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Iridoid Glycosides Isolated from *Oldenlandia diffusa* Inhibit LDL-Oxidation

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An iridoid glycoside, oldenlandoside III (**5**) was isolated from the *n*-butanol fraction of methanol extracts of the aerial parts of *Oldenlandia diffusa* Roxb. along with six others previously characterized iridoid glycosides; geniposidic acid (**1**), scandoside (**2**), feretoside (**3**), 10-*O*-benzoylscandoside methyl ester (**4**), asperulosidic acid (**6**) and deacetylasperulosidic acid (**7**). Compounds **1**, **2**, and **7** inhibited LDL-oxidation, and showed 63.3±2.0, 62.2±1.6, and 63.8±1.5% inhibition, respectively, at a concentration of 20 μg/mL.

Key words: *Oldenlandia diffusa*, Rubiaceae, Iridoid, Oldenlandoside III, LDL-Oxidation inhibition

INTRODUCTION

Since the initial report showing that oxidized low-density lipoproteins (ox-LDLs) play an important role in the early stages of atherosclerosis (Steinberg *et al.*, 1989), many antioxidants have been developed and tested for their antiatherogenic activities, as measured by inhibition of foam cell formation in animal models (Bjorkhem *et al.*, 1991). Among the chemicals tested, both natural (vitamin E) and synthetic (probucol) antioxidants are known to lower lipid levels and reduce the incidence of coronary disease (Kita *et al.*, 1987; Carew *et al.*, 1987; Nagano *et al.*, 1992; Daugherty *et al.*, 1989; Sasahara *et al.*, 1994; Rimm *et al.*, 1993).

Oldenlandia diffusa Roxb. (Rubiaceae) is a common medicinal plant endogenous to India and China. (Ho *et al.*, 1986) and has also been classified as *Hedyotis diffusa* Willd. The aerial parts of this plant have been used as a treatment for cancer, as well as inflammations such as appendicitis, urethritis, and bronchitis, due to its antibacterial activity (Kim, 1995; Jung *et al.*, 1990). The chemical constituents of *O. diffusa* have been studied by a number of researchers. For example, iridoid glycosides including asperuloside, feretoside, asperulosidic acid, geniposidic acid, and scandoside (Nishihama *et al.*, 1981; Takagi *et al.*, 1982), as well as an anthraquinone (Ho *et al.*, 1986), have been isolated from *O. diffusa*. However, the effects of iridoid glycosides from *O. diffusa* on LDL-oxidation have not yet been reported. In this study, we report the inhibition of LDL-oxidation by iridoid glycosides from the aerial parts of *O. diffusa* and describe the structures of a new iridoid glycoside as well as six previously known iridoid glycosides.

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MATERIALS AND METHODS

Plant materials

Oldenlandia diffusa Roxb. plants were purchased at an oriental drug market in Seoul, Korea in 2003 and identifications were verified by Dr. Dae-Keun Kim, Woosuk University, Jeonju, Korea. A voucher specimen (KHU 03128) has been preserved at the Laboratory of Natural Products Chemistry, KyungHee University, Suwon, Korea.

Instrumentation

Melting points were determined on a Fisher-John apparatus and uncorrected. Optical rotations were measured

on a JASCO P-1010 digital polarimeter. GC was carried out on a GC-14B and FABMS were recorded on a JEOL JMSAX 505-WA. IR spectra were run on a Perkin Elmer Spectrum One FT-IR spectrometer. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were taken on a Varian Unity Inova AS 400 FT-NMR spectrometer. All reagent grade chemicals, such as $CuSO_4$, EDTA, trichloroacetic acid (TCA), thiobarbituric acid (TBA), and malondialdehyde bis(dimethyl acetal) (MDA), were purchased from Sigma-Aldrich Korea Ltd.

Isolation of iridoid glycosides from the aerial parts of *Oldenlandia diffusa* Roxb.

The dried and powdered aerial parts of *O. diffusa* (2.9 kg) were extracted three times at room temp. with 80% aqueous methanol (MeOH (20 L×3)). The extracts were partitioned with water (2 L), ethyl acetate (EtOAc (2 L×3)), and *n*-butanol (*n*-BuOH (2 L×3)), successively. The *n*-BuOH extract (83 g) was fractionated by silica gel column chromatography (c.c.) and eluted with $CHCl_3$-MeOH (10:1 → 7:1 → 5:1 → 3:1) and analyzed by thin layer chromatography (TLC) to produce twenty four fractions (ODB1 to ODB24). ODB11 (446 mg) was again subjected NH_2-silica gel c.c. and eluted with $CHCl_3$-MeOH (11:1) to isolate compound **4** {98 mg, Rf: 0.33 on silica gel TLC in $CHCl_3$: MeOH (5 : 1)}. ODB15 (5 g), which contained considerable amounts of iridoid glycosides, was fractionated by NH_2-silica gel c.c. and eluted with $CHCl_3$-MeOH (5:1) to give eleven fractions (ODB15-1 to ODB15-11). ODB15-2 (287 mg) was fractionated by NH_2-silica gel c.c. using $CHCl_3$-MeOH (7:1) as the eluent to produce compound **1** {41 mg, Rf: 0.27 on NH_2-silica gel TLC in $CHCl_3$: MeOH (3 : 1)}. ODB15-4 (163 mg) was fractionated on an octadecyl silica gel (ODS) column with MeOH-H_2O (1:10) as the eluent, and six fractions were collected (ODB15-4-1 to ODB15-4-6). ODB15-4-3 (41 mg) was fractionated by ODS c.c., eluted with MeOH-H_2O (1:5) to produce compound **3** {20 mg, Rf: 0.47 on ODS TLC in MeOH : H_2O (1 : 3)}. ODB16 (1.7 g) was fractionated by silica gel c.c., and eluted with $CHCl_3$-MeOH (3:1) to produce nine fractions (ODB16-1 to ODB16-9). ODB16-8 (171 mg) was purified by ODS c.c using MeOH-H_2O (1:2) as the eluent to yield compound **5** {35 mg, Rf: 0.34 on silica gel TLC in $CHCl_3$: MeOH (2 : 1)}. ODB16-8-1 (90 mg) was fractionated by silica gel c.c. and eluted with $CHCl_3$-MeOH-H_2O (7:3:1) to isolate compound **6** {30 mg, Rf: 0.46 on silica gel TLC in $CHCl_3$: MeOH : H_2O (6 : 4 : 1)}. ODB19 (3.2 g), containing iridoid glycosides was purified by silica gel c.c. using $CHCl_3$-MeOH-H_2O (7:3:1→6:4:1) as the eluent to yield compound **7** {50 mg, Rf: 0.16 on silica gel TLC in $CHCl_3$: MeOH : H_2O (6 : 4 : 1)}. ODB22 (3.5 g) was fractionated by silica gel c.c., and eluted with $CHCl_3$-MeOH-H_2O (6:4:1) to produce compound **2** {15 mg, Rf: 0.14 on silica gel TLC in $CHCl_3$: MeOH : H_2O (6 : 4 : 1)}. The physical characteristics of the seven compounds are as follows

Geniposidic aicd (1)

Amorphous powder (MeOH); $[\alpha]_D$ +19.3° (MeOH; *c*0.6); UV (MeOH) λ_{max} nm (log ε): 237 (3.64); IR (Films) ν_{max} cm^{-1}: 3500, 1680, 1630; ^{1}H-NMR (pyridine-d_5, 400 MHz, d): 7.69 (1H, *s*, H-3), 5.94 (1H, br *s*, H-7), 5.67 (1H, *d*, *J* = 7.1 Hz, H-1), 5.39 (1H, *d*, *J* = 8.0 Hz, H-1'), 4.77 (1H, *d*, *J* = 15.2 Hz, H-10a), 4.51 (1H, *d*, *J* = 15.2, H-10b), 3.32 (1H, *ddd*, *J* = 16.0, 7.4, 1.2 Hz, H-5), 3.01 (1H, *dd*, *J* = 7.4, 7.1 Hz, H-9), 2.87 (1H, br *dd*, *J* = 16.0, 8.4 Hz, H-6a), 2.16 (1H, m, H-6b); pos. FABMS *m/z*: 375 $[M+H]^+$, HR-FABMS *m/z* 375.1297 (Calculated for $C_{16}H_{23}O_{10}$, 375.1291).

Scandoside (2)

White powder (MeOH); m.p. 139-143; $[\alpha]_D$ -53.3° (H_2O, *c*0.6); UV (MeOH) λ_{max} nm (log ε): 235 (4.16); IR (Films) ν_{max} cm^{-1}: 3350, 1680, 1635; ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.31 (1H, s, H-3), 5.79 (1H, br s, H-7), 4.82 (1H, *d*, *J* = 7.2 Hz, H-1), 4.58 (1H, *d*, *J* = 7.6 Hz, H-1'), 4.51 (1H, m, H-6), 4.33 (1H, δ , *J* = 15.6, H-10a), 4.14 (1H, *d*, *J* = 15.6 Hz, H-10b), 2.87 (1H, *dd*, *J* = 7.5, 7.2 Hz, H-9), 2.77 (1H, *dd*, *J* = 7.5, 7.5 Hz, H-5); pos. FABMS *m/z*: 391$[M+H]^+$, HR-FABMS *m/z*: 391.1243 (Calculated for $C_{16}H_{23}O_{11}$, 391.1240).

Feretoside (3)

Amorphous powder (MeOH); $[\alpha]_D$ -34.8° (MeOH, *c*0.2); UV (MeOH) λ_{max} nm (log ε): 238 (3.89); IR (Films) ν_{max} cm^{-1}: 3450, 1695, 1635; ^{1}H-NMR (pyridine-d_5, 400 MHz): δ 7.68 (1H, s, H-3), 6.31 (1H, br s, H-7), 5.80 (1H, *d*, *J* = 5.8 Hz, H-1), 5.38 (1H, *d*, *J* = 8.4 Hz, H-1'), 4.92 (1H, br s, H-6), 4.78 (1H, *d*, *J* = 15.6, H-10a), 4.48 (1H, *d*, *J* = 15.6 Hz, H-10b), 3.48 (1H, *dd*, *J* = 7.2, 5.8 Hz, H-9), 3.44 (1H, *dd*, *J* = 7.2, 4.0 Hz, H-5); pos. FABMS *m/z*: 427 $[M+Na]^+$, HR-FABMS *m/z*: 427.1221 (Calculated for $C_{17}H_{24}O_{11}Na$, 427.1216).

10-*O*-Benzoylscandoside methyl ester (4)

Amorphous powder ($CHCl_3$-MeOH); $[\alpha]_D$ -22.5° (MeOH, *c*1.0); UV (MeOH) λ_{max} nm (log ε): 213 (3.60); IR (Films) ν_{max} cm^{-1}: 3375, 2875, 1705, 1630; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.97 (1H x 2, *d*, *J* = 7.8 Hz, H-2', 6'), 7.49 (1H, *dd*, *J* = 7.6, 7.6 Hz, H-4'), 7.43 (1H, s, H-3), 7.36 (1H x 2, *dd*, *J* = 7.8, 7.6 Hz, H-3', 5'), 5.80 (1H, br s, H-7), 5.40 (1H, *d*, *J* = 15.4 Hz, H-10a), 4.70 (1H, *d*, *J* = 7.6 Hz, H-1''), 4.67 (1H, *d*, *J* = 8.2 Hz, H-1), 4.60 (1H, *d*, *J* = 15.4 Hz, H-10b), 4.50 (1H, br s, H-6), 3.69 (3H, s, OCH_3), 2.88 (1H, *d*, *J* = 6.8 Hz, H-5), 2.83 (1H, *dd*, *J* = 8.2, 6.8 Hz, H-9); pos. FABMS *m/z*: 531 $[M+Na]^+$, HR-FABMS *m/z:* 531.1483 (Calculated for $C_{24}H_{28}O_{12}Na$, 531.1478).

Oldenlandoside III (5)

Amorphous powder (MeOH-H_2O); $[\alpha]_D$ +7.7° (MeOH-DMSO, *c*0.6); UV (MeOH) λ_{max} nm (log ε): 228 (3.15), 204 (3.11); IR (Fimls) ν_{max} cm^{-1}: 3369, 2919, 1716, 1643, 1548; ^{1}H-NMR (pyridine-d_5, 400 MHz): δ 8.20 (1H x 2, *d*, *J* = 7.6 Hz, H-2', 6'), 7.88 (1H, s, H-3), 7.70 (1H, *dd*, *J* = 7.4, 7.4 Hz, H-4'), 7.58 (1H x 2, *dd*, *J* = 7.6, 7.4 Hz, H-3', 5'), 6.04 (1H, br s, H-7), 5.52 (1H, *d*, *J* = 7.8 Hz, H-1), 5.36 (1H, *d*, *J* = 15.8 Hz, H-10a), 5.32 (1H, *d*, *J* = 15.8 Hz, H-10b), 5.23 (1H, *d*, *J* = 7.6 Hz, H-1"), 4.84 (1H, *d*, *J* = 6.8, H-1'"), 3.61 (1H, br *dd*, *J* = 15.0, 7.2 Hz, H-5), 3.24 (1H, *dd*, *J* = 15.0, 8.0, H-6a), 2.94 (1H, *dd*, *J* = 7.8, 7.2 Hz, H-9), 2.53 (1H, m, H-6b); ^{13}C-NMR (pyridine-d_5, 100 MHz): δ 175.2 (C-11), 166.8 (C-7'), 148.9 (C-3), 138.8 (C-8), 133.6 (C-4'), 131.2 (C-7), 130.7 (C-1'), 129.9 (C-2', 6'), 129.0 (C-3', 5'), 118.3 (C-4), 105.0 (C-1'"), 100.5 (C-1"), 97.7 (C-1), 77.7 (C-5"), 77.3 (C-3"), 74.5 (C-2"), 73.9 (C-3'"), 72.0 (C-2'"), 71.5 (C-4"), 69.4 (C-6"), 69.0 (C-4'"), 66.4 (C-5'"), 63.9 (C-10), 47.2 (C-9), 40.0 (C-6), 37.4 (C-5); pos. FABMS *m/z*: 611[M+H]$^+$, HR-FABMS *m/z*: 611.1980 (Calculated for $C_{28}H_{35}O_{15}$, 611.1976).

Asperulosidic acid (6)

Amorphous powder (MeOH-H_2O); $[\alpha]_D$ +22.7° (MeOH, *c*0.6); UV (MeOH) λ_{max} nm (log ε): 201.5 (3.80), 239.1 (4.00); IR (Films) ν_{max} cm^{-1}: 3368, 2927, 1737, 1634, 1371; ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.62 (1H, s, H-3), 6.01 (1H, br s, H-7), 5.05 (1H, *d*, *J* = 8.0 Hz, H-1), 4.93 (1H, *d*, *J* = 15.4 Hz, H-10a), 4.82 (1H, br s, H-6), 4.80 (1H, *d*, *J* = 15.4 Hz, H-10b), 4.72 (1H, *d*, *J* = 7.6 Hz, H-1'), 3.01 (1H, br *dd*, *J* = 8.0, 6.4 Hz, H-5), 2.62 (1H, *dd*, *J* = 8.0, 8.0 Hz, H-9), 2.08 (3H, s, Ac-Me); ^{13}C-NMR (CD_3OD, 100 MHz) δ 172.4 (CO-AcO), 171.2 (C-11), 154.8 (C-3), 145.8 (C-8), 131.8 (C-7), 108.3 (C-4), 101.1 (C-1), 100.5 (C-1'), 78.5 (C-5'), 77.8 (C-3'), 75.4 (C-6), 74.9 (C-2'), 71.5 (C-4'), 63.8 (C-10), 62.9 (C-6'), 46.3 (C-9), 42.6 (C-5), 20.8 (Ac-Me); pos. FABMS *m/z*: 433 [M+H]$^+$, HR-FABMS *m/z*: 433.1350 (Calculated for $C_{18}H_{25}O_{12}$, 433.1346).

Deacetylasperulosidic acid (7)

Amorphous powder (MeOH-pyridine); $[\alpha]_D$ +4.13° (MeOH, *c*0.6); UV (MeOH) λ_{max} nm (log ε): 202.0 (3.70), 233.0 (3.80); IR (Films) ν_{max} cm^{-1}: 3340, 2921, 1730, 1645, 1557, 1404; ^{1}H-NMR (D_2O, 400 MHz): δ 7.49 (1H, s, H-3), 5.99 (1H, br s, H-7), 4.91 (1H, *d*, *J* = 8.0 Hz, H-1), 4.82 (1H, br s, H-6), 4.80 (1H, *d*, *J* = 8.4 Hz, H-1'), 4.41 (1H, *d*, *J* = 15.4 Hz, H-10a), 4.23 (1H, *d*, *J* = 15.4 Hz, H-10b), 3.05 (1H, br *dd*, *J* = 8.0, 6.4 Hz, H-5), 2.59 (1H, *dd*, *J* = 8.0, 8.0 Hz, H-9); ^{13}C-NMR (D_2O, 100 MHz) δ 173.5 (C-11), 152.8 (C-3), 149.4 (C-8), 128.8 (C-7), 110.0 (C-4), 100.5 (C-1), 99.1 (C-1'), 76.3 (C-5'), 75.9 (C-3'), 74.5 (C-6), 73.0 (C-2'), 69.7 (C-4'), 60.8 (C-6'), 60.4 (C-10), 44.7 (C-9), 41.4 (C-5); pos. FABMS *m/z*: 391 (M+H$^+$), HR-FABMS *m/z*: 391.1245 (Calculated for $C_{16}H_{23}O_{11}$, 391.1240).

Acetylation of compound 5

Compound **5** (15 mg) was treated with acetic anhydride (5 mL) in pyridine (5 mL) in an ice bath and then stirred at room temperature overnight. The reaction solution was treated by standard methods to produce compound **5a**.

Oldenlandoside III hexaacetate (5a)

^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.04 (each 1H, *d*, *J* = 7.6 Hz, H-2', 6'), 7.55 (1H, *dd*, *J* = 7.6, 7.6 Hz, H-4'), 7.52 (1H, s, H-3), 7.44 (each 1H, *dd*, *J* = 7.6, 7.6 Hz, H-3', 5'), 5.93 (1H, br s, H-7), 5.25 (1H, *d*, *J* = 6.0 Hz, H-1), 4.98 (1H, *d*, *J* = 15.8 Hz, H-10a), 4.93 (1H, *d*, *J* = 15.8 Hz, H-10b), 4.88 (1H, *d*, *J* = 8.0 Hz, H-1"), 4.45 (1H, *d*, *J* = 7.2 Hz, H-1'"), 3.21 (1H, br *dd*, *J*=15.6, 7.2 Hz, H-5), 2.91 (1H, *dd*, *J* = 7.2, 6.4 Hz, H-9), 2.85 (1H, *dd*, *J* = 15.6, 8.0 Hz, H-6a), 2.53 (1H, m, H-6b), 2.08 (3H, s, Ac-Me), 2.01 (3H, s, Ac-Me), 2.00 (3H, s, Ac-Me), 1.99 (3H, s, Ac-Me), 1.97 (3H, s, Ac-Me), 1.95 (3H, s, Ac-Me); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 171.7 (C-11), 170.1, 170.1, 169.9, 169.3, 169.2, 169.0 (CO-AcO×6), 166.0 (C-7'), 152.7 (C-3), 137.0 (C-8), 133.0 (C-4'), 131.0 (C-7), 130.0 (C-1'), 129.6 (C-2', 6'), 128.4 (C-3', 5'), 100.7 (C-1'"), 96.5 (C-1"), 96.0 (C-1), 74.1 (C-3"), 72.5 (C-5"), 70.7 (C-2"), 69.9 (C-2'"), 69.1 (C-3'"), 68.8 (C-4"), 67.5 (C-4'"), 67.3 (C-6"), 63.0 (C-5'"), 62.3 (C-10), 46.8 (C-9), 38.4 (C-6), 33.8 (C-5), 21.0, 20.8, 20.7, 20.7, 20.5 (Ac-Me×6); pos. FABMS *m/z*: 863 [M+H]$^+$.

LDL-isolation and -oxidation activity assay

Plasma was obtained from fasted healthy normolipidemic volunteers. LDL was isolated by a standard procedure with the following minor modifications. Plasma was centrifuged at 43,800 rpm (100,000 g) at 4°C for 20 h in a Beckman T8-M ultracentrifuge. Chylomicra and very low-density lipoprotein particles (VLDL) floated to the top of the tube and were removed. Other infranatants were collected, adjusted to δ = 1.063 g/mL with NaBr solution, and centrifuged at 43,800 rpm at 4°C for 28 h. 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 (Buege *et al.*, 1978) was used with a slight modification. An LDL solution (250 μL, 50-100 mg 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 at 95°C for 5 min, cooled

on ice, and centrifuged for 2 min at 1,000 g. The optical density of the malondialdehyde (MDA) product was measured at 532 nm. Calibration was performed using an MDA standard prepared from tetramethoxypropane (Ahn *et al.*, 2001).

RESULTS AND DISCUSSION

The MeOH extract obtained from the aerial parts of *O. diffusa* plants was successively fractionated into H_2O, EtOAc, *n*-BuOH layers. The *n*-BuOH fraction was subjected to repetitive chromatography on silica gel, ODS and NH_2 silica gel columns. Six known compounds were recovered from these fractionations and were identified as geniposidic acid (**1**) (Takeda *et al.*, 1975; El-Naggar *et al.*, 1980), scandoside (**2**) (El-Naggar *et al.*, 1980), feretoside (**3**) (Inouye *et al.*, 1974; El-Naggar *et al.*, 1980; Otsuka *et al.*, 1991), 10-*O*-benzoylscandoside (**4**) (Otsuka *et al.*, 1991), asperulosidic acid (**6**) (Kamiya *et al.*, 2002), and desasperulosidic acid (**7**) (Kamiya *et al.*, 2002). Compound **4** had not been previously isolated from *O. diffusa*.

Compound **5** showed absorbance bands due to the hydroxy (3369 cm^{-1}), carbonyl (1716 cm^{-1}) and olefine (1643 cm^{-1}) groups in the IR spectrum and a molecular ion peak $[M+H]^+$ at *m/z* 611 in the positive FABMS spectrum. The NMR spectral features were similar to those of compound **4**, except for the absence of a methoxy group bonded to a carboxylic acid and a hydroxylated methine group, the presence of one methylene group {δ_H 3.24 (*d*, *J* = 6.8 Hz), δ_H 2.53 (m); δ_C 40.0}, and an α-L-arabinopyranose δ_H 4.84 (*d*, *J* = 6.8 Hz); δ_C 105.0, 73.9, 72.0, 69.0, 66.4) (Seo *et al.*, 1978). The position of the methylene and the arabinosyl unit was confirmed to be C-6 and C-6", respectively, by gHMBC analysis. The gHMBC spectra showed cross peaks between C-6 (δ_C 40.0) and H-7 (δ_H 6.04), between C-6 and H-5 (δ_H 3.61), and between C-6" (δ_C 69.4) and H-1"' (δ_H 4.84). Additionally, acetylation of compound **5** produced a hexaacetate form (**5a**), as indicated by NMR signals characteristic of six acetyl groups. Thus, compound **5** was determined to be a new compound, and was named oldenlandoside III.

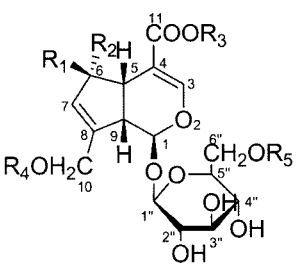
In addition, the iridoid glycosides with an oxygen atom in the R-groups at position C-6, such as compounds **2**, **3**, **4**, **6**, and **7**, showed a blue color when sprayed with H_2SO_4 and then heated on silica gel TLC, whereas the iridoid gylcosides with no oxygen in the C-6 substituents, as in compounds **1** and **5**, stained a dark brown color. This novel finding may be useful as a diagnostic tool to differentiate among glycosides.

Compounds **1-7** were examined for their ability to inhibit LDL-oxidation (Table I), since this is a standard test for the development of agents that may be useful in prevention of heart disease. Probucol, a synthetic antioxidant that has been commercially prescribed for the treatment of coronary disease incidence, was used as a positive control (Jeong *et al.*, 2004). Compounds **1**, **2**, and **7** exhibited similar levels of inhibition of LDL-oxidation, with values of 63.3 ±2.0, 62.2±1.6, and 63.8±1.5% inhibition at a concentration of 20 μg/mL. Probucol reduced LDL-oxidation by 78.1±2.8% under the same conditions (Table I). The other glycosides also showed significant inhibitory activity. Therefore, iridoid glycosides with the structural features common to compounds **1-7** may be inhibitors of LDL-oxidation. Moreover, it is rare for natural occurring components to display such high levels of inhibition of LDL-oxidation and these data suggest that the iridoid glycosides found in *O. diffusa* may be valuable agents in treatment of coronary disease.

Table I. Inhibition of LDL-oxidation by iridoid glycosides of *Oldenlandia diffusa* Roxb.

Iridoid glycoside	Inhibition of LDL-oxidation (20 μg/mL)*
Positive control (probucol)	78.1 ± 2.8 %
1	63.3 ± 2.0 %
2	62.2 ± 1.6 %
3	34.4 ± 1.2 %
4	50.8 ± 1.1 %
5	58.7 ± 1.3 %
6	55.8 ± 1.6 %
7	63.8 ± 1.5 %

The data are presented as mean ± standard deviation of three replications.
*Each compound was tested at this concentration.



	R_1	R_2	R_3	R_4	R_5
1	H	H	H	H	Glc
2	OH	H	H	H	Glc
3	OH	H	CH_3	H	Glc
4	OH	H	CH_3	benzoyl	Glc
5	H	H	H	benzoyl	Glc-Ara
5a	H	H	H	benzoyl	acetyl Glc-Ara
6	H	OH	H	Ac	Glc
7	H	OH	H	H	Glc

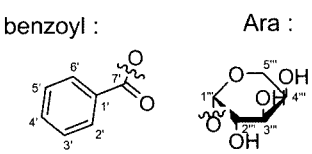


Fig. 1. Chemical structures of iridoid glycosides isolated from the aerial parts of *Oldenlandia diffusa* Roxb.

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