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Isolation of Phenolics, Nucleosides, Saccharides and an Alkaloid from the root of *Aralia cordata*

Sook Kyung Hyun[1,2], Hyun Ah Jung[1], Byung-Sun Min[3], Jee H. Jung[4], and Jae Sue Choi[1,2,*]

[1]*Division of Food Science and Biotechnology, Pukyong National University, Busan 608-737, Korea*
[2]*Blue-Bio Industry RIC, Dongeui University, Busan 614-714, Korea*
[3]*College of Pharmacy, Catholic University of Daegu, Gyeongbuk 712-702, Korea*
[4]*College of Pharmacy, Pusan National University, Busan 609-735, Korea*

Abstract – Fourteen compounds were isolated from the *n*-BuOH fraction of the roots of *Aralia cordata* (syn. = *A. continentalis*). Through spectroscopic method, the chemical structures were elucidated as: caffeic acid (**1**), protocatechuic acid (**2**), thymidine (**3**), uridine (**4**), methyl-α-D-fructofuranoside (**5**), a mixture (3 : 1) of β-D-fructopyranoside and β-D-fructofuranoside (**6**), 1-methyl 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**7**), methyl-β-D-fructofuranoside (**8**), sucrose (**9**), 5-caffeoylquinic acid (chlorogenic acid) (**10**), 3-caffeoylquinic acid (neochlorogenic acid) (**11**), 4-caffeoylquinic acid (cryptochlorogenic acid) (**12**), 3,5-di-*O*-caffeoylquinic acid (**13**), and 1-kestose [β-D-fructofuranosyl-(2 → 1)-β-D-fructofuranosyl-(2 → 1)-α-D-glucopyranoside] (**14**). Among them, compounds **5**, **7**, **8**, and **10 - 14** were isolated from this plant for the first time.
Keywords – *Aralia cordata*; Araliaceae; Phenolic acid; Nucleoside; Alkaloid; Saccharide

Introduction

Aralia cordata (Araliaceae), known as 'Dokwhal' in Korea, has been widely used in traditional Chinese medicine for analgesia and neuralgia, and as a cure for arthralgia, rheumatism, lumbago, and lameness (Perry, 1980). In previous phytochemical investigations, various diterpenes, flavonoids, saponins, and essential oils were isolated from the roots and leaves of this plant (Kang, 1997; Jung *et al*., 2009). In biological studies, a few essential oils, phenolic acid, and diterpenoid isolated from the roots have shown to have antioxidant (Kim *et al*., 1995; 1998), anti-inflammatory (Han *et al*., 1983a; 1983b, 1985; Park *et al*., 2005), analgesic (Okuyama *et al*., 1991), sedative (Wang *et al*., 1988), antifungal (Jeong *et al*., 2006), anti-thrombotic (Han *et al*., 1986), anti-cancer (Kwon *et al*., 2008), and anti-Alzheimer activities (Jung *et al*., 2009).

However, limited studies on the polar fractions of this plant have been performed. Therefore, this works deals with the isolation and characterization of fourteen known compounds, including six phenolic acids (**1**, **2**, **10 - 13**), two nucleosides (**3**, **4**), one alkaloid (**7**), and five saccharides (**5**, **6**, **8**, **9**, **14**) from the *n*-BuOH fraction of the roots of *A. cordata*.

*Author for correspondence
Tel: +82-51-629-5845; E-mail: choijs@pknu.ac.kr

Experimental

General – Melting points were measured on a Mitamura-Riken apparatus (Tokyo, Japan), and are uncorrected. The EI-MS were recorded on a Hewlett-Packard 5989B spectrometer (Agilent Technologies, CA, USA) and a JEOL JMS-700 spectrometer (Tokyo, Japan). The FAB-MS was obtained in a 3-nitrobenzyl alcohol matrix in positive ion mode on a JEOL JMS-700 spectrometer (Tokyo, Japan). The ^{1}H- and ^{13}C-NMR spectra were determined using a JEOL JNM ECP-400 spectrometer (Tokyo, Japan) at 400 MHz in deuterated solvents (D_2O, MeOH-d_4, DMSO-d_6). The 2D NMR, including HMQC, HMBC, and COSY were recorded on a JEOL JNM ECP-400 spectrometer using pulsed field gradients. Column chromatography was conducted using silica gel 60 (70-230 mesh, Merck, Darmstadt, Germany), Sephadex LH-20 (20 - 100 μm, Sigma, St. Louis, MO, USA), and MCI-gel CHP20P (75 - 150 μm, Mitsubishi Chemical Co., Tokyo, Japan), and Lichroprep® RP-18 (40 - 63 μm, Merck, Darmstadt, Germany). TLC was conducted on pre-coated Merck Kieselgel 60 F_{254} plates (20 × 20 cm, 0.25 mm), and RP-18 F_{254s} plates (5 × 10 cm, Merck, Darmstadt, Germany), using 50% H_2SO_4 as a

spray reagent.

Plant material – The root of *A. cordata* was purchased from Omni Herb Co. (Daegu, Korea), and authenticated by Prof. J. H. Lee (Dongguk University, Gyeongju, Korea). A voucher specimen (no. 20080320) was deposited in the laboratory of Prof. J. S. Choi (Pukyong National University, Busan, Korea).

Extraction, fractionation, and isolation – The roots of *A. cordata* (12 kg) were refluxed with MeOH for 3 hr, and the filtrates were concentrated to dryness *in vacuo* at 40 °C to render the MeOH extract. The MeOH extract was suspended in distilled H_2O and partitioned with, *n*-hexane, CH_2Cl_2, EtOAc, *n*-BuOH and H_2O in sequence, thus yielding five fractions. The *n*-BuOH fraction (160 g) obtained from the roots of *A. cordata* was then chromatographed over a silica gel column using CH_2Cl_2-MeOH (gradient), and generated 15 subfractions (Fr. 1 to 15). Fractions 3 (9.60 g) was chromatograped on a silica gel column using CH_2Cl_2-MeOH-H_2O (15 : 1 : 0.1) to yield 4 subfractions (Fr. 3-1 to 3-4). Fraction 3-4 (8.62 g) was chromatograped on a Sephadex LH-20 and RP-18 gel column with H_2O-MeOH (gradient) to obtain **1** (caffeic acid, 10 mg), **2** (protocatechuic acid, 200 mg), **3** (thymidine, 20 mg), **4** (uridine, 20 mg), and **5** (methyl-α-D-fructofuranoside, 210 mg), respectively. Fraction 7 (12.2 g) was continually separated by MCI-gel CHP20P column chromatography using mixed solvent (H_2O and MeOH, gradient) to give 4 subfractions (Fr. 7-1 to 7-4). Fraction 7-1 (8.49 g) was recrystallized using MeOH to yield **6** (a mixture (3 : 1) of β-D-fructopyranoside and β-D-fructofuranoside, 100 mg). Fraction 7-2 (2.55 g) was subjected to column chromatography on a silica gel column, with gradient elution using a CH_2Cl_2-MeOH-H_2O (10 : 1 : 0.1, gradient to MeOH), generating **7** (1-methyl 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, 6.5 mg). Fraction 8 (24. 6 g) was chromatograped on a MCI gel eluted with 40% MeOH to obtain **8** (methyl β-D-fructofuranoside, 30 mg). Fractions 12 and 13 (14.8 g) subjected to column chromatography on a MCI gel column, using H_2O as the eluent, to generate **9** (sucrose, 100 mg), **13** (3,5-di-*O*-caffeoylquinic acid, 10 mg), and **14** (l-kestose, 260 mg). Fractions 12 and 13 were consecutively purified, using a HPLC [(column: symmetry C18 (4.6 × 250 mm, 5 μm, USA); flow rate: 1 ml/min; detector: UV (327 nm)], eluted with 0.1% phosphoric acid-CH_3CN (89 : 11) to afford **10** (chlorogenic acid, 500 mg, t_R 9.842 min), **11** (neochlorogenic acid, 10 mg, t_R 6.092 min), and **12** (cryptochlorogenic acid, 10 mg, t_R 11.717 min). The structures of compounds **5**, **7**, **8**, and **10 - 14** are shown in Fig. 1.

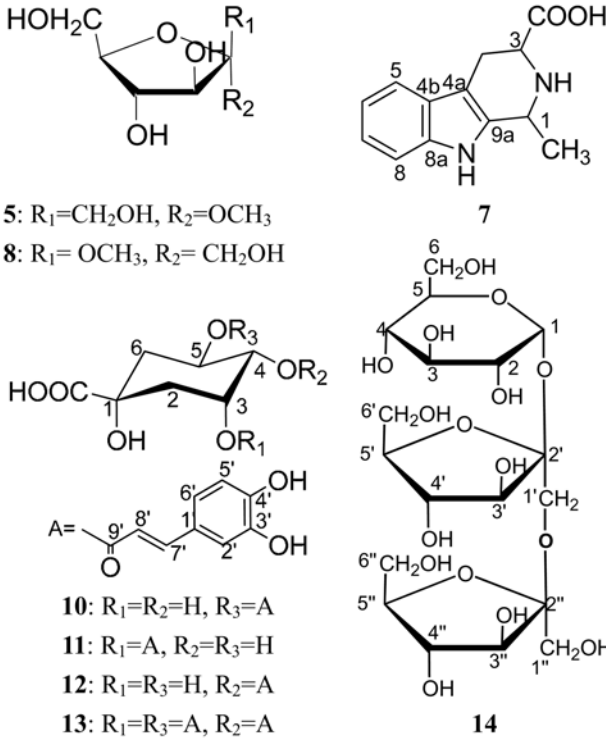

Fig. 1. Structures of compounds isolated from the roots of *A. cordata*.

Methyl-α-D-fructofuranoside (5) – white amorphous powder; ^{1}H-NMR (400 MHz, D_2O): δ 3.96 (1H, d, *J* = 2.8 Hz, H-3), 3.82 (2H, m, H-4/H-5), 3.69 (1H, dd, *J* = 12.0, 1.8 Hz, H-6b), 3.66 (1H, d, *J* = 12.0 Hz, H-1b), 3.56 (1H, dd, *J* = 12.0, 5.1 Hz, H-6a), 3.52 (1H, d, *J* = 12.0 Hz, H-1a), 3.12 (3H, s, OCH_3); ^{13}C-NMR (100 MHz, D_2O) δ: 108.4 (C-2), 83.5 (C-5), 80.2 (C-3), 77.5 (C-4), 61.4 (C-6), 57.7 (C-1), 48.3 (OCH_3).

1-Methyl 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (7) – amorphous powder; mp 290 - 292 °C; $[\alpha]_D^{20}$ –104° (*c* 0.05, 50% pyridine); IR 3297 (NH), 1645 (COOH), 1578, 741 (aromatic) cm^{-1}; EIMS *m/z* (relative intensity): 230 [M, 72.6]$^+$, 215 [M – CH_3, 57.6]$^+$, 143 [RDA fragment, 18.6]$^+$; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 11.12 (1H, s, COOH), 7.44 (1H, d, *J* = 8.0 Hz, H-5), 7.34 (1H, d, *J* = 7.5 Hz, H-8), 7.08 (1H, dd, *J* = 7.5 Hz, H-7), 7.00 (1H, dd, *J* = 8.0 Hz, H-6), 4.53 (1H, dd, *J* = 12.0, 6.0 Hz, H-1), 3.62 (1H, dd, *J* = 12.0, 5.0 Hz, H-3), 3.17 (1H, dd, *J* = 16.0, 5.0 Hz, H_{eq}-4), 2.78 (1H, dd, *J* = 16.0, 12.0 Hz, H_{ax}-4), 1.62 (3H, d, *J* = 6.8 Hz, CH_3-10); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 169.5 (COOH), 136.4 (C-8a), 132.2 (C-9a), 126.1 (C-4b), 121.4 (C-7), 118.9 (C-6), 118.0 (C-5), 111.3 (C-8), 106.7 (C-4a), 57.7 (C-3), 49.1 (C-1), 23.2 (C-4), 16.9 (CH_3).

Methyl-β-D-fructofuranoside (8) – white amorphous

powder; ^{1}H-NMR (400 MHz, D_2O): δ 4.09 (1H, d, *J* = 8.2 Hz, H-3), 3.97 (1H, dd, *J* = 7.9 Hz, H-4), 3.77 (1H, m, H-5), 3.72 (1H, dd, *J* = 12.0 Hz, H-6b), 3.64 (1H, d, *J* = 12.0 Hz, H-1b), 3.56 (2H, m, H-1a/H-6a), 3.24 (3H, s, OCH_3); ^{13}C-NMR (100 MHz, D_2O): δ 103.9 (C-2), 81.4 (C-5), 77.0 (C-3), 75.1 (C-4), 62.8 (C-6), 59.9 (C-1), 49.0 (OCH_3).

5-Caffeoylquinic acid (Chlorogenic acid, 10) – slightly yellow amorphous powder; mp 209 - 210 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.62 (1H, s, H-4'), 9.19 (1H, s, 3'-OH), 7.42 (1H, d, *J* = 16.0 Hz, H-7'), 7.03 (1H, brs, H-2'), 6.98 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 6.76 (1H, d, *J* = 8.0 Hz, H-5'), 6.15 (1H, d, *J* = 16.0 Hz, H-8'), 5.06 (1H, ddd, *J* = 10.0, 6.0 Hz, H-3), 3.92 (1H, brs, H-5), 3.42 (1H, brs, H-4), 2.03 - 1.77 (4H, m, H-2/H-6); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 175.0 (C-7), 165.8 (C-9'), 148.4 (C-4'), 145.6 (C-7'), 145.0 (C-3'), 125.6 (C-1'), 121.4 (C-6'), 115.8 (C-5'), 114.8 (C-8'), 114.3 (C-2'), 73.5 (C-1), 70.9 (C-4), 70.3 (C-5), 68.0 (C-3), 37.2 (C-6), 36.2 (C-2).

3-Caffeoylquinic acid (Neochlorogenic acid, 11) – amorphous powder; mp 218 - 219 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.53 (1H, s, 4'-OH), 9.14 (1H, s, 3'-OH), 7.46 (1H, d, *J* = 16.0 Hz, H-7'), 7.02 (1H, d, *J* = 2.0 Hz, H-2'), 6.97 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 6.76 (1H, d, *J* = 8.0 Hz, H-5'), 6.20 (1H, d, *J* = 16.0 Hz, H-8'), 5.17 (1H, m, H-3), 3.86 (1H, dd, *J* = 12.0, 4.0 Hz, H-5), 3.54 (1H, dd, *J* = 4.0Hz, H-4), 2.02 - 1.85 (4H, m, H-2/H-6); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 176.0 (C-7), 166.1 (C-9'), 148.1 (C-4'), 145.5 (C-7'), 144.4 (C-3'), 125.7 (C-1'), 121.1 (C-6'), 115.8 (C-5'), 115.0 (C-8'), 114.6 (C-2'), 72.9 (C-1), 71.2 (C-4), 71.0 (C-3), 67.2 (C-5), 39.5 (C-6), 35.1 (C-2).

4-Caffeoylquinic acid (Cryptochlorogenic acid, 12) – pale brown amorphous powder; mp 182 - 184 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.57 (1H, s, 4'-OH), 9.15 (1H, s, 3'-OH), 7.49 (1H, d, *J* = 16.0 Hz, H-7'), 7.04 (1H, d, *J* = 2.0 Hz, H-2'), 7.00 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 6.76 (1H, d, *J* = 8.0 Hz, H-5'), 6.27 (1H, d, *J* = 16.0 Hz, H-8'), 4.87 (1H, d, *J* = 5.0 Hz, H-4), 4.67 (1H, dd, *J* = 8.0, 5.0 Hz, H-3), 4.09 (1H, brs, H-5), 4.00 - 2.73 (4H, m, H-2/H-6); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 175.4 (C-7), 166.3 (C-9'), 148.3 (C-4'), 145.6 (C-7'), 144.8 (C-3'), 125.6 (C-1'), 121.2 (C-6'), 115.8 (C-5'), 114.7 (C-8'), 114.6 (C-2'), 76.9 (C-4), 74.0 (C-1), 66.4 (C-3), 64.0 (C-5), 40.7 (C-6), 37.8 (C-2).

3,5-Di-*O*-caffeoylquinic acid (13) – pale yellow amorphous powder; FAB-MS *m/z* 517 $[M + H]^+$; ^{1}H-NMR (400 MHz, MeOH-d_4): δ 7.60, 7.57 (1H each, d, *J* = 15.9 Hz, H-7/H-7'), 7.06 (2H, brs, H-2/H-2'), 6.97, 6.96 (2H, dd, *J* = 8.2, 2.0 Hz, H-6/H-6'), 6.77 (2H, dd, *J* = 7.8, 1.2 Hz, H-5/H-5'), 6.35, 6.26 (1H each, d, *J* = 15.9 Hz, H-8/H-8'), 5.45 - 5.36 (2H, m, H-3/H-5), 3.96 (1H, dd, *J* = 9.9, 3.4 Hz, H-4), 2.35 - 2.13 (4H, m, H-2/H-6); ^{13}C-NMR (100 MHz, MeOH-d_4): δ 177.3 (COOH), 168.9, 168.3 (C-9/C-9'), 149.6, 149.5 (C-4/C-4'), 147.3, 147.0 (C-7/C-7'), 146.8 (C-3/C-3'), 127.9, 127.8 (C-1/C-1'), 123.1, 123.0 (C-6/C-6'), 116.5 (C-5/C-5'), 115.5, 115.2 (C-8/C-8'), 115.1 (C-2/C-2'), 74.7 (C-1), 72.5 (C-3), 72.1 (C-5), 70.5 (C-4), 38.6 (C-6), 36.0 (C-2).

1-Kestose [β-D-fructofuranosyl-(2 → 1)-β-D-fructofuranosyl-(21)-α-D-glucopyranoside, 14] – amorphous powder; FAB-MS *m/z* 527 $[M + Na]^+$, 341 $[M - H]^-$; HR FAB-MS *m/z* 527.1585 $[M + Na]^+$ (calcd 527.1588, $C_{18}H_{32}O_{16}$); ^{1}H-NMR (400 MHz, D_2O): δ 5.29 (1H, d, *J* = 3.8 Hz, Glucose H-1), 4.13 (1H, d, *J* = 8.6 Hz, Fructose H-3'), 4.04 (1H, d, *J* = 8.6 Hz, Fructose H-3''), 3.92 (2H, m, Fructose H-4'/H-4''), 3.72 (3H, m, Glucose H-5, Fructose H-5'/H-5''), 3.71 - 3.61 (10H, m, Glucose H-6, Fructose H-1'/H-6'/H-1''/H-6''), 3.58 (1H, brs, Glucose H-3), 3.39 (1H, dd, *J* = 9.9, 3.8 Hz, Glucose H-2), 3.33 (1H, dd, *J* = 12.0 Hz, Glucose H-4); ^{13}C-NMR (100 MHz, D_2O): δ 103.8 (Fructose C-2''), 103.4 (Fructose C-2'), 92.6 (Glucose C-1), 81.3 (Fructose C-5'), 81.2 (Fructose C-5''), 76.7 (Fructose C-3'/C-3''), 74.6 (Fructose C-4''), 73.9 (Fructose C-4'), 72.7 (Glucose C-3), 72.5 (Glucose, C-5), 71.2 (Glucose C-2), 69.3 (Glucose C-4), 62.4 (Fructose C-6''), 62.3 (Fructose C-6'), 61.0 (Fructose C-1'), 60.5 (Fructose C-1''), 60.2 (Glucose C-6).

Results and Discussion

The dried roots of *A. cordata* were extracted with MeOH and partitioned as described in the Experimental section. Six phenolic acids (**1**, **2**, **10** - **13**), two nucleosides (**3**, **4**), an alkaloid (**7**), and five saccharides (**5**, **6**, **8**, **9**, **14**) were isolated by successive chromatography using silica gel and RP-18 gel from the *n*-BuOH fraction. These compounds were identified as caffeic acid (**1**, Lim *et al*., 2003), protocatechuic acid (**2**, Hur *et al*., 2001), thymidine (**3**, Spilsberg *et al*, 2006), uridine (**4**, Sierzputowska-Gracz *et al*., 1987), methyl-α-D-fructofuranoside (**5**, Angyal and Bethell, 1976), a mixture (3 : 1) of β-D-fructopyranoside and β-D-fructofuranoside (**6**, Angyal and Bethell, 1976), 1-methyl 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**7**, Kicha *et al*., 2003), methyl-β-D-fructofuranoside (**8**, Angyal and Bethell, 1976), sucrose (**9**, Hesse *et al*., 1997), 5-caffeoylquinic acid (**10**, chlorogenic acid) (Tatefuji *et al*., 1996), 3-caffeoylquinic acid (**11**, neochlorogenic acid) (Nakatani *et al*., 2000), 4-caffeoylquinic acid (**12**, cryptochlorogenic acid) (Nakatani

et al., 2000), 3,5-di-*O*-caffeoylquinic acid (**13**, Tatefuji *et al*., 1996), and 1-kestose [β-D-fructofuranosyl-(2 → 1)-β-D-fructofuranosyl-(2 → 1)-α-D-glucopyranoside] (**14**, Calub and Waterhouse, 1990), respectively, by direct comparison of the spectroscopic data with the published values. Although **5**, **7**, **8**, and **10 - 14** were previously reported, it was obtained from this plant for the first time.

Compounds **5** and **8** were obtained as amorphous white powders. Inspection of the ^{13}C-NMR spectroscopic data of **5** and **8** with the aid of DEPT and HMQC experiments showed the presence of seven well resolved carbon signals: one methyls (CH_3), two methylenes (CH_2), three methines (CH) and one quaternary carbon (see Experimental). The presence of an anomeric carbon at δ 103.9 (β-form) for **8** and δ 108.4 (α-form) for **5** and the absence of the correspondence proton indicate two compounds possess the ketonic nature. The chemical shifts in the ^{13}C-NMR spectra of **5** and **8** pointed to furanose form (Duker and Serianni, 1993). The position of methyl group in **5** and **8** was determined using HMBC experiment: HMBC correlation between the methyl protons at δ 3.12 for **5** (δ 3.24 for **8**) and the anomeric carbons at δ 108.4 for **5** (δ 103.9 for **8**). Thus, **5** and **8** were identified as methyl-α-D-fructofuranoside and methyl-β-D-fructofuranoside, respectively.

The compound **7**, possessing specific physical properties [mp 290 - 292 °C; $[\alpha]_D^{20}$ −104° (*c* 0.05, 50% pyridine)], showed absorption peaks characteristic of indole chromophores (Tschesche *et al*., 1958) at 221, 271, 278 and 288 nm in its UV spectrum and showed absorption bands at 3297 (NH), 1645 (COOH), 1578, 741 (aromatic) cm^{-1} in its IR spectrum. The EI-MS of **7** afforded a molecular ion peak at *m/z* 230 corresponding to $C_{13}H_{14}N_2O_2$. The ^{1}H NMR spectrum showed signals of four aromatic protons at δ 7.00 (1H, dd, *J* = 8.0 Hz), 7.08 (1H, dd, *J* = 7.5 Hz), 7.34 (1H, d, *J* = 7.5 Hz), and 7.44 (1H, d, *J* = 8.0 Hz), respectively, and the coupling patterns are indicative of an *ortho*-disubstituted benzene ring. The signals at δ 2.78 (dd, *J* = 16.0, 12.0 Hz, H_{ax}-4) and 3.17 (dd, *J* = 16.0, 5.0 Hz, H_{eq}-4) were assignable to the axial and equatorial protons of a methylene group, which were coupled to the vicinal methine proton appearing at δ 3.62 (1H, dd, *J* = 12.0, 5.0 Hz, H-3). A double doublet signal due to another methine proton appeared at δ 4.53 (*J* = 12.0, 6.0 Hz, H-1), and a doublet signal at δ 1.62 (*J* = 6.8 Hz, CH_3) was indicative of tertiary methyl signal attached aforementioned methine proton at δ 4.53. The ^{13}C NMR, coupled with the DEPT and HMQC spectroscopic data showed the presence of nine quaternary carbons, one methylene, two methines, and one methyl group (See Experimental). Twelve ^{13}C NMR resonances were found at chemical shift values similar to those of tetrahydro-carboline-3-carboxylic acid (Choi *et al*., 1988). One remaining resonance resulted from a methyl group. The methyl group could be located at C-1 due to correlation between the signals of δ 1.62 (CH_3) and δ 49.1 (C-1)/ 136.4 (C-8a) in the HMBC spectrum. These data together with the appearance of a peak at *m/z* 143 formed by *retro*-Diels-Alder fragmentation suggested **7** to be methyl 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid. This is the first example of its occurrence from the Aralia genus. The proposed stereostructure of **7** was confirmed finally by comparing with the authentic sample (IR, mp, MS, and NMR) obtained from Sigma-Aldrich Co. (St. Louis, MO, USA).

Compounds **10 - 12** gave similar characteristic phenolic color reactions, greenish-brown in ferric chloride solution. The MS spectra of **10 - 12** did not show a molecular ion peak but two prominent ion peaks at *m/z* 180 ($C_9H_8O_4$) and 162 ($C_9H_8O_4$-H_2O) suggesting the presence of caffeoyl moiety. The NMR spectra of **10 - 12** showed the signals of caffeic acid (See Experimental) and two methylenes, one oxygen bearing carbon with acid ascribable to cyclopolyoxycarboxylic acid, i.e. quinic acid, respectively. These spectroscopic data were in agreement with those for the structure of caffeoyl quinic acid known as chlorogenic acid isomers. The respective isomers were identified by comparison of NMR spectroscopic data with those reported in the literature (Tatefuji *et al*., 1996; Nakatani *et al*., 2000) and finally confirmed by direct comparison (HPLC) with authentic samples purchased from Chengdu Biopurify Phytochemicals Ltd. (Chengdu, Sichuan, China).

Compound **13** was obtained as a pale yellow amorphous powder with $[M + H]^+$ peak at *m/z* 527 in the FAB-MS spectrum, corresponding to dicaffeoyl quinic acid (Hung *et al*., 2008). The ^{1}H-NMR spectrum of **13** exhibited signals corresponding to two caffeic acid and a quinic acid. Four doublets with coupling constants of 15.9 Hz appeared for the *trans*-olefinic protons at H-7' and H-8' of **13**. The signals at δ 7.06 (d, *J* = 2.0 Hz, H-2'), 6.77 (d, *J* = 8.2 Hz, H-5') and δ 6.97 and 6.96 (2H, dd, *J* = 8.2, 2.0 Hz, H-6') can be assigned to two groups of three aromatic protons in a characteristic ABX system. The signals of H-5 (m) and H-3 (m) at the region of δ 5.36 - 5.45 and the doublet of doublets signal of H-4 (dd, *J* = 9.9, 3.4 Hz) at δ 3.96 of the quinic acid moiety were assigned according to their multiplicity and coupling patterns. The ^{13}C-NMR data of **13** revealed the presence of a quinic acid moiety characterized with two

methylenes at C-2 (δ 36.0) and C-6 (δ 38.6), three oxymethines at C-3 (δ 72.5), C-4 (δ 70.5) and C-5 (δ 72.1), one quaternary carbon (δ 74.7) and one carboxyl group (δ 177.3). From the above results, the structure of **13** was established to be 3,5-dicaffeoyl quinic acid.

Compound **14** showed the *pseudo*-molecular ion $[M+Na]^+$ at *m/z* 527.1585 (calcd 527.1588) in the HR FAB-MS (positive ion), suggestive of a formula as $C_{18}H_{32}O_{16}$. Inspection of the ^{1}H-NMR spectrum of **14** showed the presence of trisaccharides units, as evidenced by the easily identifiable signals for anomeric protons at δ 5.29, 3.68, and 3.68, indicating the presence of two β-fructofuranosyl and α-glucopyranosyl units, respectively, and these proton signals were correlated with anomeric carbon signals at δ 92.6, 103.4, and 103.8 in the HMQC experiment. All ^{13}C signal assignments and positions of the three glycosidic linkages in **14** were determined by detailed analysis of the 1D NMR, DEPT, ^{1}H-^{1}H COSY, HMQC, and HMBC experiments, and by comparisons with the ^{13}C-NMR data for related trisaccharides (Calub and Waterhouse, 1990). The ^{1}H-^{1}H-COSY spectrum revealed the connectivities of protons from H-1 to H-6 of the glucose, and the carbons attached to those protons were assigned from the HMQC and HMBC spectra. The HMBC correlations between δ 5.29 (Glucose H-1) and one of the quaternary carbon at δ 103.4 indicates this carbon is C-2' in the inner fructose (C2-$^1F_1$). The C-2' showed additional HMBC correlation peaks with methylene protons, which could thus be assigned as δ 3.68 (H1-$^1F_1$). The methylene signal at the H-1 in the inner fructose (H1-$^1F_1$) showed a HMBC correlation with another carbon (δ 103.8, C2-$^1F_2$) in the terminal fructose unit. Based on the above results, **14** was identified as 1-kestose [β-D-fructofuranosyl-(2 → 1)-β-D-fructofuranosyl-(2 → 1)-α-D-glucopyranoside] and isolated from this plant for the first time.

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