Short communication

A New Neolignan Glycoside from the Roots of *Acanthopanax brachypus*

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Abstract

A new neolignan glycoside, named as brachyposide A, was isolated from the EtOH extract of the roots of *Acanthopanax brachypus*, together with nine known compounds. The structure of brachyposide A was characterized by spectroscopic means as (7*S*,8*S*)-$\Delta^{7'}$-2,9,9′-trihydroxy-7-*O*-3′,8-*O*-4′-neolignan-4-*O*-*β*-*D*-apiofuranosyl-(1→6)-*β*-*D*-glucopyranoside. The known compounds were identified by comparing their spectral data with those of authentic samples or literature data.

Keywords: Araliaceae; *Acanthopanax brachypus*; brachyposide A; (7*S*,8*S*)-$\Delta^{7'}$-2,9,9′-trihydroxy-7-*O*-3′,8-*O*-4′-neolignan-4-*O*-*β*-*D*-apiofuranosyl-(1→6)-*β*-*D*-glucopyranoside

1. Introduction

The *Acanthopanax* genus of the Araliaceae family includes 37 species around the world, and is widely distributed in Korea, Japan, China and the far-eastern region of Russia. Twenty-six species and 18 varieties grow in mainland China.[1,2] The root and stem bark of these plants have been used clinically for a long time as a tonic and sedative, as well as for the treatment of rheumatism, diabetes, chronic bronchitis, hypertension, anti-stress and ischemic heart disease and gastric ulcers.[3–6] As an endangered shrub in the wild due to overharvesting and loss of habitat through deforestation, *Acanthopanax brachypus* Harms is distributed in a narrow geographical area, mostly in the loess plateau of the Northwest of China.[7,8] Research indicates that the seeds of *A. brachypus* contain many kinds of micro-elements indispensable to the human body, can relax women's menopause syndrome and exhibit immunostimulatory and anticancer activities, and its rhizomatic extracts has also been successfully used in China and Korea for the inhibition of the various allergic responses.[9–11] Nowadays, the other parts of this plant such as the roots, leaves and flowers are also employed for various therapeutic purposes.[12–14] Although, the research has so far mainly concentrated on the reproductive biology and ecology, there have been a few studies on the chemical composition and biological activity.[15,16] To further study its active constituents and provide the reference for effective utilization and quality control of the natural resources, our continuing phytochemical investigation on *Acanthopanax*

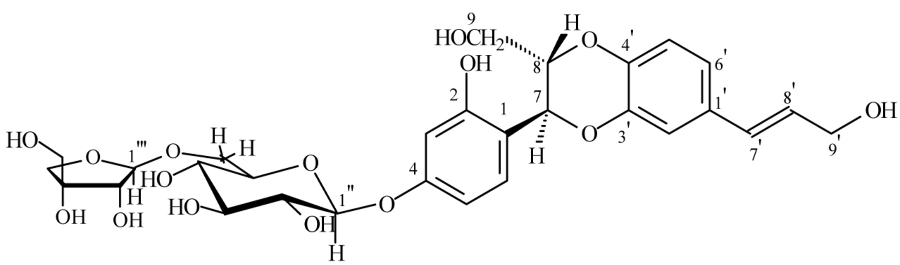

Figure 1: The structures of the isolated compound **1** from *A. brachypus*.

species[17–21] has led to the identification of a new neolignan glycoside **1**, named brachyposide A, along with nine known compounds, have been isolated for the first time from the roots of *A. brachypus*. In the present communication, we describe the isolation and structural elucidation of the new compound.

2. Results and Discussion

Compound **1** was obtained as a white amorphous powder from MeOH. Its molecular formula $C_{29}H_{36}O_{15}$ was determined by positive ion HRFAB-MS (*m/z*: 625.2138 $[M+H]^+$, calcd. 625.2132, Δ 0.6 nnu), corresponding to 12 degrees of unsaturation. The UV spectrum showed absorption bands at 209 and 266 nm, and its IR spectrum revealed the presence of hydroxy (3327 cm^{-1}), olefinic carbons (1628 cm^{-1}) and phenyl (1602, 1515 cm^{-1}) moieties. The ^1H and ^{13}C NMR spectra showed the presence of a 1,3,4-trisubstiuted benzene ring [δ_H 6.93 (1H, d, J = 1.7 Hz), 6.82 (1H, d, J = 8.2 Hz) and 6.88 (1H, dd, J = 1.7, 8.2 Hz), δ_C 110.9, 117.3 and 118.2], an *asym*-trisubstituted benzene ring [δ_H 6.43 (1H, d, J = 2.4 Hz), 6.45 (1H, dd, J = 7.9, 2.4 Hz) and 6.96 (1H, d, J = 7.9 Hz), δ_C 104.0, 108.5 and 116.7], an (*E*)-coniferyl alcohol [δ_H 4.02 (2H, br d, J = 5.7 Hz), 6.38 (1H, d, J = 15.3 Hz) and 6.19 (1H, dd, J = 15.3, 5.7 Hz), δ_C 61.6, 128.6 and 126.5],[22] two methines [δ_H 4.76 (1H, d, J = 8.0 Hz) and 4.30 (1H, dq, J = 8.0, 6.4 Hz), δ_C 80.2 and 73.8], one phenolic hydroxy [δ_H 9.70 (1H, s), δ_C 155.0] and a hydroxymethyl [δ_H 5.18 (1H, s) and 3.76 (2H, br d, J = 11.2 Hz), δ_C 60.8], and two sugar anomeric protons [δ_H 4.82 (1H, d, J = 7.5 Hz, H-1″) and 5.27 (1H, d, J = 2.2 Hz, H-1‴), the corresponding anomeric carbon signals at δ_C 104.6 (C-1″) and 111.0 (C-1‴)]. The ^{13}C NMR and DEPT spectra of **1** clearly displayed 29 carbon signals (5 × CH_2, 17 × CH, 7 × C), of which 11 could be assigned to a glucose unit (δ_C 104.6, 74.7, 77.5, 71.0, 77.1, 68.0) and an apiose unit (δ_C 111.0, 77.9, 80.4, 75.1, 65.7), and the remaining 18 carbon signals were assigned to the aglycone. Comparison of the ^1H and ^{13}C NMR data of **1** with those of eusiderin E,[23] indicated that **1** is a 7-*O*-3′,8-*O*-4′-neolignan glycoside. In the HMBC and NOESY spectra, the correlations between δ_C 145.9 (C-4) and δ_H 4.82 (H-1″)/6.43 (H-3)/6.45 (H-

Table 1: ^1H (400 MHz) and ^{13}C NMR (100 MHz) spectral data of compounds **1** (DMSO-d_6, TMS)*.

No.	δ_H (J)(Hz)	δ_C	DEPT	HMBC(H→C)	ROESY(H↔H)
1	–	131.1	C	H-3, H-5, H-6, H-7, H-8, HO-2	
2	–	155.0	C	H-3, H-6, H-7, HO-2	
3	6.43 (d, *2.4*)	104.0	CH	HO-2, H-5	
4	–	145.9	C	H-1'', H-3, H-5, H-6	
5	6.45 (dd, *7.9*, *2.4*)	108.5	CH	H-3, H-6	H-1”
6	6.96 (d, *7.9*)	116.7	CH	H-5, H-7	
7	4.76 (d, *8.0*)	80.2	CH	H-6, H-8	H-9
8	4.30 (dq, *8.0*, *6.4*)	73.8	CH	H-7, H-9	
9	3.76 (br d, *11.2*)	60.8	CH_2	H-8, HO-9	H-7
1'	–	131.3	C	H-2', H-5', H-6', H-7', H-8'	
2'	6.93 (d, *1.7*)	110.9	CH	H-6', H-7'	H-7'
3'	–	143.4	C	H-7, H-2', H-5'	
4'	–	136.7	C	H-8, H-6', H-5'	
5'	6.82 (d, *8.2*)	117.3	CH	H-6'	
6'	6.88 (dd, *1.7*, *8.2*)	118.2	CH	H-2', H-5', H-7'	H-8'
7'	6.38 (d, *15.3*)	128.6	CH	H-2', H-6', H-8'	
8'	6.19 (dd, *15.3*, *5.7*)	126.5	CH	H-7', H-9'	H-6'
9'	4.02 (br d, *5.7*)	61.6	CH_2	H-8', HO-9'	
HO-2	9.70 (s)	–	–		
HO-9	5.18 (s)	–	–		
Glc-1''	4.82 (d, *7.5*)	104.6	CH	H-2''	H-6, H-3'', H-5''
2''	3.82 (dd, *9.1*, *7.4*)	74.7	CH	H-1''	H-4''
3''	3.78 (dd, *9.1*, *8.5*)	77.5	CH		H-1'', H-5''
4''	3.94 (dd, *9.9*, *8.5*)	71.0	CH		H-2''
5''	3.81 (ddd, *9.9*, *6.0*, *1.6*)	77.1	CH		H-1'', H-3''
6''	4.06 (dd, *11.3*, *1.6*) 3.94 (dd, *11.3*, *6.0*)	68.0	CH_2	H-1'''	
Api-1'''	5.27 (d, *2.2*)	111.0	CH	H-6'', H-2'''	
2'''	3.98 (d, *2.2*)	77.9	CH	H-4''', H-5'''	
3'''	–	80.4	C	H-2'''	
4'''	3.77 (d, *9.4*)/3.95 (d, *9.4*)	75.1	CH_2		
5'''	3.68 (s)	65.7	CH_2		

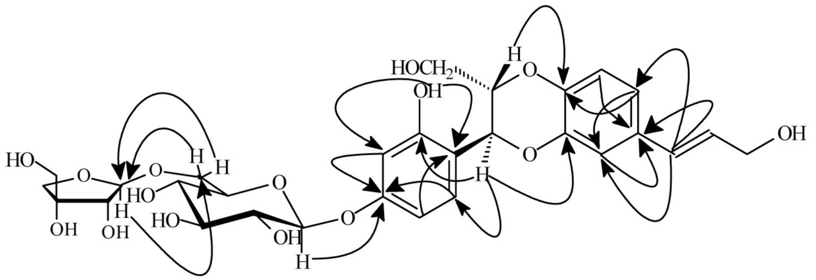

Figure 2: The key HMBC (H → C) correlations of compound **1**.

5)/6.96 (H-6), δ_C 131.3 (C-1′) and δ_H 6.38 (H-7′)/6.88 (H-6′)/6.93 (H-2′), Δ_C 155.0 (C-2) and δ_H 6.43 (H-3)/6.96 (H-6), δ_C 104.0 (C-3)/131.1 (C-1) and δ_H 9.70 (HO-2), δ_H 4.82 (H-1″) and δ_H 6.45 (H-5), Δ_H 6.88 (H-6′) and δ_H 6.19 (H-8′) as well as δ_H 6.93 (H-2′) and δ_H 6.38 (H-7′), suggested that the disaccharide chain, (*E*)-coniferyl alcohol side-chain and hydroxyl groups were connected to C-4, C-1′ and C-2 of the aglycone, respectively.

Upon acid hydrolysis, compound **1** gave *D*-glucose and *D*-apiose, according to *co*-TLC with authentic samples and rotational analysis.[24] In addition, this was also confirmed by the FAB-MS spectral observation of fragment ions at *m/z* 493 $[M+H–132]^+$ and *m/z* 331 $[M+H–132–162]^+$, arising from the elimination of an apiose and a glucose unit, indicating the apiose was terminal sugar and the glucose was attached to the aglycone. Comparison of ^{13}C NMR data of the sugar moieties with literature values,[25] revealed that the glucose was present in pyranoside form and the apiose was in furanoside form. The HMBC correlations (Figure 2) of H-1‴ (δ_H 5.27) with C- 6″ (δ_C 68.0) and H-6″ (δ_H 4.06/3.94) with C-1‴ (δ_C 111.0), suggested an apiose-(1→6)-glucose linkage. The *β*-configuration of apiose was confirmed by comparing the ^{13}C-NMR spectra of **1** with those of *α-D*-(δ_C 104.5) and *β-D*-apiofuranosides (δ_C 111.5), respectively,[26] and the glucose had the *β*-configuration according to the coupling constant (*J* = 7.5 Hz) of H-1″ of glucose. The coupling constants (*J* = 15.3 Hz) between H-7′ and H-8′ suggested that the (*E*)-coniferyl alcohol side-chain had a *trans*-configuration. The signals of H-7 (δ_H 4.76) and H-8 (δ_H 4.30) at slightly lower fields, with a larger coupling constant (*J* = 8.0 Hz), along with the NOESY correlations between δ_H 4.76 (H-7) and δ_H 3.76 (H-9), indicated a *trans*-orientation of H-7 and H-8 pair in **1**.[27] By comparison of CD value of **1** with that of the known (7*S*,8*S*)-$\Delta^{7'}$-2,4-dihydroxy-7-*O*-3′,8-*O*-4′-neolignan,[28] suggested the plausible configurations of C-7 and C-8 as *S* and *S*, respectively. On these grounds, the structure of **1** was determined as (7*S*,8*S*)-$\Delta^{7'}$-2,9,9′-trihydroxy-7-*O*-3′,8-*O*-4′-neolignan-4-*O*-*β*-*D*-apiofuranosyl-(1→6)-*β*-*D*-glucopyranoside, and named brachyposide A.

The known compounds **2–10** were identified as quercetin-3-*O*-neohesperidoside (**2**),[29] echioidin (**3**),[30] maltol *β*-*D*-glucopyranoside (**4**),[31] isoandrographolide (**5**),[32] 2-methoxyphenyl *β*-*D*-glucopyranoside (**6**),[33] (–)-syringaresinol-4,4′-bis-*O*-*β*-*D*-glucopyranoside (**7**),[34,35] acantrifoside A (**8**),[6] *β*-sitosterol (**9**)[36] and daucosterol (**10**)[37] by comparing their spectroscopic data with values reported in the literature.

3. Experimental

3. 1. General

Melting points (uncorrected) were observed with a Chinese X-4 melting point apparatus. Optical rotations were measured with Perkin-Elmer 241 digital polarimeter. UV and IR (KBr disks) spectra were obtained on Shimadzu UV-300 (double beam) and Alpha-Centari FT-IR spectrophotometers. CD spectra were recorded on a Jasco J-715 spectropolarimeter. ^{1}H and ^{13}C NMR (DEPT) spectra were recorded on a Bruker AM-400 NMR spectrometer. Mass spectra were carried out on ZAB-HS and MAT-112 mass spectrometers, respectively. Separation and purification were performed by column chromatography on silica

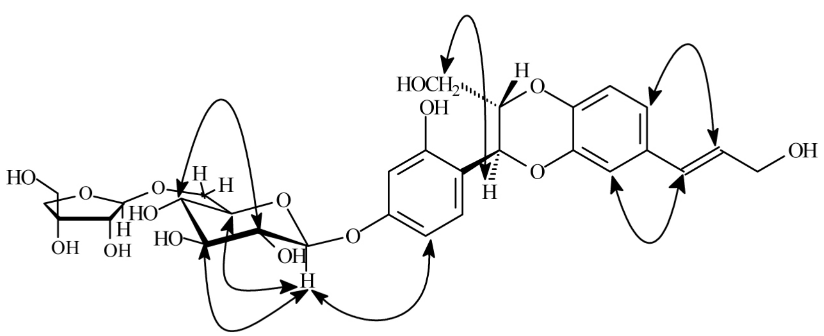

Figure 3: The key NOESY (H↔H) correlations of compound **1**.

gel (100–200, 200–300 mesh). TLC was performed on silica gel GF_{254} plates. The spots were visualized by UV (254 nm) and EtOH–H_2SO_4.

3. 2. Plant Material

The roots of *A. brachypus* were collected in August 2007 from Qingyang of Gansu Province, China, and were identified by Prof. Xiao-Qiang Guo, Department of Life Sciences, Longdong University. A voucher specimen (No. 12107) was deposited in the Herbarium of the Department of Life Sciences, Longdong University, People's Republic of China.

3. 3. Extraction and Isolation

The air-dried and powered roots of *A. brachypus* (5.5 kg) were soaked in 95% EtOH (15 L, 7 d×3) at room temperature. After removing the solvent, the extract (298 g) was suspended in warm water and partitioned with petroleum ether (60–90 °C), $CHCl_3$, EtOAc and *n*-BuOH, successively. The *n*-BuOH-soluble fraction was evaporated to give 86.7 g of residues, which was isolated on a silica gel column eluting with $CHCl_3$–MeOH (6:0→1:6) in increasing polarity and combined by monitoring with TLC to give four fractions (A, B, C and D). Fraction A (4.6 g) was further fractionated over silica gel column and eluted with $CHCl_3$–MeOH (4:1) to obtain **3** (17 mg). Fraction B (3.1 g) was purified on a silica gel column using $CHCl_3$–MeOH gradient (3:0→0:3) as eluent to afford **2** (11 mg). Fraction C (5.4 g) was rechromatographed over a silica gel column eluting with acetone–MeOH (3:0→1:3) to yield **1** (11 mg) and two subfractions (C_1–C_2). Subfraction C_1 was further purified by preparative TLC (silica gel) and developed with $CHCl_3$–MeOH (1:1) to provide compounds **4** (8 mg) and **5** (10 mg). Subfraction C_2 was further purified on silica gel column eluting stepwise with $CHCl_3$–MeOH (from 5:1 to 1:8, V/V), and then on Sephadex LH-20 eluting with MeOH to obtain compounds **6** (17 mg) and **7** (9 mg), respectively. Fraction D (5.4 g) was purified on a silica gel column using $CHCl_3$–MeOH gradient (2:1→1:8) as eluent to afford three subfractions (D_1–D_3). Subfraction D_1 was rechromatographed over silica gel and further purified by preparative TLC (MeOH/$CHCl_3$/*n*-hexane, 1:5:2, V/V) to afford compounds **8** (24 mg) and **9** (11 mg). Subfraction D_3 was chromatographed on Sephadex LH-20 with MeOH-H_2O (1:1, V/V) to afford compound **10** (19 mg).

3. 4. Characterization Data

Compound **1**: White amorphous powder (MeOH), mp. 212–215°C; $[\alpha]_D^{20}$ –10.8` (*c* 0.45, MeOH); HRFAB-MS: *m/z* 625.2138 $[M+H]^+$ ($C_{29}H_{36}O_{15}$ calcd. 625.2132, Δ 0.6 nnu); UV λ_{max}^{MeOH} (nm): 209, 266; CD (MeOH, *c* 2.45 × 10^{-5} g/mL), $\Delta\varepsilon^{18}$ (nm): +10.58 (223.5), 0 (237.5), –2.43 (258.5); IR ν_{max}^{KBr} (cm^{-1}): 3327 (OH), 1628 (olefinic C=C), 1602, 1515 (phenyl); FAB-MS: *m/z* 625 $[M+H]^+$, 493 $[M+H–132]^+$ and 331 $[M+H–132–162]^+$; the ^1H and ^{13}C NMR data see Table **1**.

4. Acknowledgements

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Povzetek

Novi, neolignanski glikozid imenovan brahipozid A, je bil skupaj s še devetimi, že znanimi spojinami, izoliran iz EtOH ekstrakta korenin *Acanthopanax brachypus*. Struktura brahipozida A je bila s spektroskopskimi metodami določena kot (7*S*,8*S*)-$\Delta^{7'}$-2,9,9′-trihidroksi-7-*O*-3′,8-*O*-4′-neolignan-4-*O*-*β*-*D*-apiofuranozil-(1→6)-*β*-*D*-glukopiranozid. Ostale znane spojine so bile identificirane s pomočjo primerjave njihovih spektroskopskih podatkov s podatki za avtentične vzorce ali literaturnimi vrednostmi.