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Chemical Constituents of *Morinda citrifolia* Fruits Inhibit Copper-Induced Low-Density Lipoprotein Oxidation

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The oxidative modification of low-density lipoprotein (LDL) plays an important role in the genesis of arteriosclerosis. The present study focused on the effects of the fruits of *Morinda citrifolia* on preventing arteriosclerosis. The MeOH extract and $CHCl_3$-, EtOAc-, *n*-BuOH-, and H_2O-soluble phases derived from the fruits of *M. citrifolia* were evaluated for their inhibitory activity on copper-induced LDL oxidation by the thiobarbituric acid-reactive substances (TBARS) method. The MeOH extract and EtOAc-soluble phase showed 88 and 96% inhibition, respectively. Six lignans were isolated by repeated column chromatography from the EtOAc-soluble phase. These compounds were determined by spectroscopic analysis to be 3,3′-bisdemethylpinoresinol (**1**), americanol A (**2**), americanin A (**3**), americanoic acid A (**4**), morindolin (**5**), and isoprincepin (**6**), of which **4** and **5** are novel compounds. These compounds inhibited copper-induced LDL oxidation in a dose-dependent manner. **1**, **2**, **5**, and **6** exhibited remarkably strong activities, which were the same or better than that of the known antioxidant 2,6-di-*tert*-butyl-*p*-cresol. The IC_{50} values for **1**, **2**, **5**, and **6** were 1.057, 2.447, 2.020, and 1.362 μM, respectively. The activity of these compounds is mainly due to their number of phenolic hydroxyl groups.

KEYWORDS: ***Morinda citrifolia*; Rubiaceae; noni; arteriosclerosis; low-density lipoprotein; lignan; spectroscopic analysis**

INTRODUCTION

Morinda citrifolia L. (Rubiaceae) is a small tree that grows widely across Polynesia. The common name is "noni" in the Hawaiian and Tahitian islands and "pace" in Java. The roots, barks, stems, leaves, and fruits have been used traditionally as a folk medicine for the treatment of many diseases including diabetes, hypertension, and cancer. The hypotensor activity of *M. citrifolia* roots was supported by its lowering effect on the blood pressure of dogs (*1*), and the anticancer activity of *M. citrifolia* fruits was supported by the prolonged life span of C57BL/6 mice infected with Lewis lung carcinoma (*2*). Furthermore, today "noni juice", which is made of the fruits of this plant, is widely drunk for the purported prevention of lifestyle-related diseases such as diabetes, hypertension, and cardiopathy and cerebral apoplexy caused by arteriosclerosis. In previous studies on the chemical constituents of *M. citrifolia*, several anthraquinones have been isolated from the roots (*3*), heartwoods (*4*), and flowers (*5*). Fatty acid derivatives were found in the seeds (*6*) and in the fruits (*7*). Iridoid glycosides were found in the fruits (*7*) and in the leaves (*8*). Flavonol glycosides were also found in the fruits (*7*), leaves (*8*), and flowers (*9*). In addition, sterol derivatives (*10*) in the leaves and volatile compounds (*11*) in the fruits have been reported. In the present study, we focused on the effects of the fruits of *M. citrifolia* on preventing arteriosclerosis. The development of arteriosclerosis is closely related to the oxidation of low-density lipoprotein (LDL) (*12*). LDL particles are susceptible to oxidation in the presence of free radicals and metal transition ions such as copper. Oxidatively modified LDL particles can be efficiently endocytosed by macrophages via scavenger receptors (*13*), thereby forming foam cells, which are the key component of the fatty streak lesions of arteriosclerosis. The extract of *M. citrifolia* leaves was tested previously for copper-induced LDL oxidative activity, which showed only 5% inhibition (*14*). However, the fruits of *M. citrifolia* had not been tested for such activity. We therefore investigated the inhibitory effect of *M. citrifolia* fruits used as noni juice on copper-induced LDL oxidation and now report the isolation and structural elucidation of biologically active compounds that inhibit copper-induced LDL oxidation from the fruits of *M. citrifolia*.

MATERIALS AND METHODS

General Procedures. Optical rotations were measured using a Jasco DIP-1000 digital polarimeter. CD spectra were recorded in MeOH on

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Table 1. ^{1}H NMR Spectroscopic Data for Compounds **1**–**6** in CD_3OD[a]

	1	2	3	4	5	6
H-2	6.81 (d, 2.1)	6.86 (d, 1.9)	6.87 (d, 1.9)	6.88 (d, 2.0)	6.82 (d, 1.8)	6.79 (d, 2.0)
H-5	6.75 (d, 8.1)	6.81 (d, 8.2)	6.82 (d, 8.0)	6.83 (d, 8.1)	6.75 (d, 8.3)	6.73 (d, 8.1)
H-6	6.67 (dd, 8.1, 2.1)	6.76 (dd, 8.2, 1.9)	6.78 (dd, 8.0, 1.9)	6.77 (dd, 8.1, 2.0)	6.72 (dd, 8.3, 1.8)	6.68 (dd, 8.1, 2.0)
H-7	4.62 (d, 4.4)	4.80 (d, 8.0)	4.85 (d, 8.1)	4.82 (d, 7.9)	5.51 (d, 6.0)	4.63 (d, 4.1)
H-8	3.06 (m)	3.99 (ddd, 8.0, 4.8, 2.6)	4.08 (ddd, 8.1, 4.5, 2.5)	4.04 (ddd, 7.9, 4.6, 2.5)	3.49 (br q, 6.2)	3.09 (m)
H-9	3.78 (dd, 9.1, 3.7)	3.47 (dd, 12.3, 4.8)	3.50 (dd, 12.4, 4.5)	3.49 (dd, 12.3, 4.6)	3.78 (dd, 11.2, 6.9)	3.82 (m)
	4.18 (dd, 9.1, 6.9)	3.67 (dd, 12.3, 2.6)	3.71 (dd, 12.4, 2.5)	3.70 (dd, 12.3, 2.5)	3.83 (dd, 11.2, 5.8)	4.21 (m)
H-2′	6.81 (d, 2.1)	6.95 (d, 1.9)	7.25 (d, 2.0)	7.14 (d, 2.0)	7.06 (d, 1.7)	6.92 (d, 2.2)
H-5′	6.75 (d, 8.1)	6.88 (d, 8.5)	7.03 (d, 8.3)	6.97 (d, 8.3)		6.93 (d, 8.5)
H-6′	6.67 (dd, 8.1, 2.1)	6.92 (dd, 8.5, 1.9)	7.23 (dd, 8.3, 2.0)	7.11 (dd, 8.3, 2.0)	6.97 (d, 1.7)	6.87 (dd, 8.5, 2.2)
H-7′	4.62 (d, 4.4)	6.48 (d, 15.8)	7.57 (d, 15.8)	7.56 (d, 15.9)	7.56 (d, 15.9)	4.69 (d, 4.7)
H-8′	3.06 (m)	6.20 (dt, 15.8, 5.8)	6.63 (dd, 15.8, 7.8)	6.30 (d, 15.9)	6.26 (d, 15.9)	3.09 (m)
H-9′	3.78 (dd, 9.1, 3.7)	4.18 (dd, 5.8, 1.5)	9.58 (d, 7.8)			3.82 (m)
	4.18 (dd, 9.1, 6.9)					4.21 (m)
H-2″						6.85 (d, 1.9)
H-5″						6.80 (d, 8.1)
H-6″						6.76 (dd, 8.1, 1.9)
H-7″						4.79 (d, 7.8)
H-8″						3.98 (ddd, 7.8, 4.9, 2.7)
H-9"						3.47 (dd, 12.3, 4.9)
						3.67 (dd, 12.3, 2.7)

[a] Coupling patterns and coupling constants (*J*) in hertz are given in parentheses.

a Jasco J-725 spectrometer. HR-FAB-MS and HR-EI-MS were performed with a JEOL JMS-BU 20 spectrometer. IR and UV spectra were measured on a Shimadzu FT-IR 8300 infrared spectrometer and a Hitachi U-3000 spectrometer, respectively. The NMR spectra were recorded in CD_3OD on a Bruker DPX-400 instrument. TLC was performed on precoated 70−230 mesh kieselgel 60F_{254}, RP-18 F_{254} TLC plates (Merck). Column chromatography was conducted with 70−230 mesh kieselgel 60 (Merck) and Sephadex LH-20 (Pharmacia). Medium-pressure liquid chromatography (MPLC) using a micropump KP-7 (Kusano Scientific Co., Tokyo, Japan) was carried out on an ODS C-18 CIG column (Kusano Scientific Co.).

Plant Materials and Chemicals. The fruits of *M. citrifolia* were purchased from JAMU factory in Jakarta, and the plant was identified by Dr. Asmanizar, University of Indonesia. The herbarium specimen has been deposited at the Botanical Museum of Kobe Gakuin University.

Trichloroacetic acid (TCA) and 2,6-di-*tert*-butyl-*p*-cresol (BHT) were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). 2-Thiobarbituric acid (TBA) and phosphate-buffered saline tablets were purchased from Sigma-Aldrich Co. (St. Louis, MO). LDL from human plasma was purchased from Funakoshi Co. (Tokyo, Japan). All other chemicals and solvents used in this study were of reagent or HPLC grade.

Extraction and Isolation. The dried fruits of *M. citrifolia* (1.3 kg) were extracted with hot MeOH (4 L × 6) for 6 h. The solvent was evaporated off under reduced pressure to yield the MeOH extract (89 g). The MeOH extract was suspended in a H_2O/MeOH (1:3, 1 L) mixture and was extracted successively with $CHCl_3$, EtOAc, and *n*-BuOH (each 1 L × 3). Each solvent was evaporated off under reduced pressure to yield $CHCl_3$ (44 g), EtOAc (3.5 g), *n*-BuOH (9.7 g), and H_2O (23 g) soluble phases. The EtOAc-soluble phase was chromatographed on Sephadex LH-20 (40 mm i.d. × 1000 mm) using MeOH to give fraction A (2.07 g), fraction B (520 mg), fraction C (460 mg), fraction D (260 mg), and fraction E (190 mg). Lignan-containing fractions (fractions B−D) were subjected repeatedly to RP-18 column chromatography using 25% MeOH and to silica gel column chromatography (22 mm i.d. × 100 mm) using $CHCl_3$/MeOH (4:1) and hexane/EtOAc (1:2). Compounds **1** (69 mg), **2** (21 mg), and **3** (12 mg) were obtained from fraction B, compounds **4** (39 mg) and **5** (10 mg) were obtained from fraction C, and compound **6** (14 mg) was obtained from fraction D.

Compound Identification. *3,3′-Bisdemethylpinoresinol (1):* pale yellow amorphous powder; $[\alpha]_D^{24}$ −3.4° (*c* 0.3, MeOH); HR-EI-MS, *m/z* $[M]^+$ 330.1071 (calcd for $C_{18}H_{18}O_6$ 330.1103); IR ν_{max}^{KBr} cm^{-1} 3400, 1605, 1520, 1445, 1285, 973, 864; UV λ_{max}^{MeOH} nm (log ϵ) 283 (3.86), 223 (4.12), 210 (4.40); CD $[\theta]_{196}$ +7910, $[\theta]_{227}$ −2970, $[\theta]_{247}$ −896; ^{1}H NMR (400 MHz, CD_3OD), **Table 1**; ^{13}C NMR (100 MHz, CD_3OD), **Table 2**.

Table 2. ^{13}C NMR Spectroscopic Data for Compounds **1**–**6** in CD_3OD

no.	1	2	3	4	5	6
1	133.83	129.58	129.13	129.26	134.59	133.86
2	114.47	115.55	115.57	115.60	113.93	114.48
3	145.94	146.61	147.28	147.14	146.93	146.07
4	146.30	147.10	148.16	147.17	146.44	146.44
5	116.27	116.37	116.41	116.45	116.26	116.26
6	118.91	120.41	120.46	120.50	118.63	118.88
7	87.34	77.59	77.57	77.53	89.50	87.43
8	55.15	80.04	80.50	80.28	55.08	55.28
9	72.49	62.11	61.95	62.00	64.86	72.68
1′	133.83	132.05	129.15	129.24	129.59	135.69
2′	114.47	115.60	118.15	117.46	117.83	115.78
3′	145.94	144.55	145.73	145.53	146.52	144.45
4′	146.30	145.28	146.70	146.62	142.66	145.28
5′	116.27	117.95	118.63	118.45	131.01	117.96
6′	118.91	120.84	123.69	123.21	116.80	120.21
7′	87.34	131.38	155.24	146.22	151.10	87.11
8′	55.15	128.16	127.81	117.19	116.03	55.44
9′	72.49	63.76	196.04	170.76	171.05	72.60
1″						129.59
2″						115.56
3″						146.65
4″						147.14
5″						116.36
6″						120.41
7″						77.64
8″						79.98
9″						62.13

Americanol A (2): pale yellow amorphous powder; $[\alpha]_D^{24}$ +24.0° (*c* 0.7, MeOH); HR-EI-MS, *m/z* $[M]^+$ 330.1113 (calcd for $C_{18}H_{18}O_6$, 330.1103); IR ν_{max}^{KBr} cm^{-1} 3400, 1606, 1585, 1506, 1441, 1273, 965, 816; UV λ_{max}^{MeOH} nm (log ϵ) 268 (4.10), 220 (4.41); CD $[\theta]_{196}$ +6221, $[\theta]_{226}$ −1156, $[\theta]_{234}$ +74; ^{1}H NMR (400 MHz, CD_3OD), **Table 1**; ^{13}C NMR (100 MHz, CD_3OD), **Table 2**.

Americanin A (3): pale yellow amorphous powder; $[\alpha]_D^{24}$ +35.0° (*c* 0.3, MeOH); HR-FAB-MS, *m/z* $[M+H]^+$ 329.0996 (calcd for $C_{18}H_{17}O_6$, 329.1025); IR ν_{max}^{KBr} cm^{-1} 3400, 1659, 1605, 1580, 1504, 1443, 1288, 970, 814; UV λ_{max}^{MeOH} nm (log ϵ) 333 (4.12), 313 (4.08), 290 (4.04), 224 (4.25), 204 (4.63); CD $[\theta]_{198}$ +7213, $[\theta]_{217}$ +1049, $[\theta]_{227}$ +1749, $[\theta]_{258}$ +131; ^{1}H NMR (400 MHz, CD_3OD), **Table 1**; ^{13}C NMR (100 MHz, CD_3OD), **Table 2**.

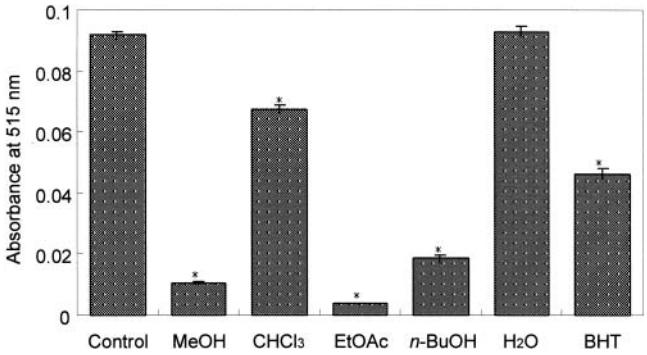


Figure 1. Inhibitory effects of the MeOH extract and derived soluble phases of *M. citrifolia* fruits on copper-induced LDL oxidation. LDL (100 μg/mL) was incubated for 6 h at 37 °C in PBS containig 25 μM $CuSO_4$ in the presence of each sample (50 μg/mL) and BHT (0.53 μg/mL). At the end of the incubation period TBARS were determined fluorometrically according to the method of Dousset et al. (*15*). Control (buffer), positive control (BHT, amount of IC_{50}). Values are means ± SEM of triplicate incubations ($n = 3$). Asterisks indicate significant difference compared to control at $p < 0.001$.

*Americanoic acid A (**4**):* pale yellow amorphous powder; $[\alpha]_D^{24}$ +26.2° (*c* 0.7, MeOH); HR-EI-MS, *m*/*z* $[M]^+$ 344.0923 (calcd for $C_{18}H_{16}O_7$, 344.0896); IR ν_{max}^{KBr} cm^{-1} 3400, 1676, 1633, 1606, 1583, 1507, 1437, 1272, 973, 815; UV λ_{max}^{MeOH} nm (log ϵ) 318 (4.10), 287 (4.18), 234 (4.24), 218 (4.33); CD $[\theta]_{197}$ +7645, $[\theta]_{227}$ −661, $[\theta]_{247}$ −115; ^{1}H NMR (400 MHz, CD_3OD), **Table 1**; ^{13}C NMR (100 MHz, CD_3OD), **Table 2**.

*Morindolin (**5**):* pale yellow amorphous powder; $[\alpha]_D^{24}$ +36.0° (*c* 0.3, MeOH). HR-EI-MS, *m*/*z* $[M]^+$ 344.0869 (calcd for $C_{18}H_{16}O_7$, 344.0896); IR ν_{max}^{KBr} cm^{-1} 3400, 1681, 1660, 1606, 1562, 1506, 1445, 1285, 979, 854; UV λ_{max}^{MeOH} nm (log ϵ) 324 (4.21), 224 (4.34), 209 (4.48), 206 (4.47); CD $[\theta]_{190}$ +4213, $[\theta]_{229}$ −2366, $[\theta]_{243}$ −443; ^{1}H NMR (400 MHz, CD_3OD), **Table 1**; ^{13}C NMR (100 MHz, CD_3OD), **Table 2**.

*Isoprincepin (**6**):* pale yellow amorphous powder; $[\alpha]_D^{26}$ +27.8° (*c* 0.4, MeOH); HR-FAB-MS, *m*/*z* $[M - H]^-$ 493.1476 (calcd for $C_{27}H_{25}O_9$, 493.1498); IR ν_{max}^{KBr} cm^{-1} 3400, 1608, 1508, 1445, 1282, 816; UV λ_{max}^{MeOH} nm (log ϵ) 283 (4.04), 210 (4.65); CD $[\theta]_{197}$ +11519, $[\theta]_{227}$ −2246, $[\theta]_{253}$ −1104, $[\theta]_{288}$ +279; ^{1}H NMR (400 MHz, CD_3OD), **Table 1**; ^{13}C NMR (100 MHz, CD_3OD), **Table 2**.

Copper-Induced Oxidation of LDL. LDL (100 μg/mL) was incubated in phosphate-buffered saline (pH 7.4, 1 mL) containing 25 μM $CuSO_4$, in the absence or presence of test samples for 6 h at 37 °C. After the incubation, the inhibition of LDL oxidation were measured as described below. BHT was used as a reference compound.

Evaluation of the Inhibitory Effects against LDL Oxidation. The extent of LDL oxidation was assessed using a modified method of the thiobarbituric acid (TBA) assay (*15*). TBARS reagent (1 mL) (15% TCA, 0.375% TBA, and 0.25 N hydrochloric acid) was added into the above reaction mixtures. The mixtures were heated in an oil bath for 30 min at 95 °C. After cooling, the absorbance of the pink chromophore was measured at 515 nm.

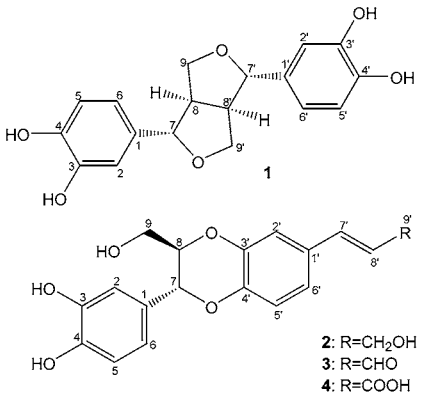
RESULTS AND DISCUSSION

The ability of the MeOH extract and $CHCl_3$-, EtOAc-, *n*-BuOH-, and H_2O-soluble phases obtained from the fruits of *M. citrifolia* to inhibit copper-mediated LDL oxidation was measured by using the TBARS method. As shown in **Figure 1**, the MeOH extract and the EtOAc- and *n*-BuOH-soluble phases exhibited remarkable activity. The most efficient EtOAc-soluble phase was then purified by repeated column chromatography using Sephadex LH-20, RP-18, and silica gel to afford six lignans (**1**−**6**) (**Figure 2**).

Compound **1** was obtained as a pale yellow amorphous powder. The molecular formula of **1** was determined by HR-EI-MS to be $C_{18}H_{18}O_6$. The ^{13}C NMR and DEPT spectra showed nine signals corresponding to one methylene, five methine, and three quaternary carbons. This represents half of the total number of carbon atoms estimated from the molecular formula, suggesting that compound **1** may consist of two identical units. The IR spectrum showed absorption bands assignable to a hydroxyl group (3400 cm^{-1}) and an aromatic ring (1605, 1520, and 1445 cm^{-1}). In the ^{1}H NMR spectrum, the presence of 1,3,4-trisubstituted phenyl groups was deduced from signals at δ 6.81 (d, $J = 2.1$ Hz), 6.75 (d, $J = 8.1$ Hz), and 6.67 (dd, $J = 8.1$, 2.1 Hz). Furthermore, we observed an oxygen-bearing methine proton (H-7) at δ 4.62 and methylene protons (H-9) at δ 3.78 and 4.18, which were coupled to a methine proton (H-8) at δ 3.06. From these observations, **1** was identified as (7*S*,7′*S*,8*R*,8′*R*)-7,9′:7′,9-diepoxylignan-3,3′,4,4′-tetraol (3,3′-bisdemethylpinoresinol), which has been reported as a metabolite of sesamin in rat bile (*16*).

Compounds **2** and **3** were deduced to have the molecular formulas $C_{18}H_{18}O_6$ and $C_{18}H_{16}O_6$ from HR-EI-MS, respectively. The ^{1}H NMR spectra of **2** and **3** resembled each other; the presence of two 1,3,4-trisubstituted phenyl groups and 1,4-dioxane ring linked to hydroxymethylene were observed. Differences between **2** and **3** were as follows: a trans double bond linked to hydroxymethylene was observed in **2** and a trans double bond linked to an aldehyde was observed in **3**. From these spectroscopic data, compound **2** was identified as americanol A (*17*) and compound **3** as americanin A (*18*), which were previously isolated from the seeds of *Phytolacca americana* L.

From HR-EI-MS, compound **4** was deduced to have the molecular formula $C_{18}H_{16}O_7$, which is 16 mass units higher than the molecular weight of **3**. The IR spectrum showed an absorption band due to a conjugated carboxylic acid (1676 cm^{-1}), together with the absorption bands observed in **2**. The ^{1}H and ^{13}C NMR spectra of **4** showed patterns similar to those of **3**, except that the aldehyde proton resonance was not present. In the ^{1}H NMR spectrum, the presence of two 1,3,4-trisubstituted phenyl groups was indicated from the two ABC coupling systems [δ 6.88 (d, $J = 2.0$ Hz), 6.83 (d, $J = 8.1$ Hz), and 6.77 (dd, $J = 8.1$, 2.0 Hz); δ 7.14 (d, $J = 2.0$ Hz), 6.97 (d, $J = 8.3$ Hz), and 7.11 (dd, $J = 8.3$, 2.0 Hz)]. Furthermore, the presence of a trans double bond was suggested from the ^{1}H resonances of δ 7.56 and 6.30 (d, $J = 15.9$ Hz). Moreover, we observed an oxygen-bearing methine proton signal (δ 4.04), which was coupled to an oxygen-bearing methine proton signal (δ 4.82) and hydroxymethylene proton signals (δ 3.49 and 3.70). These observations suggested the presence of the partial structures (a−d) shown in **Figure 3**. The proton−carbon long-range coupling correlations derived from the HMBC spectrum allowed us to determine the planar structure (**Figure 3**). The oxygen-bearing methine proton at δ 4.82 (H-7) was correlated with the 1,3,4-trisubstituted phenyl carbons at δ 115.60 (C-2) and 120.50 (C-6) and the aromatic carbon at δ 146.62 (C-4′). Furthermore, the aromatic carbon at δ 146.62 (C-4′) and the olefinic carbon at δ 146.22 (C-7′) were correlated with the 1,3,4-trisubstituted phenyl protons at δ 7.14 (H-2′) and 7.11 (H-6′). Further correlation was observed between the olefinic proton at δ 7.56 (H-7′) and the carboxyl carbon at δ 170.76 (C-9′). The combination of all these data identified a 1,4-benzodioxan-type lignan. From the coupling constant of $J_{7,8} = 7.9$ Hz, the relative configuration of C-7 and C-8 in the dioxane ring was trans. The absolute configuration of C-7 and C-8 was determined by the CD spectrum: the positive Cotton effect at 196 nm and the negative Cotton effect at 226 nm suggested a configuration of 7*R* and 8*R* (*19*). Consequently, the structure of **4** was determined



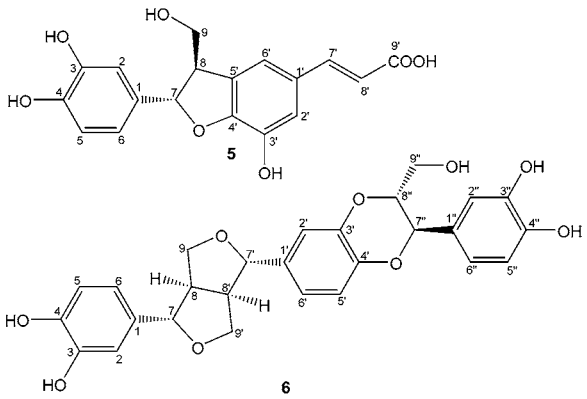


Figure 2. Chemical structures of compounds **1**–**6**.

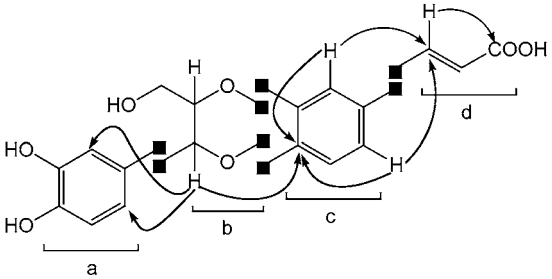


Figure 3. Partial structures (a–d) and significant HMBC correlation (arrows) for **4**.

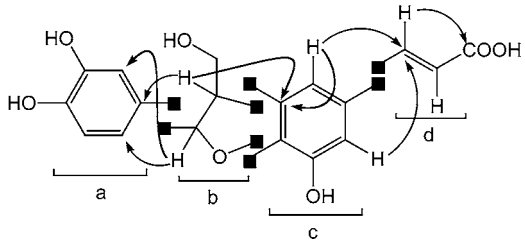


Figure 4. Partial structures (a–d) and significant HMBC correlation (arrows) for **5**.

as (7′*E*),(7*R*,8*R*)-3,4,9,-trihydroxy-4′,7-epoxy-8,3′-oxyneolign-7′-en-9′-oic acid, which is an oxidative derivative of **3** and has been named americanoic acid A.

Compound **5** was deduced to have the molecular formula $C_{18}H_{16}O_7$ from HR-EI-MS. The IR spectrum showed absorption bands due to a conjugated carboxylic acid (1681 cm^{-1}) and a trans double bond (1660 and 979 cm^{-1}), as well as the absorption bands observed in **1**. The ^1H NMR spectrum showed an ABC coupling system [δ 6.82 (d, J = 1.8 Hz), 6.75 (d, J = 8.3 Hz), and 6.72 (dd, J = 8.3, 1.8 Hz)] due to the 1,3,4-trisubstituted phenyl group and meta-coupled proton signals [δ 7.06 and 6.97 (d, J = 1.7 Hz)]. Furthermore, in the aliphatic region of **5**, we observed trans double bond signals at δ 7.56 and 6.26 (d, J = 15.9 Hz) and an oxygen-bearing methine signal at δ 5.51 and methylene signals at δ 3.78 and 3.83, which were coupled to a methine signal at δ 3.49. These observations suggested the presence of the partial structures (a−d) shown in **Figure 4**. Each partial structure was constructed by HMBC analysis (**Figure 4**). The trans double bond proton at δ 7.56 (H-7′) was correlated with the carboxyl carbon at δ 171.05 (C-9′); in addition, the meta-coupling protons at δ 7.06 (H-2′) and 6.97 (H-6′) were correlated with the trans double bond carbon at δ 151.10 (C-7′). Further correlations were observed between the oxygen-bearing methine proton at δ 5.51 (H-7) and the 1,3,4-trisubstituted phenyl carbons at δ 113.93 (C-2) and 118.63 (C-6), and between the methine proton at δ 3.49 (H-8) and the aromatic carbons at δ 134.59 (C-1) and 131.01 (C-5′). The combination of all these data identified a benzofuran-type lignan. From the coupling constant of $J_{7,8}$ = 6.0 Hz, the relative configuration of C-7 and C-8 in the furan ring was trans. The absolute configuration at the chiral centers C-7 and C-8 was deduced from the CD spectrum: the positive Cotton effect at 190 nm and the negative Cotton effect at 229 and 243 nm suggested a configuration of 7*S* and 8*R* (*20*). On the basis of the above evidence, the structure of **5** was characterized as (7′*E*),(7*S*,8*R*)-3,3′,4,9-tetrahydroxy-4′,7-epoxy-8,5′-neolign-7′-en-9′-oic acid, which is a new neolignan. We named compound **5** morindolin.

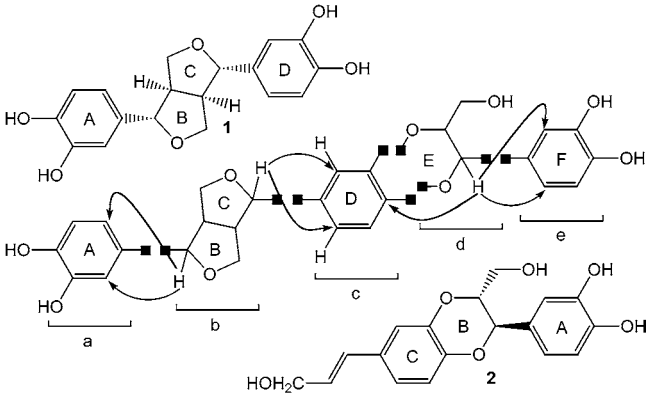


Figure 5. Partial structures (a–e) and significant HMBC correlation (arrows) for **6**.

Compound **6** was obtained as a pale yellow amorphous powder and was found to have the molecular formula $C_{27}H_{26}O_9$ from HR-negative-FAB-MS. In the ^1H NMR spectrum, three ABC coupling systems were observed [δ 6.79 (d, J = 2.0 Hz), 6.73 (d, J = 8.1 Hz), and 6.68 (dd, J = 8.1, 2.0 Hz); δ 6.92 (d, J = 2.2 Hz), 6.93 (d, J = 8.5 Hz), and 6.87 (dd, J = 8.5, 2.2 Hz); and δ 6.85 (d, J = 1.9 Hz), 6.80 (d, J = 8.1 Hz), and 6.76 (dd, J = 8.1, 1.9 Hz)], which indicated the presence of three 1,3,4-trisubstituted aromatic rings (partial structures a, c, and e). These findings suggested that **6** was a sesquineolignan. Furthermore, partial structures (b and d) in the aliphatic region were deduced with the help of ^1H, $^1H-^{13}C$, and ^1H-COSY spectra. To investigate the connectivities of the partial structures, an HMBC experiment was carried out (**Figure 5**). In the HMBC spectrum, the oxygen-bearing methine proton signal at δ 4.63 (H-7) was correlated with aromatic carbons at δ 114.48 (C-2) and 118.88 (C-6), whereas the other oxygen-bearing methine proton signal at δ 4.69 (H-7′) was correlated with aromatic carbons at δ 115.78 (C-2′) and 120.21 (C-6′). Further correla-

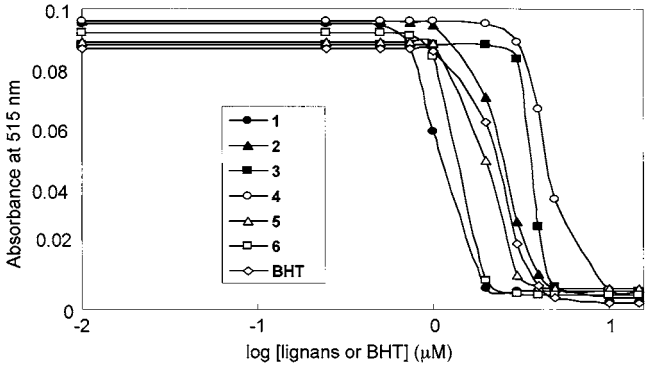


Figure 6. Concentration dependence of the inhibition of copper-induced LDL oxidation by lignans **1**–**6** and BHT. LDL (100 μg/mL) was incubated for 6 h at 37 °C in PBS containing 25 μM $CuSO_4$ in the presence of increasing concentrations of compounds **1**–**6** and BHT.

tions were observed between the oxygen-bearing methine proton signal at δ 4.79 (H-7″) and aromatic carbon signals at δ 145.28 (C-4′), 115.56 (C-2″), and 120.41 (C-6″). Moreover, cross-peaks were observed between the carbon signal at δ 145.28 (C-4′) and proton signals at δ 6.92 (H-2′) and 6.87 (H-6′). From these results, the planar structure of **6** was established, and **6** was deduced to be a sesquineolignan comprising pinoresinol-type and 1,4-benzodioxan-type moieties. This conclusion was supported by a comparison of ^{13}C NMR data for **1** and **2**. The carbon signals of the A-, B-, C-, and D-rings of **6** were quite close to those of **1**, and the carbon signals of the E- and F-rings of **6** were also similar to those of the B- and A-rings of **2**, respectively. Moreover, the relative stereochemistry at C-7 and C-8, C-7′ and C-8′, and C-7″ and C-8″ was deduced as trans from the coupling constants of 4.1, 4.7, and 7.8 Hz, respectively. Finally, the absolute configurations at the C-7, C-7′, C-7″, and C-8″ chiral centers were defined as *S*, *S*, *R*, and *R*, respectively, from the positive Cotton effect at 197 nm and the negative Cotton effects at 227 and 253 nm of the CD spectrum (*19*, *20*). Accordingly, the structure of **6** was characterized as (7*S*,7′*S*, 7″*R*,8*R*,8′*R*,8″*R*)-4′,7″:7,9′:7′,9-triepoxy-8″,3′-oxy-8,8′-sesquineolignan-3,3″,4,4″,9″-pentaol. This compound has been previously isolated from the seeds of *Joannesia princeps* Vellozo and has been named isoprincepin (*21*). However, the absolute configuration at the C-7″ and C-8″ positions was not previously determined by Waibel et al. (*21*).

Six lignans were isolated from the biologically active EtOAc-soluble phase. This appears to be the first report of lignans from the genus *Morinda* and the plant *M. citrifolia* (noni). These lignans were tested for their inhibitory activity against copper-induced LDL oxidation by measuring the decrease in TBARS formation. All lignans (**1**−**6**) showed a sigmoidal dose−response inhibition of LDL oxidation (**Figure 6**) and varied in their ability to inhibit oxidation in the order **1** > **6** > **5** > **2** > **3** > **4**. Compounds **1**, **2**, **5**, and **6** exhibited remarkably strong activity, which was the same or better than that of the known antioxidant BHT. The IC_{50} values for **1**, **2**, **5**,and **6** were 1.057, 2.447, 2.020, and 1.362 μM, respectively. The inhibitory activity of **1**−**6** was dependent on the number of phenolic hydroxyl groups. Furthermore, for the 1,4-benzodioxan-type lignans with two phenolic hydroxyl groups (**2**−**4**), the inhibitory activity decreased with the increasing degree of oxidation at C-9 (CH_2OH > CHO > COOH) (**Table 3**).

To our knowledge, the present work is the first to compare systematically lignans to BHT in their ability to inhibit the LDL oxidation process leading to arteriosclerosis. In summary, these observations suggest that the fruits of *M. citrifolia* are partially involved in the prevention of arteriosclerosis and that their active components are lignans.

Table 3. Inhibition of Copper-Induced LDL Oxidation by Compounds **1**–**6** and BHT

compound	no. of phenolic hydroxyl group	IC_{50} (μM)
3,3′-bisdemethylpinoresinol (**1**)	4	1.057 ± 0.06
americanol A (**2**)	2	2.447 ± 0.15
americanin A (**3**)	2	3.704 ± 0.21
americanoic acid A (**4**)	2	4.498 ± 0.23
morindolin (**5**)	3	2.020 ± 0.09
isoprincepin (**6**)	4	1.362 ± 0.09
BHT	1	2.382 ± 0.12

[a] Values are means ± SEM of triplicate determinations.

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