Arch Pharm Res Vol 27, No 6, 593-599, 2004

Archives of Pharmacal Research
http://apr.psk.or.kr

Isolation of Flavonoids and a Cerebroside from the Stem Bark of *Albizzia julibrissin*

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(Received April 19, 2004)

From the EtOAc fraction of the MeOH extract of *Albizzia julibrissin* (Leguminosae), a rare 5-deoxyflavone (geraldone, **1**), isookanin (**2**), luteolin (**3**), an isoflavone (daidzein, **4**), five prenylated flavonoids [sophoflavescenol (**5**), kurarinone (**6**), kurarinol (**7**), kuraridin (**8**) and kuraridinol (**9**)], a cerebroside (soya-cerebroside I, **10**), and (-)-syringaresinol-4-O-β-D-glucopyranoside (**11**) were isolated and characterized on the basis of spectral data. Compounds **2**, **3**, and **11**, showed 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity.

Key words: *Albizzia julibrissin*, Geraldone, Isookanin, Soya-cerebroside I, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radicals

INTRODUCTION

The dried stem bark of *Albizzia julibrissin* Durazz (Leguminosae) has been used to treat insomnia, diuresis, sthenia, ascaricide, and contusion in China, Japan, and Korea (Kim, 1996). In a previous study, we reported the antioxidative effect of *A. julibrissin* and active components such as sulfuretin and 3',4',7-trihydroxyflavone isolated from the EtOAc soluble fraction of this plant (Jung *et al.*, 2003). In our continuous study on this plant, we isolated geraldone (**1**), isookanin (**2**), luteolin (**3**), daidzein (**4**), five prenylated flavonoids [sophoflavescenol (**5**), kurarinone (**6**), kurarinol (**7**), kuraridin (**8**), and kuraridinol (**9**)], soya-cerebroside I (**10**), and (-)-syringaresinol-4-O-β-D-glucopyranoside (**11**). The present paper describes the structural characterization and scavenging activity on DPPH radicals of these compounds.

MATERIALS AND METHODS

General experimental procedures

The EI and GC mass data were recorded using a GC-MS QP-5050A spectrometer. The LR- and HR-FAB mass data were recorded using JMS-HX110A/HX110A Tandem mass spectrometer (JEOL). Optical rotations were recorded on a Perkin-Elmer 341 polarimeter. The ^{1}H- and ^{13}C-NMR spectra were measured using a JEOL JNM-ECP 400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) spectrometer. The chemical shifts were referenced to the respective residual solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD, δ_H 2.50 and δ_C 39.5 for DMSO-d_6, and δ_H 7.19, 7.55, and 8.17 and δ_C 123.5, 135.5, and 149.5 for pyridine-d_5). The DEPT, HMQC, HMBC, and COSY spectra were recorded on a JEOL JNM-EPC 400 using pulsed field gradients. Column chromatography was carried out using Si gel (Merck, 70-230 mesh), Sephadex LH-20 (Sigma, 25-100 μm), and RP-18 gel (Merck, 40-63 μm). The TLC was performed on a precoated Merck Kieselgel 60 F_{254} plate (0.25 mm) and a Merck RP-18 F_{254s} plate (5×10 cm). 50% H_2SO_4 was used as the spray reagent.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl and L-ascorbic acid were purchased from Sigma Chemical Company (St. Louis, MO, USA).

Plant materials

The stem bark of *Albizzia julibrissin* Durazz (Leguminosae) was purchased from the herbal medicine co-operative association in Busan Province, in August 2001. A voucher

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specimen (no. 20010820) was deposited at the Faculty of Food Science and Biotechnology, Pukyong National University.

Isolation of compounds 1-11

The stem bark (18.2 kg) of *A. julibrissin* was refluxed with MeOH for three hours (18 L×3). The total filtrate was concentrated to dryness *in vacuo* at 40°C to render the MeOH extract (2.97 kg), and this extract was suspended in distilled water and partitioned with CH_2Cl_2 (932 g), EtOAc (86 g), *n*-BuOH (650 g), and H_2O (1182 g) in sequence. The EtOAc (86 g) fraction was chromatographed on a Si gel column using CH_2Cl_2-MeOH (gradient) to yield 29 subfractions. Fractions 14-18 (8.5 g) were separately purified by Sephadex LH-20, with MeOH, to obtain compounds **1** (18 mg), **4** (40 mg), **5** (7 mg), and **8** (6 mg). The fraction 19 (14.6 g) was further chromatographed on a Sephadex LH-20 and RP-18 gel column with H_2O-MeOH (gradient) to give compounds **2** (20 mg), **3** (20 mg), and **6** (7 mg). Fraction 20 (17.5 g) was subjected to column chromatography over Sephadex LH-20 and RP-18, eluted with H_2O-MeOH (gradient) to afford 10 subfractions. Fraction 20-1 (6.4 g) was chromatographed on the Si gel column with CH_2Cl_2-MeOH-H_2O (12:1:0.1) to give compound **9** (10 mg). Fraction 20-4 (2.4 g) was further chromatographed on a Sephadex LH-20 and RP-18 gel column with H_2O-MeOH (gradient) to obtain compounds **7** (16 mg), **10** (120 mg), and **11** (50 mg), respectively.

Geraldone (1)

Amorphous yellow powder; UV λ_{max} (MeOH) (log ε) : 237 (4.16), 313 (sh. 4.04), 337 (4.14); +NaOMe : 254 (4.12), 319 (sh. 3.92), 3.70 (4.00); +$AlCl_3$: 237 (4.15), 315 (sh. 4.04), 337 (4.13); +$AlCl_3$+HCl : 236 (4.15), 315 (sh. 4.03), 337 (4.12), 408 (sh. 3.17); +NaOAc : 246 (4.11), 352 (4.08); +NaOAc+H_3BO_3 : 236 (4.15), 315 (sh. 4.03), 338 (4.12) nm; $C_{16}H_{12}O_5$; EIMS *m/z* (rel. int.) δ : 284 ($[M]^+$, 100), 256 ($[M\text{-}CO]^+$, 23.0), 253 ($[M\text{-}OMe]^+$, 7.3), 148 ($[B_1]^+$, 28.1), 137 ($[A_1+H]^+$, 54.8); ^{1}H-NMR (400 MHz, DMSO-d_6) δ : 7.86 (1H, d, *J* = 8.6 Hz, H-5), 7.54 (1H, d, *J* = 2.2 Hz, H-2'), 7.54 (1H, dd, *J* = 8.9, 2.2 Hz, H-6'), 6.99 (1H, d, *J* = 2.2 Hz, H-8), 6.93 (1H, d, *J* = 9.0 Hz, H-5'), 6.90 (1H, dd, *J* = 8.7, 2.2 Hz, H-6), 6.83 (1H, s, H-3), 3.89 (3H, s, OCH_3); ^{13}C-NMR (100 MHz, DMSO-d_6) δ : 176.3 (C-4), 162.6 (C-7), 162.3 (C-2), 157.4 (C-9), 150.2 (C-4'), 148.0 (C-3'), 126.4 (C-5), 122.1 (C-1'), 119.9 (C-6'), 116.1 (C-10), 115.7 (C-5'), 114.7 (C-6), 110.0 (C-2'), 104.8 (C-3), 102.5 (C-8), 55.9 (OCH_3).

Isookanin (2)

Amorphous orange powder; $[\alpha]_D^{20}$ -2° (*c* 0.10, MeOH); UV λ_{max} (MeOH) (log ε) : 233 (sh. 4.24), 287 (4.13), 375 (3.75); +NaOMe : 253 (4.04), 291 (sh. 3.77), 337 (4.20), 437 (3.57); +$AlCl_3$: 249 (3.99), 294 (sh. 3.71), 313 (3.83), 352 (sh. 3.19), 476 (2.77); +$AlCl_3$+HCl : 232 (sh. 4.24), 285 (4.11), 424 (3.80); +NaOAc : 232 (sh. 4.22), 258 (4.13), 288 (4.05), 332 (3.99), 379 (3.77); +NaOAc+H_3BO_3 : 243 (sh. 4.23), 297 (4.06), 308 (4.06), 396 (3.71) nm; ^{1}H-NMR (400 MHz, CD_3OD) δ : 7.29 (1H, d, *J* = 8.7 Hz, H-5), 6.98 (1H, d, *J* = 1.7 Hz, H-2'), 6.85 (1H, dd, *J* = 8.2, 1.7 Hz, H-6'), 6.78 (1H, d, *J* = 8.2 Hz, H-5'), 6.51 (1H, d, *J* = 8.7 Hz, H-6), 5.36 (1H, dd, *J* = 12.4, 3.0 Hz, H-2), 3.06 (1H, dd, *J* = 16.9, 12.4 Hz, H-3_{ax}), 2.72 (1H, dd, *J* = 16.9, 3.0 Hz, H-3_{eq}); ^{13}C-NMR (100 MHz, CD_3OD) δ : 194.8 (C-4), 154.8 (C-7), 153.5 (C-9), 147.7 (C-4'), 147.3 (C-3'), 134.8 (C-8), 132.7 (C-1'), 120.4 (C-6'), 120.1 (C-5), 117.0 (C-5'), 116.5 (C-10), 115.8 (C-2'), 111.7 (C-6), 82.4 (C-2), 45.9 (C-3).

Luteolin (3)

Amorphous yellow powder; UV λ_{max} (MeOH) (log ε) : 254 (4.20), 268 (sh. 4.16), 348 (4.24); +NaOMe : 230 (sh. 4.65), 266 (4.65), 328 (sh. 4.27), 403 (4.63); +$AlCl_3$: 273 (4.29), 300 (sh. 3.90), 424 (4.35); +$AlCl_3$+HCl : 267 (sh. 4.18), 275 (4.19), 294 (4.01), 360 (4.16), 390 (4.18); +NaOAc 269 (4.22), 360 (4.14); +NaOAc+H_3BO_3 : 260 (4.31), 371 (4.27) nm; ^{1}H-NMR (400 MHz, DMSO-d_6) δ : 12.97 (1H, s, OH-5), 10.80 (1H, s, OH-7), 9.90 (1H, s, OH-4'), 9.39 (1H, s, OH-3'), 7.41 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 7.39 (1H, d, *J* = 2.0 Hz, H-8), 6.88 (1H, d, *J* = 8.0 Hz, H-5'), 6.66 (1H, s, H-3), 6.43 (1H, d, *J* = 2.0 Hz, H-6), 6.18 (1H, d, *J* = 2.0 Hz, H-2'); ^{13}C-NMR (100 MHz, DMSO-d_6); 181.6 (C-4), 164.1 (C-2), 163.9 (C-7), 161.5 (C-5), 157.3 (C-9), 149.7 (C-4'), 145.7 (C-3'), 121.5 (C-6'), 119.0 (C-1'), 116.0 (C-5'), 113.4 (C-2'), 103.7 (C-10), 102.9 (C-3), 98.8 (C-6), 93.8 (C-8).

Daidzein (4)

Colorless needles; $C_{15}H_8O_4$; EIMS *m/z* (rel. int.) : 254 ($[M]^+$, 100), 137 ($[A_1+H]^+$, 56), 118 ($[B_1]^+$, 44); ^{1}H-NMR (400 MHz, CD_3OD) δ : 8.12 (1H, s, H-2), 8.05 (1H, d, *J* = 9.0 Hz, H-5), 7.36 (2H, d, *J* = 8.0 Hz, H-2', 6'), 6.93 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 6.84 (1H, d, *J* = 2.0 Hz, H-8), 6.83 (2H, d, *J* = 8.0 Hz, H-3', 5'); ^{13}C-NMR (100 MHz, CD_3OD) δ : 179.0 (C-4), 165.5 (C-7), 160.6 (C-9), 159.5 (C-4'), 155.4 (C-2), 132.2 (C-2', C-6'), 129.3 (C-5), 126.8 (C-1'), 125.1 (C-3), 119.0 (C-10), 117.3 (C-6), 117.0 (C-3', C-5'), 104. 0 (C-8).

Sophoflavescenol (5)

Amorphous yellow powder; ^{1}H-NMR (400 MHz, DMSO-d_6) δ : 10.58 (1H, s, OH-7), 9.99 (1H, s, OH-4'), 7.98 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.91 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.44 (1H, s, H-6), 5.18 (1H, t-like, H-2"), 3.80 (OCH_3), 3.46 (2H, brd, *J* = 6.7 Hz, H-1"), 1.75 (3H, s, H-5"), 1.62 (3H, s, H-4"); ^{13}C-NMR (100 MHz, DMSO-d_6) δ : 171.2 (C-4), 159.5

(C-7), 158.5 (C-4'), 157.9 (C-5), 155.4 (C-9), 141.8 (C-2), 136.8 (C-3), 130.9 (C-3"), 128.6 (C-2', 6'), 122.7 (C-2"), 122.3 (C-1'), 115.4 (C-3', 5'), 106.8 (C-8), 105.2 (C-10), 95.4 (C-6), 55.7 (OCH_3), 25.4 (C-4"), 21.5 (C-1"), 17.8 (C-5").

Kurarinone (6)

Amorphous orange powder; $[\alpha]_D^{20}$ +12° (*c* 0.10, MeOH); ^{1}H-NMR (400 MHz, DMSO-d_6) δ : 10.32 (1H, s, OH-7), 9.55 (1H, s, OH-2'), 9.30 (1H, s, OH-4'), 7.22 (1H, d, *J* = 8.4 Hz, H-6'), 6.33 (1H, d, *J* = 2.2 Hz, H-3'), 6.26 (1H, dd, *J* = 8.4, 2.2 Hz, H-5'), 6.12 (1H, s, H-6), 5.42 (1H, dd, *J* = 13.3, 2.4 Hz, H-2), 4.90 (1H, m, H-4"), 4.55 (1H, brs, H-9"), 4.48 (1H, d, *J* = 2.0 Hz, H-9"), 3.70 (3H, s, OCH_3), 2.81 (1H, dd, *J* = 16.5, 13.2 Hz, H-3_{ax}), 2.50 (2H, m, H-1"), 2.48 (1H, m, H-2"), 2.45 (1H, dd, *J* = 16.5, 2.5 Hz, H-3_{eq}), 1.95 (2H, m, H-3"), 1.58 (3H, s, H-10"), 1.53 (3H, s, H-7"), 1.43 (3H, s, H-6"); ^{13}C-NMR (100 MHz, DMSO-d_6) δ : 188.9 (C-4), 162.4 (C-5), 162.0 (C-7), 159.6 (C-9), 158.1 (C-4'), 155.2 (C-2'), 147.9 (C-8"), 130.6 (C-5"), 127.2 (C-6'), 123.4 (C-4"), 116.3 (C-1'), 110.7 (C-9"), 106.9 (C-8), 106.2 (C-5'), 104.3 (C-10), 102.3 (C-3'), 92.4 (C-6), 73.5 (C-2), 55.2 (OCH_3), 46.3 (C-2"), 44.3 (C-3), 30.7 (C-3"), 26.9 (C-1"), 25.5 (C-6"), 18.6 (C-10"), 17.5 (C-7").

Kurarinol (7)

Colorless needles; $[\alpha]_D^{20}$ -3° (*c* 0.012, MeOH); UV λ_{max} (MeOH) (log ε) : 223 (4.38), 287 (4.23), 317 (3.76), 386 (3.27); +NaOMe : 248 (4.03), 287 (3.79), 330 (4.39), 439 (3.37); +$AlCl_3$: 224 (4.35), 286 (4.20), 318 (3.73), 338 (3.23); +$AlCl_3$+HCl : 223 (4.35), 286 (4.19), 321 (3.69), 402 (3.19); +NaOAc : 222 (4.37), 287 (4.20), 324 (3.77), 389 (3.27); +NaOAc+H_3BO_3 : 223 (4.38), 287 (4.21), 319 (3.75), 390 (3.25) nm; HR-FABMS : $[M+H]^+$ Found *m/z* 457.2223; calc for $C_{26}H_{33}O_7$ 457.2226; ^{1}H-NMR (400 MHz, DMSO-d_6) δ : 7.21 (1H, d, *J* = 8.4 Hz, H-6'), 6.32 (1H, d, *J* = 2.3 Hz, H-3'), 6.25 (1H, dd, *J* = 8.4, 2.3 Hz, H-5'), 6.12 (1H, s, H-6), 5.41 (1H, dd, *J* = 13.2, 2.7 Hz, H-2), 4.56 and 4.48 (each 1H, brs, H-9"), 3.70 (3H, s, 5-OMe), 2.80 (1H, dd, *J* = 16.0, 13.2 Hz, H-3_{ax}), 2.50 (1H, m, H-3_{eq}), 2.49 (2H, m, H-1"), 2.30 (1H, m , H-2"), 1.56 (3H, s, H-10"), 1.23 (2H, m, H-3"), 1.00 (2H, m, H-4"), 0.95 (6H, s, H-6", 7"); ^{13}C-NMR (100 MHz, DMSO-d_6) δ : 188.9 (C-4), 162.4 (C-9), 162.0 (C-7), 159.5 (C-5), 158.0 (C-4'), 155.2 (C-2'), 148.1 (C-8"), 127.2 (C-6'), 116.3 (C-1'), 110.8 (C-9"), 107.0 (C-8), 106.2 (C-5'), 104.3 (C-10), 102.3 (C-3'), 92.4 (C-6), 73.5 (C-2), 68.6 (C-5"), 55.2 (OCH_3), 46.5 (C-2"), 41.4 (C-4"), 29.4 (C-6"), 28.9 (C-7"), 27.4 (C-1"), 26.4 (C-3"), 18.0 (C-10"); ^{1}H-NMR (400 MHz, CD_3OD) δ : 7.29 (1H, d, *J* = 8.3 Hz, H-6'), 6.33 (1H, dd, *J* = 8.3, 2.3 Hz, H-5'), 6.32 (1H, d, *J* = 2.3 Hz, H-3'), 6.10 (1H, s, H-6), 5.53 (1H, dd, *J* = 13.0, 2.3 Hz, H-2), 4.60 and 4.53 (2H, dd, *J* = 2.4, 1.3 Hz, H-9"), 3.79 (3H, s, OCH_3), 2.86 (1H, dd, *J* = 16.0, 13.0 Hz, H-3_{ax}), 2.69 (1H, dd, *J* = 16.0, 2.3 Hz, H-3_{eq}), 2.62 (2H, d, *J* = 7.0 Hz, H-1"), 2.39 (1H, m, H-2"), 1.63 (3H, s, H-10"), 1.38 (2H, m, H-3"), 1.28 (2H, m, H-4"), 1.06 (3H, s, H-6"), 1.05 (3H, s, H-7"); ^{13}C-NMR (100 MHz, CD_3OD) δ : 194.7 (C-4), 165.7 (C-8), 165.5 (C-9), 162.7 (C-5), 160.3 (C-4'), 157.4 (C-2'), 150.4 (C-8"), 129.3 (C-6'), 119.2 (C-1'), 112.4 (C-9"), 110.3 (C-7), 108.4 (C-5'), 106.6 (C-10), 104.2 (C-3'), 94.1 (C-6), 76.3 (C-2), 72.3 (C-5"), 56.7 (OCH_3), 49.2 (C-2"), 46.4 (C-3), 43.6 (C-4"), 30.0 (C-6"), 29.7 (C-7"), 29.5 (C-1"), 28.9 (C-3"), 19.5 (C-10").

Kuraridin (8)

Amorphous orange powder; $[\alpha]_D^{20}$ +1.2° (*c* 0.025, MeOH); UV λ_{max} (MeOH) (log ε) : 289 (3.92), 369 (4.19); +NaOMe : 332 (4.20), 456 (4.34); +$AlCl_3$: 292 (3.90), 390 (4.12); +$AlCl_3$+HCl : 295 (3.84), 399 (4.07); +NaOAc : 291 (3.93), 386 (4.19); +NaOAc+H_3BO_3 : 291 (3.93), 386 (4.19) nm; HR-FABMS : $[M+H]^+$ Found *m/z* 439.2121; calc for $C_{26}H_{31}O_6$ 439.2121; EIMS *m/z* (rel. int.) δ : 438 ($[M]^+$, 100), 315 ($[M-C_9H_{15}]^+$, 20), 288 ($[A_1]^+$, 17), 165 ($[A_1-C_9H_{15}]^+$, 79), 150 ($[B_1]^+$, 17); ^{1}H-NMR (400 MHz, CD_3OD) : 7.99 (1H, d, *J* = 15.7 Hz, H-β), 7.92 (1H, d, *J* = 15.7 Hz, H-α), 7.39 (1H, d, *J* = 8.2 Hz, H-6), 6.33 (1H, dd, *J* = 8.2, 2.4 Hz, H-5), 6.32 (1H, d, *J* = 2.4 Hz, H-3), 5.99 (1H, s, H-3'), 5.03 (1H, t, *J* = 7.0 Hz, H-4"), 4.55 (2H, m, H-9"), 3.88 (3H, s, OCH_3), 2.62 (1H, d, *J* = 2.7 Hz, H-1"a), 2.60 (1H, s, H-1"b), 2.54 (1H, m, H-2"), 2.05 (2H, t, *J* = 7.5 Hz, H-3"), 1.69 (3H, s, H-10"), 1.62 (3H, s, H-7"), 1.55 (3H, s, H-6"); ^{13}C-NMR (100 MHz, CD_3OD) δ : 195.6 (C=O), 167.4 (C-6'), 164.8 (C-4'), 163.2 (C-2'), 163.0 (C-4), 161.1 (C-2), 150.6 (C-8"), 140.6 (C-β), 132.6 (C-5"), 132.4 (C-6), 126.2 (C-α), 125.8 (C-4"), 117.0 (C-1), 112.0 (C-9"), 109.7 (C-5'), 109.6 (C-5), 107.3 (C-1'), 104.4 (C-3), 92.3 (C-3'), 56.8 (OCH_3), 48.8 (C-2"), 33.2 (C-3"), 28.9 (C-1"), 26.7 (C-7"), 19.8 (C-10"), 18.7 (C-6").

Kuraridinol (9)

Amorphous orange powder; ^{1}H-NMR (400 MHz, DMSO-d_6) δ : 14.84 (1H, s, OH-2'), 10.39 (1H, s, OH-4'), 10.18 (1H, s, OH-4), 9.93 (1H, s, OH-2), 7.94 (1H, d, *J* = 15.6 Hz, H-β), 7.85 (1H, d, *J* = 15.6 Hz, H-α), 7.43 (1H, d, *J* = 8.6 Hz, H-6), 6.37 (1H, d, *J* = 2.4 Hz, H-3), 6.32 (1H, dd, *J* = 8.5, 2.4 Hz, H-5), 6.03 (1H, s, H-5'), 4.56 (1H, s, H-9"), 4.47 (1H, d, *J* = 2.2 Hz, H-9"), 3.83 (3H, s, OCH_3), 2.55 (2H, m, H-1"), 2.35 (1H, m, H-2"), 1.64 (3H, s, H-10"), 1.35 (2H, m, H-3"), 1.19 (2H, m, H-4"), 1.02 (3H, s, H-6"), 1.01 (3H, s, H-7"); ^{13}C-NMR (100 MHz, DMSO-d_6) δ : 192.0 (C=O), 165.3 (C-4'), 162.6 (C-2'), 161.1 (C-4), 160.3 (C-6'), 159.0 (C-2), 148.0 (C-8"), 138.6 (C-β), 130.3 (C-6), 122.8 (C-α), 113.8 (C-1), 110.9 (C-9"), 108.1 (C-5), 106.8 (C-3'), 104.5 (C-1'), 102.6 (C-3), 90.7 (C-5'), 68.7 (C-5"), 55.5 (OCH_3), 46.4 (C-2"), 41.6 (C-4"), 29.5 (C-6"), 29.0 (C-7"), 27.1 (C-1"), 26.7 (C-3"), 17.9 (C-10").

Soya-cerebroside I (10)

Amorphous white powder; LR-FABMS : *m/z* 759 $[M+2Na]^+$, 736 $[M+Na]^+$, 482 [long-chain base+glucose+Na]$^+$; ^{1}H-NMR (400 MHz, pyridine-d_5) δ : 8.34 (1H, d, *J* = 8.7 Hz, NH), 5.98 (1H, dd, *J* = 15.4, 6.0 Hz, H-5), 5.90 (1H, dd, *J* = 15.8, 5.3 Hz, H-4), 5.48 (2H, t-like, H-8, 9), 4.90 (1H, d, *J* = 7.7 Hz, H-1"), 4.79 (1H, m, H-2), 4.75 (1H, t, *J* = 6.0 Hz, H-3), 4.69 (1H, dd, *J* = 10.5, 5.8 Hz, H-1b), 4.56 (1H, dd, *J* = 7.7, 3.6 Hz, H-2'), 4.50 (1H, dd, *J* = 11.7, 2.1 Hz, H-6"a), 4.34 (1H, dd, *J* = 11.7, 5.1 Hz, H-6"b), 4.22 (1H, dd, *J* = 10.4, 3.7 Hz, H-1a), 4.20 (2H, d, *J* = 9.1 Hz, H-3", 4"), 4.01 (1H, t, *J* = 8.1 Hz, H-2"), 3.89 (1H, m, H-5"), 2.13 (4H, m, H-6, 10), 1.99 (2H, m, H-7), 1.32 [brs, $(CH_2)_n$], 1.24 [brs, $(CH_2)_n$], 0.84 (6H, t-like, *J* = 6.5 Hz, H-18, 16'); ^{13}C-NMR (100 MHz, pyridine-d_5) δ : 175.6 (C-1'), 132.0 (C-5), 131.9 (C-4), 131.0 (C-9), 129.8 (C-8), 105.6 (C-1"), 78.5 (C-5"), 78.4 (C-3"), 75.1 (C-2"), 72.4 (C-2'), 72.2 (C-3), 71.5 (C-4"), 70.1 (C-1), 62.6 (C-6"), 54.5 (C-2), 35.6 (C-3'), 32.9 (C-7), 32.8-32.6 (C-6, 10), 32.0 (C-16, 14'), 29.5-29.9 (C-11-15, 4'-13'), 22.9 (C-17, 15'), 14.2 (C-18, 16').

Methanolysis of compound 10

Compound **10** (10 mg) was refluxed with 0.9 N HCl in 82 % aqueous MeOH (3 mL) for 18 h. The resulting solution was extracted with *n*-hexane, and the combined organic phase was dried over Na_2SO_4. Evaporation of the hexane yielded a fatty acid methyl ester. The H_2O layer was neutralized with NH_4OH and extracted with ether. The ether layer was dried over Na_2SO_4, filtered, and then concentrated to yield a long-chain base. The *n*-hexane layer was removed and analyzed by GC. The retention times (t_R) of the peaks were 24.958 min for fatty acid methyl ester. The fatty acid methyl ester was identified as 2-hydroxy hexadecanoic acid methyl ester.

[(-)-Syringaresinol-4-*O*-β-D-glucopyranoside] (11)

Amorphous powder; $[\alpha]_D^{20}$ -2° (*c* 0.10, MeOH); ^{1}H-NMR (400 MHz, CD_3OD) δ : 6.70 (2H, s, H-2, 6), 6.64 (2H, s, H-2', 6'), 4.84 (1H, s, H-1"), 4.75 (1H, d, *J* = 3.3 Hz, H-7), 4.70 (1H, d, *J* = 3.8 Hz, H-7'), 4.27 (2H, dd, *J* = 15.2, 6.5 Hz, H-9'), 3.90 (2H, dd, *J* = 9.0, 2.5 Hz, H-9), 3.84 (6H, s, OCH_3 ×2), 3.83 (6H, s, OCH_3 ×2), 3.76 (1H, dd, *J* = 12.2, 2.3 Hz, H-6"a), 3.65 (1H, dd, *J* = 11.9, 5.1 Hz, H-6"b), 3.47 (1H, m, H-2"), 3.41 (1H, d, *J* = 2.4 Hz, H-4"), 3.39 (1H, d, *J* = 2.4 Hz, H-3"), 3.19 (1H, m, H-5"), 3.12 (2H, brs, H-8, 8'); ^{13}C-NMR (100 MHz, CD_3OD) δ : 155.2 (C-3, 5), 150.1 (C-3', 5'), 140.3 (C-1), 137.0 (C-4'), 136.4 (C-4), 133.9 (C-1'), 106.1 (C-1"), 105.7 (C-2, 6), 105.3 (C-2', 6'), 88.4 (C-7'), 88.0 (C-7), 79.1 (C-5"), 78.6 (C-3"), 76.5 (C-2"), 73.7 (C-9), 73.6 (C-9'), 72.1 (C-4"), 63.4 (C-6"), 57.9 (OCH_3 ×2), 56.6 (OCH_3 ×2), 56.5 (C-8), 56.3 (C-8').

Measurement of DPPH radical scavenging activity

The DPPH radical scavenging effect was evaluated as previous described by Blois (Blois, 1958) with minor modifications. A hundred sixty microliters (μL) of MeOH solution of various sample concentrations was added to 40 μL DPPH methanol solution (1.5×10^{-4} M). After mixing gently and leaving for 30 min at room temperature, the optical density was measured at 520 nm using a microplate reader spectrophotometer VERSAmax (Molecular Devices, CA, USA). The antioxidant activity of each sample was expressed in terms of IC_{50} (μM required to inhibit DPPH radical formation by 50%) and calculated from the log-dose inhibition curve.

RESULTS AND DISCUSSION

The EtOAc-soluble fraction of the MeOH extract of the stem bark of *A. julibrissin* was repeatedly chromatographed over Si gel, Sephadex LH-20, and RP-18 to yield compounds **1~11** (Fig. 1). Among them, compounds **3~9** and **11** were identified as luteolin (**3**) (Jung *et al.*, 2001; Watanabe, 1999), daidzein (**4**) (Agrawal and Bansal, 1989), sophoflavescenol (**5**) (Woo *et al.*, 1998), kurarinone (**6**), kurarinol (**7**), kuraridin (**8**), kuraridinol (**9**) (Ryu *et al.*, 1997; Ryu *et al.*, 1996; Kyogoku *et al.*, 1973), and (-)-syringaresinol-4-*O*-β-D-glucopyranoside (**11**) (Kinjo *et al.*, 1991a; Higuchi *et al.*, 1992; Kinjo *et al.*, 1991b), respectively, by analysis of 1D and 2D NMR data including DEPT, COSY, HMQC, and HMBC techniques as well as by comparison with those of the literature values and/or by the direct comparison with the authentic samples. Compounds **3~9** are the first report from this plant.

Compound **1** was isolated as a yellow amorphous powder. The molecular formula of **1** was deduced as $C_{16}H_{12}O_5$ based on the NMR and EIMS spectral data. Its UV spectrum exhibited characteristic absorbance bands of flavones at 237 nm and 337 nm. The presence of a hydroxyl group at C-7 was suggested from the bathochromic shift of band II with NaOAc (Mabry *et al.*, 1970). The EIMS spectrum of **1** showed a molecular ion peak at *m/z* 284 and the *retro* Diels-Alder fragment ion peaks at *m/z* 137 $[A_1+H]^+$ and *m/z* 148 $[B_1]^+$ consistent with the presence of one hydroxyl group in ring-A, and both methoxy and hydroxyl groups in ring-B, respectively. The ^{1}H-NMR spectrum of **1** showed signals due to a methoxy group at δ 3.89 (3H, s), H-3 at δ 6.83 (1H, s), and two aromatic ABX coupled systems at δ 7.86 (1H, d, *J* = 8.6 Hz), 6.90 (1H, dd, *J* = 8.7, 2.2 Hz), and 6.99 (1H, d, *J* = 2.2 Hz) assigned to H-5, -6, and -8, and δ 7.54 (1H, d, *J* = 2.2 Hz), 7.54 (1H, dd, *J* = 8.9, 2.2 Hz), and 6.93 (1H, d, *J* = 9.0 Hz) assigned to H-2', -6', and -5', confirming the presence of mono substitution in ring-A and disubstitution in ring-B. The methoxy group at δ 3.89 was found to be

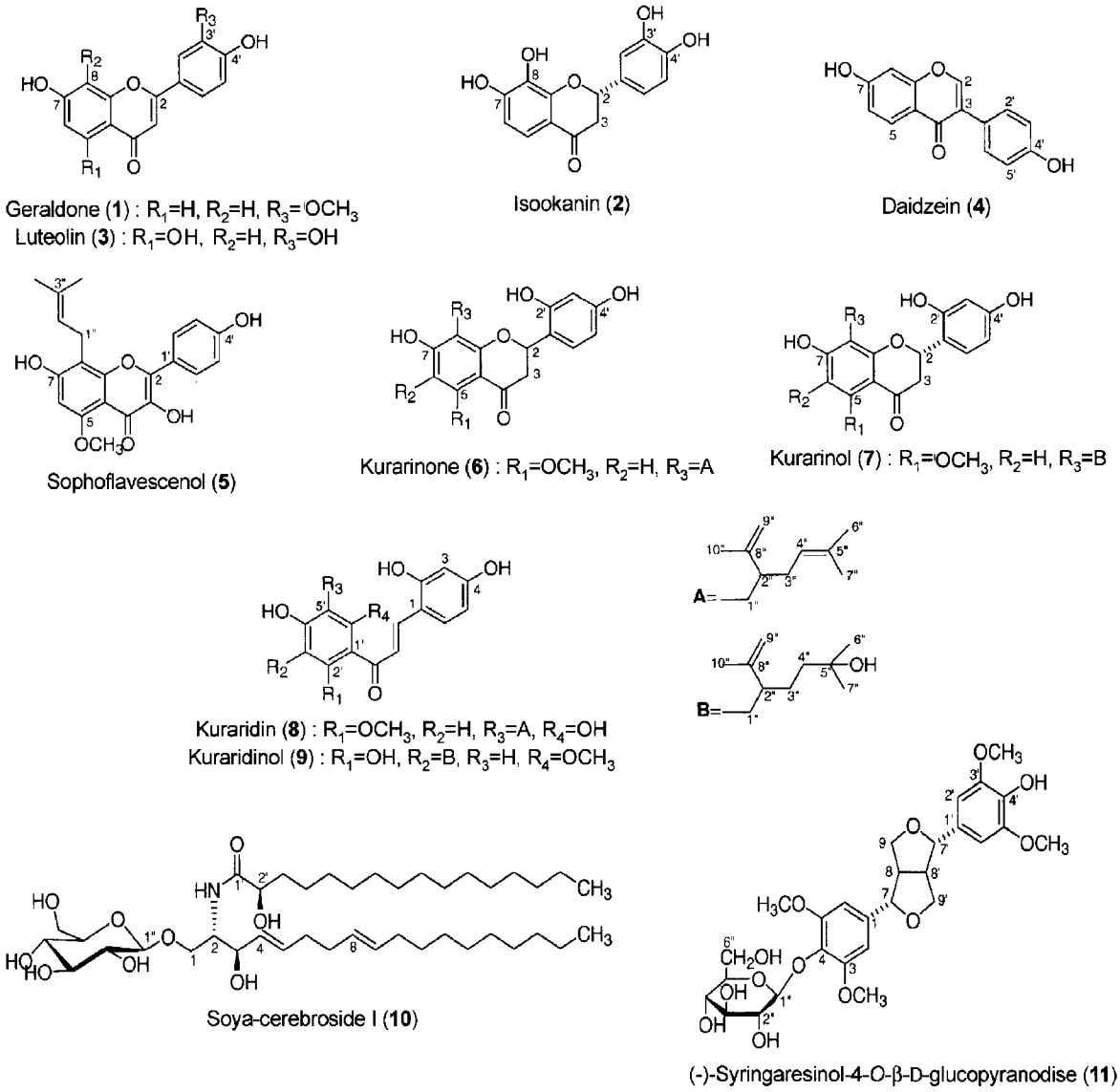

Fig. 1. Structures of compounds **1-11**

attached to C-3' according to long-range C-H coupling between OCH_3 and C-3' at δ 148.0 in the HMBC experiment. On the basis of the above evidences, compound **1** was determined as 3'-methoxy-7,4'-dihydroxyflavone (geraldone) and was further confirmed by the assignment of NMR data which was not previously reported, utilizing HMBC and HMQC experiments. Although geraldone has been previously isolated from *Trifolium subterraneum* L. (Wong and Francis, 1968), *Qualea parviflora, Q. grandiflora, Salvertia convallariodora, Vochysia tucanorum*, and *V. cinnamomea* (Lopes *et al.*, 1979), it is the first time to be isolated from *Albizzia* genus.

Compound **2** was obtained as an orange amorphous powder. The UV spectrum exhibited characteristic absorbance bands of flavanones at 233 (sh.) and 287 nm (Markham, 1982). Free hydroxyl groups at C-7 and 4' were suggested by the presence of shift of band II and I with NaOAc and NaOMe, respectively (Mabry *et al.*, 1970). The ^{1}H- and ^{13}C-NMR spectral data also exhibited characteristic signals for the ABX system at δ_H 2.72 (dd, J = 16.9, 3.0 Hz, H-3_{eq}) and 3.06 (dd, J = 16.9, 12.4 Hz, H-3_{ax}) and δ_H 5.36 (dd, J =12.4, 3.0 Hz, H-2), along with an oxygen bearing methine at δ_C 82.4 (C-2), a carbonyl group at δ_C 194.8 (C-4) and a methylene at δ_C 45.9 (C-3), assigned to positions 2 and 3 on a flavanone (Marby *et al.*, 1970). The chemical shift value at δ_C 194.8, corresponding to a carbonyl resonance, indicated the absence of the hydroxyl group in position 5 (Ngadjui *et al.*, 2002). In the aromatic region of the ^{1}H-NMR spectrum of **2**, two aromatic protons at δ 7.29 and 6.51 (each 1H, d, J = 8.7 Hz) were assigned to H-5 and -6 on the A-ring of flavanone skeleton, and one aromatic ABX spin coupled systems at δ 6.98 (1H, d, J = 1.7 Hz), 6.85 (1H, dd, J = 8.2, 1.7 Hz) and 6.78 (1H, d, J = 8.2 Hz) revealed the presence of H-2',

-6', and -5', respectively. Chemical shifts and signal patterns in the NMR spectra indicated that the hydroxy groups were at C-7, -8, -3', and -4'. On the basis of the above evidences and the comparison of data with those of literature (Foo, 1987; Clark-Lewis and Porter, 1972), compound **2** was determined as (-)3',4',7,8-tetrahydroxyflavanone (isookanin). This is the first report of its occurrence in *Albizzia* genus.

Compound **10** was obtained as an amorphous powder. The molecular formula was deduced as $C_{40}H_{75}NO_9$ based on a molecular ion at *m/z* 736 $[M+Na]^+$ in the FAB-MS as well as the ^{13}C-NMR and DEPT spectrum. The NMR data of **10** indicated the presence of β-D-glucose (δ_H 4.90, 1H, d, J = 7.7 Hz, anomeric H; δ_C 105.6), an amide linkage (δ_H 8.34, 1H, d, J = 8.7 Hz, N-H; δ_C 175.6) and two long chain aliphatic moieties which was essentially identical to those of cerebrosides from *Arisaema amurense* (Jung *et al.*, 1996), suggesting a sphingosine-type cerebroside nature (Jung *et al.*, 1996; Inagaki *et al.*, 1998). The positive FAB-MS specstrum of **10** showed an ion [long-chain base+glucose+Na]$^+$ peak at *m/z* 482 typical for amide bond cleavage in cerebrosides (Kang *et al.*, 2001; Inagaki *et al.*, 1998). Therefore, **10** was expected to be a sphingosine-type cerebroside having 2-hydroxypalmitic acid β-D-glucopyranose residue. The amide signal at δ 8.34 gave a cross peak with the H-2 multiplet signal at δ 4.79 in the ^{1}H-^{1}H COSY spectrum of **10**, which in turn showed cross peaks with methylene protons (H-1) at δ 4.22, 4.69, and δ 4.75 (H-3). The latter correlated with two olefinic proton signals at δ 5.98 (H-5) and 5.90 (H-4). The double bond at C-4 (5) was found to be *trans* (*E*), as evidenced by the large coupling constant (J = 15.4 Hz). These results were in good agreement with those of known (2*S*,3*R*,4*E*)-sphingosine-type cerebrosides (Jung *et al.*, 1996; Inagaki *et al.*, 1998), which were further supported by the ^{13}C-NMR data. The chemical shift of three methylene carbons (C-6, 7 and 10) adjacent to the olefinic carbons were observed at δ 32.0-33.0, supporting the *trans* (*E*) double bond at C-8 and 9 (Kang *et al.*, 2001; Inagaki *et al.*, 1998). The relative configurations of C-2, 3 and C-2' of **10** were established on the basis of ^{13}C-NMR data [δ 54.5 (C-2), 72.2 (C-3) and δ 72.4 (C-2')], which were in good agreement with those published for 2*S*,3*R*,2'*R* configuration (Jung *et al.*, 1996; Inagaki *et al.*, 1998). The methanolysis with HCl in MeOH of **10** yielded 2-hydroxy-hexadecanoic acid methyl ester, which was identified by the GC-MS analysis. In light of the above evidences, the structure of **10** was deduced to be 1-*O*-β-D-glucopyranosyl-(2*S*, 3*R*,4*E*,8*E*)-2-[(2*R*)-2-hydroxyhexadecanoylamino]-4,8-octadecadiene-1,3-diol. This compound was found to be identical with the known soya-cerebroside I, which has been previously isolated from *Phaseolus angularis* (Ohnishi and Fujino, 1981), soybean (Shibuya *et al.*, 1990), *Tetragonia tetragonoides* (Okuyama and Yamazaki, 1983), *Pisum sativum* (Ito *et al.*, 1985), *Acer negundo* (Inoue *et al.*, 1992), *Prunus jamasakura* (Yoshioka *et al.*, 1990), *Allium sativum* (Inagaki *et al.*, 1998), *Dimocarpus fumatus* (Voutquenne *et al.*, 1999), *Momordica charantia* (Xiao *et al.*, 2000), and *Trichosanthes kirilowii* (Kim *et al.*, 2001). This seems to be the first instance of the isolation of soya-cerebroside I from *Albizzia* genus.

The compounds **2** and **3** exhibited strong antioxidative activity on the DPPH radical with IC_{50} values of 3.89 and 1.70 μM, respectively. Their IC_{50} values were two times and four times lower than the IC_{50} value of 8.46 μM for L-ascorbic acid, respectively. Also compound **11** showed scavenging activity on the DPPH radical with IC_{50} value of 10.46 μM, while other compounds did not show scavenging activity on the DPPH radical.

ACKNOWLEDGEMENTS

The high resolution positive ion FAB-MS spectra were provided by the Korea Basic Science institute. This study was supported by a grant from the Ministry of Health & Welfare, Republic of Korea (01-PJ2-PG6-01NA01-0002).

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