Phenolic Glycosides from the Stem Bark of *Albizzia julibrissin*

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From the stem bark of *Albizzia julibrissin* DURAZZ (Leguminosae), two new phenolic glycosides (albibrissinosides A and B) were isolated. Their structures were determined by spectroscopic analysis. The albibrissinoside B was found to be a radical scavenger on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.

Key words phenolic glycoside; albibrissinoside A; albibrissinoside B; radical scavenger; 1,1-diphenyl-2-picrylhydrazyl radical; *Albizzia julibrissin*

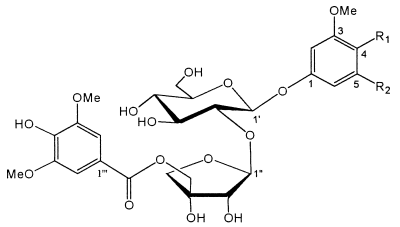
The dried stem bark of *Albizzia julibrissin* DURAZZ (Leguminosae) has been used in Chinese herbal medicine to treat insomnia, diuresis, sthenia, ascaricide, and confusion.[1)] Earlier phytochemical investigations on this species resulted in the isolation of saponins,[2—5)] lignans,[6—8)] phenolic glycosides,[6)] triterpenes,[9)] flavonoids and others.[10—15)] Previously, we reported that the methanolic extract of the stem bark of *A. julibrissin* exerts an radical scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.[16)] From this methanolic extract, 3′,4′,7-trihydroxyflavone, isookanin, luteolin and syringaresinol glucoside were isolated as the active principles.[17)] In a course of continuous work on this plant, two new phenolic glycosides named albibrissinosides A and B were isolated and characterized.

The dried methanolic extract of *A. julibrissin* was subjected to solvent partitioning between hexane, CH_2Cl_2, EtOAc, *n*-BuOH, and H_2O phases. Further chromatographic isolation was carried out with the EtOAc and *n*-BuOH fractions, which were found to be biologically active in the DPPH radical scavenging assay. The EtOAc fraction was subjected silica gel column chromatography to yield albibrissinoside A (**1**). The *n*-BuOH fraction was successive column chromatographed over silica gel, Sephadex LH-20, RP-18 and MCI gel to afford albibrissinoside B (**2**) that is also structurally related to **1**.

Compound **1** was isolated as an amorphous powder with a molecular formula of $C_{29}H_{38}O_{17}$ on the basis of a *pseudo*-molecular ion peak at *m*/*z* 681.2006 $[M+Na]^+$ in its high resolution (HR)-FAB mass spectrum. The UV spectrum showed absorption maximum at 276 nm for phenolic. The IR spectrum showed a characteristic absorption bands attributable to an ester carbonyl (1713 cm^{-1}), hydroxyl (3391 cm^{-1}), aromatic (1603, 1506 cm^{-1}) groups, as well as glycosidic linkage (1075 cm^{-1}), respectively. Acid hydrolysis of **1** afforded apiose and glucose as the sugar components identified on TLC analysis by comparison with authentic samples.

Absolute configurations for apiose and glucose were determined to be the D-form by their specific rotation showing +7.2° and +29°, respectively.[18,19)] The ^{1}H- and ^{13}C-NMR spectra (Table 1) revealed signals for a 1,3,4,5-tetrasubstituted phenolic acid with a glycosidic and a 1,3,4,5-tetrasubstituted benzoyl group. The ^{1}H-NMR signals at δ 6.26 (2H, s, H-2,6), 3.65 (6H, s, OMe-3,5), and 3.53 (3H, s, OMe-4) due to the aglycon, showed the characteristic pattern of a symmetrically 3,4,5-trimethoxy phenolic derivative.[6)] This was supported by the ^{13}C-NMR spectrum, which showed the signals for a tetrasubstituted phenolic moiety at δ 153.6 (C-1), 153.0 (C-3,5), 132.4 (C-4), and 94.0 (C-2,6). The ^{1}H-NMR spectrum also revealed the presence of two glycosyl moieties, two anomeric proton signals [δ 4.84 (1H, d, J=7.5 Hz) for glucose; 5.42 (1H, s) for apiose], the proton signals due to the sugar moieties between δ 3.12—5.42 including one symmetrically tetrasubstituted benzoyl group at δ 7.15 (2H, s, H-2‴ and H-6‴), and 3.77 (6H, OMe-3‴, 5‴), in close correspondence to the signals of authentic syringic acid. Furthermore, the coupling constants (J=7.5 Hz) of the anomeric proton signal of the D-glucosyl moiety as well as the chemical shift (δ 108.6) of the anomeric carbon of the D-apiosyl moiety, demonstrated that both sugar moieties have β-anomeric configurations.[20)] In ^{13}C-NMR spectrum, the downfield shift of a methylene carbon (C-5″ at δ 67.1) along with the upfield shift of a neighboring carbon (C-4″ at δ 73.9) of an apiose indicated that the syringoyl group is attached at C-5″ of the apiose.

Detailed analysis of the NMR data, aided by ^{1}H–^{1}H correlation spectroscopy (COSY), nuclear Overhauser enhancement exchange spectroscopy (NOESY), heteroatom multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) spectra and comparison with those reported for 3,4,5-trimethoxyphenol 1-*O*-β-D-apiofuranosyl(1→2)-β-D-glucopyranoside[6)] isolated from this plant, allowed establishment of the structure of **1**. The protonated carbons and their corresponding protons were unambiguously assigned by the HMQC experiment of **1**, and the connectivities of carbons in **1** were established by the HMBC experiments. In the HMBC spectrum, the important correlations between the syringoyl carbonyl group (δ 165.3) and the



	R_1	R_2
1	OMe	OMe
2	OH	H

Fig. 1

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Table 1. NMR Spectral Data of **1** and **2**

No.	**1**[a]		**2**[b]	
	^{13}C	^{1}H	^{13}C	^{1}H
1	153.6	—	153.1	—
2	94.0	6.26 s	103.7	6.60 d (2.6)
3	153.0	—	149.9	—
4	132.4	—	143.4	—
5	153.0	—	116.7	6.50 d (8.6)
6	94.0	6.26 s	110.1	6.39 dd (2.6, 8.6)
1′	99.2	4.84 d (7.5)	102.5	4.77 d (7.2)
2′	77.2	3.50 br d (7.0)	79.1	3.61 d (7.5)
3′	75.9	3.47 br d (9.0)	79.6	3.57 d (7.5)
4′	70.4	3.12 d (9.7)	72.5	3.34 br s
5′	77.2	3.34 d (7.5)	78.8	3.34 br s
6′	60.8	3.74 m	63.4	3.65 dd (12.2, 5.4), 3.84 dd (12.2, 2.0)
1″	108.6	5.42 s	111.3	5.50 s
2″	76.9	3.91 s	79.5	4.07 br s
3″	77.5	—	80.0	—
4″	73.9	3.85 d (9.4), 4.11 d (9.4)	76.1	3.90 m, 4.29 d (9.8)
5″	67.1	4.22 d (11.0), 4.27 d (11.0)	68.9	4.26 d (11.5), 4.42 d (11.5)
1‴	119.1	—	121.8	—
2‴, 6‴	107.1	7.15 s	109.2	7.24 s
3‴, 5‴	147.4	—	149.6	—
4‴	140.9	—	142.9	—
C=O	165.3	—	168.5	—
OMe-3	55.5	3.65 s	57.0	3.71 s
OMe-4	60.0	3.53 s	—	—
OMe-5	55.5	3.65 s	—	—
OMe-3‴, 5‴	56.1	3.77 s	57.7	3.84 s

a) DMSO-d_6, *b*) CD_3OD.

H-5″ signals and of C-2′ and H-1′ (δ 77.2, δ 5.42) revealed the syringoyl group to be at C-5″ of β-apiofuranosyl moiety and β-apiofuranosyl moiety at C-2′ of β-glucopyranosyl moiety. These spectral data established **1** to be the new natural compound 3,4,5-trimethoxyphenyl-1-*O*-β-D-(5-*O*-syringoyl)-apiofuranosyl-(1→2)-β-D-glucopyranoside, and was given a trivial name, albibrissinoside A.

Compound **2** was isolated as an amorphous powder with a molecular formula of $C_{27}H_{34}O_{16}$ on the basis of a pseudo-molecular ion peak at *m/z* 637.1745 $[M+Na]^+$ in its HR-FAB mass spectrum. Its IR and UV spectrum were almost identical to that of **1**, indicating their similar nature. The ^{1}H-NMR spectrum of **2** was very similar to that of **1** (Table 1), except for the absence of the proton signal corresponding to the C-5 methoxy group at δ 3.65 (3H), and the replacement of the methoxy group at the C-4 position in **1** by a hydroxyl group. The mass spectrum of **2**, which had a molecular ion peak 44 mass units less than that of **1**, further indicated the presence of a hydroxyl group at the C-4 position and the absence of methoxy group at the C-5 position in **2**. The methoxy group at δ 3.71 could be located at C-3 by a NOESY cross signal between OCH_3/H-2 and HMBC correlation between OCH_3 and C-3. Acid hydrolysis of **2** afforded apiose and glucose as the sugar components identified on TLC analysis by comparison with authentic samples. Detailed analysis of the NMR data, aided by ^{1}H–^{1}H COSY, NOESY, HMQC and HMBC spectra and comparison with **1**, allowed establishment of the structure of **2**. Thus the structure of **2** was elucidated as 4-hydroxy-3-methoxyphenyl-1-*O*-β-D-(5-*O*-syringoyl)-apiofuranosyl-(1→2)-β-D-glucopyranoside, and was given a trivial name, albibrissinoside B.

Albibrissinoside B showed a strong DPPH radical scavenging activity with an IC_{50} (50% inhibition concentration) value of 10.2 μM (positive control, L-ascorbic acid IC_{50} 10.4 μM), whereas albibrissinoside A showed a weak scavenging activity with an IC_{50} value of 253 μM.

Experimental

General ^{1}H- and ^{13}C-NMR spectra were determined on a JNM ECP-400 spectrometer using CD_3OD and DMSO-d_6 with tetramethylsilane (TMS) as an internal standard. HMQC and HMBC spectra were recorded using pulsed field gradients. FAB-MS, IR and optical rotation were taken with a JEOL JMS-HX110A/HX110A Tandem mass spectrometer, a Perkin-Elmer 2000 FT-IR spectrometer, and a Perkin-Elmer 341 polarimeter, respectively. Column chromatography was done with silica gel 60 (70—230 mesh, Merck, Germany), MCI gel (Mitsubishi Chem. Co., Japan), RP-18 Lichroprep (Merck), and Sephadex LH-20 (Sigma, St. Louis, MO, U.S.A.). TLC was carried out on precoated Merck Kieselgel 60 F_{254s} plates (0.25 mm) and RP-18 F_{254s} plates (Merck) and the spots were detected under UV light using 50% H_2SO_4 reagent. All the solvent for column chromatography was of a reagent grade from commercial sources.

Plant Material The stem bark of *A. julibrissin* was purchased from the herbal medicine cooperative association in Busan Province, Korea in August 2001. A voucher specimen (no. 20010820) has been deposited in author's laboratory (J. S. Choi).

Extraction and Isolation The stem bark (9.1 kg) of *A. julibrissin* was refluxed with MeOH (3×18 l) for 3 h. The extract (1.50 kg) was suspended in distilled water and partitioned with CH_2Cl_2 (465 g), EtOAc (43 g), *n*-BuOH (325 g), and H_2O (510 g) in sequence. The EtOAc fraction (43 g) was first chromatographed over the silica gel (1 kg) column (12 cm×1.5 m). The column was eluted using mixtures of CH_2Cl_2/MeOH under gradient conditions (10 : 1—1 : 1) to yield the 29 subfractions (F001—F029). The F021 and F022 (2.5 g) were subjected to column chromatography over RP-18 gel column with MeOH–H_2O (6 : 4 to MeOH) to give 12 subfractions (F030—F041). Compound **1** (20 mg) was obtained from the RP-18 column chromatography (50% MeOH) of F038 (0.8 g). The *n*-BuOH fraction (65 g) was first chromatographed over the silica gel (1.5 kg) column (12 cm×1.5 m). The column was eluted using mixture of CH_2Cl_2/MeOH under gradient conditions (10 : 1—1 : 1) to yield the 18 subfractions (F001—F018). The F005 (5.2 g) was subjected to column chromatography over MCI gel column with

MeOH–H_2O (0 : 100 to 100 : 0) to give 4 subfractions (F005-1 to F005-4). Compound **2** (15 mg) from F005-2 (2 g) was achieved by RP-18 column chromatography using a 30% MeOH, then purified by Sephadex LH-20 column chromatography with MeOH as a solvent, respectively.

Albibrissinoside A (**1**): Amorphous powder. $[\alpha]_D^{20}$ +3.70° (c=0.015, MeOH). IR ν_{max} cm^{-1}: 3391, 2942, 1713, 1603, 1506, 1464, 1426, 1336, 1228, 1126, 1075, 818, 764. UV λ_{max} (MeOH) nm (log ε): 276 (4.04). UV λ_{max} (MeOH+NaOMe): 285 (sh. 3.68), 327 (4.25). HR-FAB-MS m/z: 681.2006 (Calcd for $C_{29}H_{38}O_{17}Na$ m/z 681.2006). ^{1}H- and ^{13}C-NMR: see Table 1.

Albibrissinoside B (**2**): Amorphous powder, $[\alpha]_D^{20}$ +3.29° (c=0.002, MeOH). IR ν_{max} cm^{-1}: 3390, 2942, 1715, 1604, 1506, 1465, 1425, 1335, 1228, 1125, 1075, 818, 765. UV λ_{max} (MeOH) nm (log ε): 279 (4.40). UV λ_{max} (MeOH+NaOMe): 289 (sh. 3.89), 323 (4.14). HR-FAB-MS m/z: 637.1740 (Calcd for $C_{27}H_{34}O_{16}Na$ m/z 637.1745). ^{1}H- and ^{13}C-NMR: see Table 1.

Acidic Hydrolysis of 1 and 2 Compounds **1** and **2** (each 11 mg) were dissolved in 5% aqueous HCl (5 ml) and refluxed for 1 h, separately. The sugar components were identified by TLC on Si gel (developing solvent EtOAc/MeOH/H_2O/AcOH=13 : 3 : 3 : 4) as apiose (Rf 0.50) and glucose (Rf 0.30) in comparison with authentic samples. The reaction mixtures were also chromatographed on Si gel eluting with $CHCl_3$–MeOH–H_2O (6 : 4 : 1) to yield D-apiose ($[\alpha]_D^{20}$ +7.2°) and D-glucose ($[\alpha]_D^{20}$ +29°), respectively. Authentic D-apiose was obtained from cassitoroside, a naphthalene glycoside embracing D-apiofuranosyl unit through the same procedure as described above.[21]

DPPH Radical Scavenging Effect The DPPH radical scavenging effect was evaluated as previously described by Blois[22] with minor modifications. A methanolic sample solution of 160 μl at several concentrations and 40 μl of the DPPH methanolic solution (1.5×10^{-4} M) were added to a 96-well microplate, in a total volume of 200 μl. After standing the reaction mixture at room temperature for 30 min, its absorbance was determined at 520 nm, in a microplate reader (VERSA max, Molecular device, CA, U.S.A.).

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