine inhibit both ex vivo LDL oxidation and atherosclerosis in animals.[5,6] The role of antioxidants as potential antiatherogenic compounds has been summarized in a recent review.[7]

Lignans are widely distributed secondary metabolites containing two phenylpropane units connected by 8,8′ bonds, and their biosynthesis and function have been reviewed.[8] Many studies have demonstrated that lignans and polyphenolic flavonoids derived from plants used in the human diet medicinally possess antioxidative properties. *Saururus chinensis* Baill. (Saururaceae) is a traditional Chinese medicine used as an antipyretic, diuretic, and antiinflammatory agent.[9] By screening selected plant extracts to search for LDL-antioxidants for their potential use to treat atherosclerosis, we found that the total methanol extract of the underground parts of *S. chinensis* showed significant LDL-antioxidant activity. Subsequent activity-guided fractionation of the methanolic extracts led to the isolation of a new diarylbutane-type lignan, saururin A (**1**), and a known 8-*O*-4′-type neolignan, machilin D (**2**).[10,11] This paper describes the isolation and characterization of two antioxidative constituents of *S. chinensis* (**1** and **2**).

Bioassay-guided fractionation using an LDL-antioxidant assay[12] of the underground parts of *S. chinensis* using Si gel normal-phase and reversed-phase column chromatography led to the isolation of compounds **1** and **2**. Compound **2** was identified as machilin D by comparison of its physical and spectral data with those previously reported.[10,11] Its structure was confirmed by the conversion of compound **2** to its methyl ether **3** (virolin), which was in good accord with the published spectral data.[10] The absolute configuration of **2** was determined as 7*S*,8*S* by comparing the specific rotation of the methylated lignan **3** ($[\alpha]^{25}_{D}$ −32.0°) with a previously reported value ($[\alpha]^{25}_{D}$ −29°).[13]



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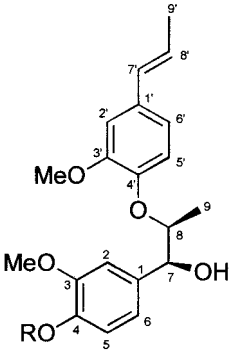

2 R = H

3 R = Me

Compound **1** was obtained as a pale brown solid. A molecular formula of $C_{20}H_{22}O_6$ was determined by HREIMS ($[M]^+$, *m*/*z* 358.1441, calcd 358.1416 for $C_{20}H_{22}O_6$). Compound **1** showed a green color on treatment with an ethanolic ferric chloride solution. Its IR spectrum showed a strong hydroxyl absorption band at 3390 cm^{-1}. The NMR spectrum of **1** showed signals to indicate four aromatic protons (δ 6.34, 6.29, each 2H, s), two methylenedioxy protons (δ 5.79, 4H, s), two hydroxyl protons (δ 4.84, 2H, brs, D_2O exchangeable), four benzyl protons [(δ 2.50, 2H, dd, J = 5.5, 13.9), (δ 2.26, 2H, dd, J = 8.4, 13.9)], and two CH−CH_3 protons [(δ 1.72 2H, m), (δ 0.77, 6H, d, J = 6.6)]. The mass spectrum showed a base peak at *m*/*z* 358 [M^+] and a 98% intensity peak at *m*/*z* 152 (2-hydroxy-4,5-methylenedioxybenzyl ion or its equivalent). These analyti-

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cal and spectral data were characteristic of a structurally symmetrical 2,3-dimethyl-1,4-diarylbutane-type neolignan.[11] Furthermore, two singlet protons on a benzene ring suggested the presence of a 2-hydroxy-4,5-methylenedioxy group in the molecule. Compound **1** showed no optical activity and a large coupling constant (J = 6.6 Hz) for the C-9, C-9′ methyl protons, as observed in machilin A possessing the same relative stereochemistry as compound **1**.[11] Thus, compound **1** was assumed to have the 8*S* and 8′*R* configurations (*meso*-form). The ^{13}C NMR spectrum of **1** showed 10 signals indicating the presence of a symmetrical 2,3-dimethyl-1,4-diarybutane-type neolignan. The position of the methylene group in compound **1** was determined by the HMBC NMR experiment, which showed a long-range correlation between the methylene proton signal at δ_H 5.79 and both C-4 at δ_C 146.4 and C-5 at δ_C 141.9. On the basis of the above data, the structure of **1** was concluded to be *rel*-(8*S*,8′*R*)-2,2′-dihydroxy-4,5:4′,5′-bis-(methylenedioxy)-8,8′-neolignan, for which the name saururin A is proposed.[14]

Among diarylbutane-type lignans, the mother substance of the mace lignans, nor-dihydroguaiaretic acid (NDGA), has been used as an antioxidant to protect fats and oils. Lee et al. reported that lignans carrying aromatic methylenedioxy and hydroxyl moieties demonstrated potent antioxidant activity.[15] This phenomenon was also observed in sesamolinol, which is furofuran-type lignan carrying a similar aryl moiety as the mace lignans.[16] Compounds **1** and **2**, with IC_{50} values of 8.5 and 2.9 μM, respectively, exhibited potent activities as LDL-antioxidants in the thiobarbituric acid-reactive substance (TBARS) assay.[12] Virolin (**3**), the methyl ether of **2** using diazomethane,[13] also exhibited potent LDL-antioxidant activity under the same conditions with an IC_{50} of 4.3 μM. Probucol (IC_{50} 1.3 μM) was used as a positive control substance in this assay.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. The IR spectra were measured with a JASCO IR report-100 infrared spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker DMX 600 (KBSI) and Bruker AMX 500 NMR spectrometers using TMS as internal reference. Low- and HREIMS were recorded on a VG high-resolution GC/MS (model, Autospec-Ultima) spectrometer. Silica gel 60 (Merck No. 5715 and 5744) and RP-18 (Merck No. 5385) precoated plates were used for thin-layer chromatography. Silica gel 60 (230−400 mesh, Merck No. 9385) was used for normal-phase column chromatography and LiChroprep RP-18 (40−63 μm, Merck No. 13900) for reversed-phase column chromatography.

Plant Material. The underground parts of *S. chinensis* were collected at an herbal garden (Sambufarm), Geochang City, Gyeongsang Province, Korea, in May 1998, and identified by B.-T.A. A voucher sample has been deposited at the Herbarium of the College of Pharmacy of Chungbuk National University (CPNU 3053).

Extraction and Isolation. The dried plant material (2 kg) was cut into small pieces and extracted repeatedly with 80% MeOH (3 × 6 L). The combined methanolic extracts were concentrated in vacuo, and the resulting aqueous suspension was partitioned with *n*-hexane, $CHCl_3$, EtOAc, and BuOH, successively. The $CHCl_3$ fraction (20 g) exhibited a potent low-density lipoprotein (LDL)-antioxidant effect and was chromatographed on Si gel (230−400 mesh, 600 g) using hexane with increasing concentrations of EtOAc in *n*-hexane to afford 2.0 g of an active fraction in the LDL-antioxidant assay (see below). The active fraction was further fractionated using reversed-phase column chromatography (40−63 μm, 40 g) eluting with 70% MeOH−H_2O to obtain 0.9 g of a further active fraction. The active constituents were finally purified by Si gel column chromatography eluting with a step gradient of $CHCl_3$−MeOH (100% $CHCl_3$, 99:1, 49:1) to obtain compounds **1** (10 mg) and **2** (15 mg). Virolin (**3**) was prepared by treatment of compound **2** with diazomethane.[13]

Saururin A (1): pale brown solid; $[\alpha]^{25}_D$ 0° (*c* 0.45, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 208 (3.15), 243 (2.90), 296 (2.87) nm; IR ($CHCl_3$) ν_{max} 3390, 2905, 1608, 1503 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.34 (2H, s, H-6, 6′), 6.29 (2H, s, H-3, 3′), 5.79 (4H, s, −O−CH_2−O−), 4.84 (2H, brs, OH), 2.50 (2H, dd, J = 5.5, 13.9 Hz, Ha-7, 7′), 2.26 (2H, dd, J = 8.4, 13.9 Hz, Hb-7, 7′), 1.72 (2H, m, H-8, 8′), 0.77 (6H, d, J = 6.6 Hz, H-9, 9′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 148.6 (s, C-2, 2′), 146.6 (s, C-4, 4′), 141.9 (s, C-5, 5′), 119.9 (s, C-1, 1′), 110.7 (d, C-6, 6′), 101.4 (t, −OCH_2−O−), 98.9 (d, C-3, 3′), 38.0 (d, C-8, 8′), 36.0 (t, C-7, 7′), 15.1 (q, C-9, 9′); EIMS *m*/*z* 358 $[M]^+$ (100), 340 (55), 152 (98), 122 (54), 93 (74), 77 (51); HREIMS *m*/*z* 358.1441 (calcd for $C_{20}H_{22}O_6$ 358.1416).

Machilin D (2): colorless oil; $[\alpha]^{25}_D$ −88° (*c* 4.0, $CHCl_3$) [lit. $[\alpha]^{25}_D$ 38.1° (*c* 0.07, $CHCl_3$)];[11] IR, ^{1}H NMR, ^{13}C NMR, and EIMS data, consistent with literature values.[10,11]

Machilin D methyl ether (virolin) (3): brown solid; $[\alpha]^{25}_D$ −32.0° (*c* 1.0, $CHCl_3$) [lit. $[\alpha]^{25}_D$ −29° (*c* 1.0, $CHCl_3$)];[13] IR, ^{1}H NMR, ^{13}C NMR, and EIMS data, consistent with literature values.[13]

Low-Density Lipoprotein Isolation and Oxidation Assay. Plasma was obtained from fasted healthy normalipidemic volunteers. Low-density lipoprotein (LDL) was isolated by a standard procedure with a slight modification.[17] Plasma was centrifuged at 43 800 rpm (100 000*g*) for 20 h at 4 °C in a Beckman T8-M ultracentrifuge. Chylomicron and very low-density lipoprotein (VLDL) floated to the top of the tube and were removed. Other infranatants were collected, adjusted to d = 1.063 g/mL with NaBr solution, and centrifuged at 43 800 rpm for 28 h at 4 °C. The top layer of LDL (1.006 < d < 1.063 g/mL) was collected and dialyzed at 4 °C with phosphate-buffered saline (PBS, 10 mM; pH 7.4). Dialyzed LDL was stored under argon at 4 °C and was used within 4 weeks. The TBARS assay of Buege and Aust[12] was used with a slight modification. Thus, a LDL solution (250 μL, 50−100 μg of protein) in BPS (10 mM, pH 7.4, 0.15 M NaCl) was supplemented with 10 μM $CuSO_4$. The oxidation was performed in a screw-capped 5 mL glass vial at 37 °C in a shaking water bath. After 4 h incubation, the reaction was terminated by addition of 1 mL of 20% trichloroacetic acid. Following precipitation, 1 mL of 0.67% TBA in 0.05 N NaOH was added and vortexed, and the final mixture was heated for 5 min at 95 °C, cooled on ice, and centrifuged for 2 min at 1000*g*. The optical density of the produced malondialdehyde (MDA) was measured at 532 nm. Calibration was done with a MDA standard prepared from tetramethoxypropane.[18]

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