Phenolic Glycosides from Nuo-Mi-Xang-Cao, a Chinese Acanthaceous Herb

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From leaves of nuo-mi-xang-cao, a Chinese acanthaceous herb collected at Xi-shuang-ban-na, Yunnan, two new phenolic glycosides called nuomioside A and isonuomioside A were isolated and determined to be composed of 3,4-dihydroxyphenethylalcohol-(3'-O- β -D-apiosyl-4'-O-caffeoyl)- β -D-glucopyranoside and 3,4-dihydroxy-phenethylalcohol-3'-O- β -D-apiosyl-6'-O-caffeoyl)- β -D-glucopyranoside, respectively. Together with these compounds, known phenolic glycosides, acteoside, isoacteoside, crassifolioside, apigenin 7-O- β -D-glucuronide and its methyl ester were also identified in the leaves.

Keywords nuo-mi-xang-cao; Acanthaceae; *Strobilanthes* species; Chinese herb; acteoside; isoacteoside; nuomioside; phenethyl alcohol glycoside

Leaves of nuo-mi-xang-cao (糯米香草), an herb growing at Xi-shuang-ban-na (西双版纳), South Yunnan, China are well-known to have an odor like glutinous rice and have been used as a flavoring for tea in this district. This plant seems to belong to *Strobilanthes* genus, Acanthaceae, however, due to its sparse blooming pattern to the fact that it blooms very rarely, a scientific species name has yet been proposed. As part of China–Japan joint studies on traditional Chinese plant drugs, we have conducted chemical investigations of this plant and report now the identification of phenolic glycosides.

The leaves were extracted with methanol and an aqueous suspension of the methanolic extract was successively extracted with hexane, ether and then 1-butanol (saturated with water). The butanolic extract was subjected to repeated chromatography to give seven compounds, I–VII. Based on inspection of the proton and carbon-13 nuclear magnetic resonance (¹H- and ¹³C-NMR) spectra, compounds I, II and V were identified as known caffeoyl glycosides of 3,4-dihydroxyphenethyl alcohol: I: acteoside¹⁾ from *Digitalis purpurea*,²⁾ *Syringa vulgaris*,³⁾ *Plantago major*,⁴⁾ etc., II: isoacteoside⁵⁾ from *Leucospermum japonicum* etc. and V: crassifolioside⁶⁾ from *Plantago crassifolia*. It is noteworthy that certain biological activities such as antiviral activity⁴⁾ and enzyme inhibitory activities⁷⁾ have been reported for I and/or the congeners.

A new glycoside III, C₂₈H₃₄O₁₅ from high resolution fast atom bombardment mass spectrometry (HR-FAB-MS) afforded D-glucose and D-apiose on acid hydrolysis. Inspection of the ¹H- and ¹³C-NMR signals due to sugar

moieties indicated the presence of a β -D-apiosyl moiety^{8,9)} in III. All of the carbon and proton signals of I were assigned (Tables I and II) by means of the ¹H–¹H correlated spectroscopy (¹H–¹H COSY) and ¹³C–¹H COSY spectra. Comparison of the spectrum of III with that of I revealed that all of the carbon signals due to the caffeoyl, glucosyl and dihydroxyphenethyl moieties of I appeared at almost the same positions in the spectrum of III (Table I). This indicates that III can be formulated as 3,4-dihydroxyphenethylalcohol-(3'-O- β -D-apiosyl-4'-O-caffeoyl)- β -D-glucopyranoside (= 3'-O- β -D-apiosyl-desrhamno-acteoside). The location of the apiosyl moiety in III was further substantiated as follows. Identification of all of the proton signals of III was established by the ¹H–¹H COSY spectrum, and the subsequent ¹H–¹H nuclear Overhauser effect correlated spectroscopy (NOESY) spectrum revealed the presence of NOE between 3-H of the glucosyl moiety and H-1 of the apiosyl moiety (Chart 1). The name nuomioside A is proposed for the new glycoside, III.

Another new glycoside, IV, C₂₈H₃₄O₁₅ from HR-FAB-MS (an isomer of III), yielded D-glucose and D-apiose on acid hydrolysis. The carbon and proton signals of IV indicated the presence of a terminal β -D-apiosyl moiety (Tables I and II). Comparison of the ¹³C-NMR spectrum of IV with that of II (Table I) showed that IV can be formulated as 3',4'-dihydroxyphenethyl-(3'-O- β -D-apiosyl-6'-O-caffeoyl)- β -D-glucopyranoside (= 3'-O- β -D-apiosyl-desrhamno-isoacteoside). The name isonuomioside A is proposed for IV. The location of the apiosyl moiety was confirmed by the ¹H–¹H COSY spectrum and the

Chart 1

TABLE I. ^{13}C -NMR (δ in $\text{DMSO}-d_6$, 100 MHz) of Phenolic Glycosides

C	I	II	III	IV	V
Caffeoyl moiety					
1	125.4	125.4	125.4	125.4	125.4
2	114.5	114.7	114.7	114.8	114.4
3	145.5	145.3	145.5	145.5	145.5
4	148.5	148.2	148.3	148.3	148.4
5	115.7	115.6	115.7	115.7	115.7
6	121.4	121.3	121.2	121.3	121.3
7	145.2	145.1	145.2	145.2	145.3
8	113.4	113.8	113.8	113.2	113.6
9	165.6	166.4	165.6	165.4	165.7
Aglycone					
1	129.1	129.0	129.1	129.1	128.6
2	116.2	116.1	116.2	116.2	116.2
3	144.9	144.8	144.9	144.9	145.0
4	143.5	143.3	143.5	143.5	143.5
5	115.4	115.3	115.4	115.4	115.4
6	119.4	119.4	119.4	119.4	119.4
7	34.9	35.0	35.0	35.1	34.7
8	70.3	70.2	70.3	70.4	70.2
Glucose					
G-1	102.2	102.5	102.9	102.4	100.7
G-2	74.4	74.4	74.4	73.6	79.1
G-3	79.0	80.7	78.9	81.3	78.8
G-4	69.0	68.0	69.0	68.4	69.3
G-5	74.4	73.6	73.6	73.4	74.4
G-6	60.6	63.3	60.7	63.2	60.6
Rhamnose					
R-1	101.1	100.5			101.5
R-2	70.4	70.4			70.4 ^{a)}
R-3	70.2	70.2			70.1 ^{b)}
R-4	71.6	71.9			71.5
R-5	68.6	68.4			68.7
R-6	18.1	17.3			18.1
Apiose					
A-1			109.5	109.2	
A-2			76.0	76.0	
A-3			78.5	79.0	
A-4			73.6	73.6	
A-5			63.8	63.6	
Rhamnose					
R'-1					101.9
R'-2					70.5 ^{a)}
R'-3					70.2 ^{b)}
R'-4					71.8
R'-5					69.2
R'-6					18.1

a, b) Interchangeable.

subsequent ^1H - ^1H NOESY spectrum in a similar manner to III (Chart 1).

Inspection of the ultraviolet spectrum (UV), infrared spectrum (IR), ^1H - and ^{13}C -NMR spectra disclosed that VI and VII are identical to the known flavonoid-glycosides, apigenin 7-*O*- β -D-glucuronide¹⁰⁾ and its methyl ester,¹¹⁾ respectively. The methyl ester VII might be an artifact which is formed from VI during the process of the extraction with methanol.

Experimental

NMR spectrum were recorded on a JEOL JNM GX-400 spectrometer in deuterio-dimethyl sulfoxide ($\text{DMSO}-d_6$) using tetramethylsilane as an internal standard. FAB-MS spectra were taken on a JEOL JMS SX-102 spectrometer. The plants were collected at Xi-shuang-ban-na, Yunnan, China and identified by the Kunming Institute of Botany, Chinese Academy of Science. A specimen has been deposited in the herbarium of this Institute.

Acid Hydrolysis of Glycosides and Identification of the Resulting

TABLE II. ^1H -NMR (δ in $\text{DMSO}-d_6$; 400 MHz) of III, IV

H	III	IV
Caffeoyl moiety		
2	7.10 (d, 1.4)	7.07 (d, 2.0)
5	6.83 (d, 8.2)	6.76 (d, 8.0)
6	7.05 (dd, 1.4, 8.2)	6.97 (dd, 2.0, 8.0)
7	7.53 (d, 15.8)	7.47 (d, 15.7)
8	6.29 (d, 15.8)	6.29 (d, 15.7)
Aglycone		
2	6.69 (d, 1.9)	6.61 (d, 2.0)
5	6.70 (d, 8.1)	6.58 (d, 8.0)
6	6.57 (dd, 1.9, 8.1)	6.46 (dd, 2.0, 8.0)
7	2.77 (ddd, 7.2, 8.8, 9.0)	2.69 (ddd, 6.8, 8.9, 9.0)
	2.75 (ddd, 7.2, 8.8, 9.0)	2.71 (ddd, 6.8, 8.9, 9.0)
8	3.71 (ddd, 7.2, 8.8, 8.8)	3.61 (ddd, 6.8, 8.9, 9.0)
	3.95 (ddd, 7.2, 8.8, 8.8)	3.79 (ddd, 6.8, 8.9, 9.0)
Glucose		
1	4.36 (d, 7.8)	4.31 (d, 7.7)
2	3.27 (dd, 7.8, 9.3)	3.18 (dd, 7.7, 8.4)
3	3.74 (dd, 9.3, 9.5)	3.41 (dd, 8.4, 9.3)
4	4.68 (dd, 9.5, 9.6)	3.24 (dd, 8.7, 9.3)
5	3.53 (ddd, 1.7, 6.0, 9.6)	3.49 (ddd, 1.6, 6.1, 8.7)
6	3.42 (dd, 6.0, 10.7)	4.20 (dd, 6.1, 12.0)
	3.47 (dd, 1.7, 10.7)	4.39 (dd, 1.6, 12.0)
Apiose		
1	5.26 (d, 1.7)	5.23 (d, 2.1)
2	3.77 (d, 1.7)	3.82 (d, 2.1)
4	3.49 (d, 9.0)	3.63 (d, 9.1)
	3.70 (d, 9.0)	4.01 (d, 9.1)
5	3.25 (d, 10.8)	3.40 (2H, s)
	3.28 (d, 10.8)	

Values in parenthesis are coupling constant in Hz.

Monosaccharides A solution of a few mg of III or IV in 3.5% HCl (in 50% dioxane, 1 ml) was heated at 80 °C for 3 h in a sealed microtube. The mixture was washed with CHCl_3 and neutralized with Amberlite MB-3. Monosaccharides in the aqueous layer were identified by comparison of thin layer chromatography (TLC) and gas-liquid chromatography (GC) with a respective authentic sample. TLC: on a silica gel plate with CHCl_3 -MeOH- H_2O (6:4:1) was visualized with 2,3,4-triphenyltetrazolium chloride. Identification of monosaccharides including absolute configuration was conducted according to Oshima, Kumanotani and Watanabe's procedure.¹²⁾ A solution of the reaction product and the L-amine-reagent (*vide infra*) in H_2O (50 μl) was heated at 40 °C for 4 h. After addition of a few drops of AcOH, the mixture was concentrated to dryness. The residue was treated with a few drops of *N*-trimethylsilylimidazole at 40 °C for 8 h and the mixture was extracted with *n*- C_6H_{14} . The product was subjected to GC to compare derivatives prepared from authentic monosaccharides with the L-amine-reagent or the DL-amine-reagent in the same way as above. L-amine-reagent: a solution of L- α -methylbenzylamine (400 mg) and NaBH_3CN (100 mg) in EtOH (2.5 ml), DL-amine-reagent: DL- α -methylbenzylamine (400 mg) and NaBH_3CN (100 mg) in EtOH (2.5 ml). Condition of GC: on a capillary glass column, coated with Carbowax PEG 20M, WCOT type, 0.25 mm $\times$ 25 m, carrier gas: He, flow rate 3 ml/min, column temp.: 150 °C, injection and detector temp.: 190 °C, detection: FID.

Isolation and Properties of Glycosides The dried leaves (800 g) were extracted with MeOH. An aqueous suspension of the MeOH extract was successively extracted with *n*- C_6H_{14} , Et_2O and then 1-BuOH (saturated with H_2O). The BuOH extract was chromatographed on silica gel with CHCl_3 -MeOH- H_2O (30:10:1, 10:5:1, 6:4:1 and then 3:2:1) to give nine fractions, frs. 1–9. Fraction 6 was subjected to chromatography on LiChroprep RP-8 (Merck) with MeOH- H_2O (1:2) followed by high-performance liquid chromatography (HPLC) on TSK gel ODS 120T (Tosoh, 21.5 mm i.d. $\times$ 30 cm) with 10% 2-PrOH (in MeOH)- H_2O (1:2) (flow rate: 6 ml/min, detection: RI and UV 254 nm) to give I, II, III and IV in yields of 0.1, 0.005, 0.01 and 0.006%, respectively. Fraction 7 was chromatographed on LiChroprep RP-8 with MeOH- H_2O (3:7) and then with MeOH to give fractions, frs. 7-1 and 7-2. Fraction 7-1 was purified by HPLC on TSK gel ODS 120T (21.5 mm i.d. $\times$ 30 cm) with 10% 2-PrOH (in MeOH)- H_2O (1:2), (flow rate: 6 ml/min, detection: RI and UV

254 nm) to afford V in a yield of 0.025%. Fraction 7-2 was subjected to chromatography on silica gel with CHCl_3 -MeOH- H_2O (10:5:1) and then on Sephadex LH-20 with MeOH- H_2O (1:4) followed by HPLC TSK gel Amide-80 (Tosoh, 21.5 mm i.d. $\times$ 30 cm) with CH_3CN - H_2O (9:1) (flow rate: 6 ml/min, detection: RI and UV 254 nm) to give VI and VII in yields of 0.008 and 0.004%, respectively.

Compound I: A pale yellow powder. $[\alpha]_D^{25} -87.5^\circ$ ($c=0.71$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 244 (sh, 4.05), 291 (4.07), 344 (4.19).

Compound II: A pale yellow powder. $[\alpha]_D^{20} -52.3^\circ$ ($c=0.58$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 245 (sh, 4.05), 290 (4.10), 329 (4.19).

Compound III: A pale yellow powder. $[\alpha]_D^{21} -52.3^\circ$ ($c=0.53$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 289 (sh, 4.92), 290 (5.00), 329 (4.65). HR-FAB-MS: Calcd for $[\text{C}_{28}\text{H}_{34}\text{O}_{15} + \text{H}]^+$ 611.1975. Found: m/z 611.1893.

Compound IV: A pale yellow powder. $[\alpha]_D^{22} -54.7^\circ$ ($c=0.53$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 291 (sh, 4.92), 338 (5.00), 378 (4.71). HR-FAB-MS: Calcd for $[\text{C}_{28}\text{H}_{34}\text{O}_{15} + \text{H}]^+$ 611.1975. Found: m/z 611.1989.

Compound V: A pale yellow powder. $[\alpha]_D^{21} -74.9^\circ$ ($c=2.67$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 291 (sh, 4.91), 334 (5.07), 379 (4.72). HR-FAB-MS: Calcd for $[\text{C}_{35}\text{H}_{46}\text{O}_{19} + \text{H}]^+$ 771.2711. Found: m/z 771.2759.

Compound VI: Pale yellow needles from MeOH. mp 253–254 °C. $[\alpha]_D^{19} -64.9^\circ$ ($c=0.60$, MeOH). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 269 (4.36), 334 (4.39); $\lambda_{\text{max}}^{\text{EtOH} + \text{NaOEt}}$ nm 269, 392; $\lambda_{\text{max}}^{\text{EtOH} + \text{NaOAc}}$ nm 269, 334; $\lambda_{\text{max}}^{\text{EtOH} + \text{NaOAc} + \text{HBO}_3}$ nm 269, 333; $\lambda_{\text{max}}^{\text{EtOH} + \text{AlCl}_3}$ nm 270, 382. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : aglycone moiety 7.92 (2H, d, $J=8.5$ Hz, H-2', 6'), 6.95 (2H, d, $J=8.5$ Hz, H-3', 5'), 6.86 (1H, s, H-3), 6.84 (1H, d, $J=1.5$ Hz, H-8), 6.46 (1H, d, $J=1.5$ Hz, H-6), glucuronide moiety 5.19 (1H, d, $J=7.8$ Hz, H-1), 3.92 (1H, d, $J=7.8$ Hz, H-5), 3.2–3.5 (3H, br, H-2, 3, 4).

Compound VII: Pale yellow needles from MeOH. mp 242–244 °C. $[\alpha]_D^{19} -72.3^\circ$ ($c=0.30$, MeOH). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 269 (4.34), 334 (4.36); $\lambda_{\text{max}}^{\text{EtOH} + \text{NaOEt}}$ nm 269, 392; $\lambda_{\text{max}}^{\text{EtOH} + \text{NaOAc}}$ nm 269, 332; $\lambda_{\text{max}}^{\text{EtOH} + \text{NaOAc} + \text{HBO}_3}$ nm 269, 334; $\lambda_{\text{max}}^{\text{EtOH} + \text{AlCl}_3}$ nm 270, 383. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : aglycone moiety 7.95 (2H, d, $J=8.8$ Hz, H-2', 6'), 6.94 (2H, d, $J=8.8$ Hz, H-3', 5'), 6.86 (1H, s, H-3), 6.85 (1H, d, $J=2.2$ Hz, H-8), 6.47 (1H, d, $J=2.2$ Hz, H-6), glucuronide moiety 5.32 (1H, d, $J=7.1$ Hz, H-1), 3.92 (1H, d, $J=9.3$ Hz, H-5), 3.67 (3H, s, $-\text{OCH}_3$), 4.32 (1H, dd, $J=8.4, 9.3$ Hz, H-4), 3.35 (1H, dd, $J=8.4, 8.4$ Hz, H-3), 3.32 (1H, dd, $J=7.1, 8.4$ Hz, H-2). A solution of VII (30 mg) in 0.5N aqueous NaOH (5 ml) was allowed to stand at room temperature for 30 min. The reaction mixture was acidified (pH 3.0) with 0.5N aqueous HCl and extracted with EtOAc. The EtOAc

extract was purified by HPLC on TSK gel Amide-80 with CH_3CN - H_2O (9:1) (*vide supra*) to give VI (25 mg) which was identified by comparison of $^{13}\text{C-NMR}$ spectrum with that of an authentic sample of VI.

Acknowledgements We are grateful to Professor J. Zhou, Kunming Institute of Botany for his encouragement and to Mr. Guo-da Tao, Xi-shuang-ban-na Tropical Botanic Garden, Academia Sinica for the collection of the plant material.

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