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A NEW FLAVONOID GLYCOSIDE AND FOUR OTHER CHEMICAL CONSTITUENTS FROM *VISCUM COLORATUM* AND THEIR ANTIOXIDANT ACTIVITY

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Abstract – A new flavonoid glycoside, identified as rhamnazin-3-*O*-*β*-D-(6″-*β*-hydroxy-*β*-methylglutaryl)-*β*-D-glucoside-4′-*O*-*β*-D-glucoside (**1**), along with four known compounds, shikimic acid (**2**), quebrachitol (**3**), abscisic acid (**4**), 3,5-dihydroxy-1,7-bis(4-hydroxyphenyl)heptane (**5**), were isolated from the branches and leaves of *Viscum coloratum*. Their structures were elucidated on the basis of MS, 1D NMR, and 2D NMR techniques. All compounds were isolated in the family *Loranthaceae* for the first time. This paper describes the isolation, structural elucidation as well as *in vitro* antioxidant activity of these isolates.

The *Viscum coloratum*, also referred to as “mistletoe”, is an evergreen, semi-parasitic plant which grows on the branches or stems of deciduous trees. The branches and leaves of mistletoe are used in the treatment of hypertension, atherosclerosis, rheumatism and neuralgia.[1-3] The activity of mistletoe has been ascribed to the presence of antioxidative constituents.[4,5] Therefore, the bioactive constituents of *V. coloratum* are of our interest. The ethanol extract of mistletoe showed potent antioxidative properties in our screening program searching for antioxidative natural substances, and further bioassay-guided analysis led to the isolation of a new flavonoid glycoside and four known compounds from the active antioxidative extract. All the five compounds were characterized in the family *Loranthaceae* for the first time. In this paper, we report the isolation, structure elucidation and antioxidative activities of these

compounds. Their antioxidant activity was tested *in vitro* by the DPPH scavenging method.

The air-dried stems and leaves of mistletoe, collected in Liaoning province of China, were extracted exhaustively with 95% EtOH under reflux. Repeated chromatographic separation of the combined extracts led to the purification and subsequent characterization of a new flavonoid glycoside (**1**), along with known compounds shikimic acid (**2**), quebrachitol (**3**), abscisic acid (**4**), and 3,5-dihydroxy-1,7-bis(4-hydroxyphenyl)heptane (**5**).

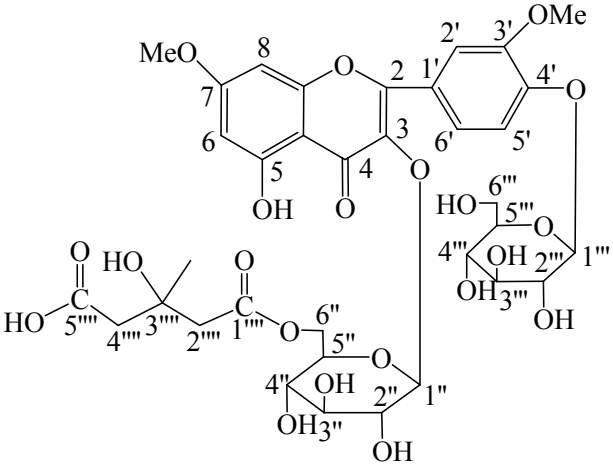

Figure 1. The structure of compound **1**

Compound **1** was obtained as yellow powder. The structure was shown in **Figure 1**. Its HR-ESI-MS spectrum displayed a $[M + H]^+$ ion peak at *m/z* 799.2295, indicating a molecular formula of $C_{35}H_{42}O_{21}$ (calcd. for 799.2297) (fifteen degrees of unsaturation). Its IR spectrum indicated the presence of hydroxy (3420 cm^{-1}), methyl (2924 cm^{-1}), carbonyl (1729 cm^{-1}) and phenyl (1654 cm^{-1}, 1600 cm^{-1}, 1071 cm^{-1}) groups. The ^1H and ^{13}C NMR signals of compound **1** were assigned by different 2D NMR analysis. The ^1H NMR spectrum of compound **1** showed the presence of three protons at δ 1.14 (3H, s) predicting 3′′′′-Me, four protons at δ 2.42 (2H, m) and 2.39 (2H, m) corresponding to H_2-2′′′′ and H_2-4′′′′, two methyl signals at δ 3.94 (3H, s) and δ 3.88 (3H, s) corresponding to 3′-OMe and 7-OMe respectively. Anomeric protons resonating as a doublet at δ 5.33 (1H, d, J = 5.4 Hz) (δ 5.50 (1H, d, J = 7.2 Hz in DMSO-d_6)) and another at δ 5.05 (1H, d, J = 7.2 Hz) indicated the presence of β-glucose, respectively. The presence of a flavonol A-ring was confirmed by two mutually coupled protons at δ 6.33 (1H, s, H-6) and δ 6.61 (1H, s, H-8). The presence of flavonol B-ring was confirmed by three mutually coupled protons at δ 7.92 (1H, s, H-2′), 7.25 (1H, d, J = 13.2 Hz, H-5′) and 7.69 (1H, d, J = 8.4 Hz, H-6′). There were 35 carbon signals in the ^{13}C NMR spectrum of compound **1**. The signals at δ 172.3 (C-1′′′′), 46.4 (C-2′′′′), 70.6 (C-3′′′′), 46.1 (C-4′′′′), 175.8 (C-5′′′′) and 27.6 (3′′′′-C) corresponded to the carbon signals of 3-hydroxy-3-methylglutarate. The

signals at δ 55.6 and δ 57.0 corresponded to the -OMe of C-7 and C-3′, respectively. The signals at δ 103.7 and δ 102.1 were C-1″and C-1‴ signals of glucose. The carbon signals left were corresponded to the flavone skeleton and glucose. In the HMBC spectrum (**Figure 2**), the proton at δ 1.14 (3H, s, 3⁗-Me) showed 3*J*-correlation with C-2⁗ and C-4⁗; the 2*J*-correlation with C-3⁗ indicated that the methyl group was situated at C-3⁗. The proton at δ 4.20 (1H, d, 6″-H) and δ 4.09 (1H, dd, 6″-H) showed 3*J*-correlation with C-1⁗. The proton at δ 5.33 (1H, d, 1″-H) and δ 5.05 (1H, d, 1‴-H) showed correlation with C-3 and C-4′ respectively. The TOCOSY correlation of H-1″/H-4″and the HMBC correlation from H-6″ (δ_H 4.09) to C-4″ indicated that the 3-hydroxy-3-methylglutarate group was situated at glucose 1. The ^{1}H and ^{13}C NMR spectroscopic data were listed in **Table 1**. The complete acid hydrolysis of compound **1** yielded arious products, and in the hydrolysate separated from the aglycone parts, the sugar was identified as glucose by the thin layer chromatography (TLC) comparison with the standard sample. The D-glucose could be confirmed by the optical rotation of $[\alpha]_D^{20}$+12 (*c* 0.02, H_2O).[6] Consequently, the structure of compound **1** was assigned as rhamnazin-3-*O*-*β*-D-(6″-*β*-hydroxy-*β*-methylglutaryl)-*β*-D-glucoside-4′-*O*-*β*-D-glucoside.

Table 1. The ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectral data and HMBC correlations of compound **1** (in methanol-d_4, δ ppm, *J* in Hz)

Position	δ_H	δ_C	HMBC(H→C)
2		158.5	
3		135.9	
4		179.5	
5		162.8	
6	6.33	99.3	C-5, 7, 8, 10
7		167.5	
8	6.61	93.3	C-6, 7, 9, 10
9		158.4	
10		106.7	
1′		123.2	
2′	7.92	114.9	C-2, 1′, 3′, 4′, 5′

3′		150.0	
4′		150.4	
5′	7.25 (d, 13.2)	116.6	C-3′, 4′, 6′
6′	7.69 (d, 8.4)	125.8	C-2, 2′, 4′, 5′
7-OCH_3	3.88	57.0	C-7
3′-OCH_3	3.94	56.6	C-3′
Glc 1			
1″	5.33 (d, 5.4)	103.7	C-3
2″	3.45 (m)	75.8	C-3″
3″	3.45 (m)	77.8	C-1″, 2″, 5″
4″	3.28 (m)	71.6	C-2″, 3″, 5″
5″	3.45 (m)	75.8	C-3″
6″	4.20 (d, 12)	64.4	C-1‴′, 4″, 5″
	4.09 (dd, 10.8, 6)		
Glc 2			
1‴	5.05 (d, 7.2)	102.1	C-4′
2‴	3.55 (m)	74.8	C-1‴, 3‴
3‴	3.45 (m)	77.8	
4‴	3.43 (m)	71.3	C-2‴
5‴	3.50 (m)	78.3	C-2‴, 3‴, 4‴
6‴	3.91 (m)	62.5	C-4‴, 5‴
	3.72 (m)		
3-Hydroxy-3-methylglutarate			
1‴′		172.3	
2‴′	2.42 (m)	46.4	C-1‴′, 3‴′, 4‴′, 5‴′
3‴′		70.6	
4‴′	2.39 (m)	46.1	C- 2‴′, 3‴′, 5‴′
5‴′		175.8	
3‴′-CH_3	1.14	27.6	C-3‴′, 4‴′

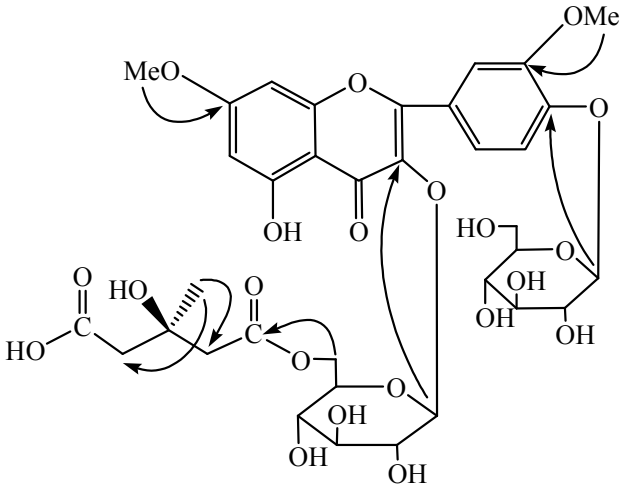

Figure 2. Key HMBC correlations of compound **1**

The four known compounds were determined as shikimic acid,[7] quebrachitol,[8] abscisic acid,[9] 3,5-dihydroxy-1,7-bis(4-hydroxyphenyl)heptane,[10,11] by comparison of their spectral data with literature values.

The potential antioxidant activity of the compounds was assessed *in vitro* and expressed as EC_{50} values by the DPPH scavenging method. The results of DPPH radical scavenging activities for all isolates are summarized in **Table 2**. All tested compounds showed moderate antioxidant activity.

Table 2. The results of the antioxidant activities of the five compounds and Vitamin C

Compounds	VC	1	2	3	4	5
EC_{50} values (μ mol/mL)	0.068	0.440	0.612	1.550	0.365	0.417

EXPERIMENTAL

General

UV absorption spectra were measured on Shimadzu UV-1700 Spectrophotometer and Perkin Elmer Lambda 35 UV/VIS Spectrometer. IR absorption spectra were recorded on a Bruker IFS 55 Infrared spectrophotometer. NMR spectra were measured with 600 MHz Bruker FTNMR Ultra ShieldTM spectrometers in methanol-d_4. Chemical shifts (δ ppm) were given relative to TMS as internal standard. HR-ESI-MS was performed on Waters Xevo G2 QTOF MS. Optical rotations were measured with a Perkin-Elmer 241 MC polarimeter. Column chromatography was performed using silica gel (Qingdao

Haiyang Chemical Group Co., Ltd., Qingdao, Shandong, China), polyamide (Luqiao Sijia Biochemical plastic factory, Taizhou, Zhejiang, China), Sephadex LH-20 (GE Healthcare, Piscataway, NJ, USA) and ODS (Phenomenex Inc., Torrance, CA, USA).

Plant Material

The stems and leaves of mistletoe were collected in Liaoning province of China and identified by associate Dr. Ying Jia (Department of Traditional Chinese Medicine, Shenyang Pharmaceutical University, Shenyang, China).

Extraction and Isolation

Air-dried stems and leaves of mistletoe (10 kg) were extracted exhaustively with 95% EtOH under reflux. The extract was concentrated under reduced pressure to get a brown syrup (1.1 kg). The syrup was suspended in H_2O and partitioned with petroleum ether, EtOAc, and *n*-BuOH, successively. The EtOAc extract was concentrated to dryness (105 g), and was subjected to silica gel column with a gradient of CH_2Cl_2-MeOH (100:0-0:100) to afford 18 fractions (F1-F18). F5 (CH_2Cl_2-MeOH 100:3) and F8 (CH_2Cl_2-MeOH 100:7) were further purified using a Sephadex LH-20 column to yield compounds **4** (17 mg) and **5** (20 mg), respectively. The *n*-BuOH extract (240 g) was eluted over polyamide column with water, 25% EtOH, 50% EtOH, 75% EtOH and 95% EtOH, consecutively. The 75% EtOH eluate was concentrated to dryness (30 g), and was subjected to silica gel column with a gradient of CH_2Cl_2-MeOH (100:0-0:100) to afford 16 fractions (F1-F16). F7 (CH_2Cl_2-MeOH 100:5) was chromatographed on a silica gel column using a gradient mixture of CH_2Cl_2-MeOH (100:0-0:100). Subfraction (CH_2Cl_2-MeOH 10:1) further purified by passage through a Sephadex LH-20 column to yield compound **2** (25 mg). F9 (CH_2Cl_2-MeOH 100:7) yielded compound **3** (150 mg) after recrystallization from MeOH, respectively. F13 (CH_2Cl_2-MeOH 100:30) was subjected to chromatography on a silica gel column using a gradient mixture of CH_2Cl_2-MeOH (100:0-0:100). Subfractions (CH_2Cl_2-MeOH 100:30) from F13 were further purified using a Sephadex LH-20 column to yield compound **1** (17 mg).

Rhamnazin-3-*O*-*β*-*D*-(6′′-*β*-hydroxy-*β*-methylglutaryl)-*β*-D-glucoside-4′-*O*-*β*-D-glucoside (1): a yellow powder; $[\alpha]_D^{20}$-72.8 (*c* 0.16, MeOH); UV (MeOH, λ_{max}, nm): 205, 251, 267, 346; IR banks (KBr) ν_{max} (cm^{-1}): 3420, 2924, 1729, 1654, 1600, 1071, 863, 802 and 620; ^1H and ^{13}C NMR data (methanol-d_4, 600 and 150 MHz, respectively) provided in **Table 1**. HR-ESI-MS (positive ion mode) *m/z* 799.2295 $[M+H]^+$ (calcd. for $C_{35}H_{42}O_{21}$, 799.2297). The fragments of positive ESI-MS at *m/z* 637.1769 $[M-C_6H_{11}O_5+H]^+$, 493.1346 $[M-C_6H_{11}O_5-C_6H_9O_4+3H]^+$, 331.0821 $[M-C_6H_{11}O_5-C_{12}H_{19}O_9+3H]^+$.

Acid Hydrolysis of Compound 1

The sample (1.0 mg) was refluxed with 1 N HCl (2.0 mL) for 5 h and then neutralized with $BaCO_3$. The precipitate was filtered off and the filtrate was used for detection. The sugar components were identified as glucose by TLC over silica gel (EtOAc/EtOH/H_2O/AcOH, 6:4:1:1) in comparison with authentic sugars. The mixtures were further purified using a Sephadex LH-20 column. The specific rotation of glucose revealed that it was a D-sugar.

Antioxidant Activity Determination

The potential antioxidant activity of these compounds was assessed on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. Different concentrations (5-500 μg/mL in MeOH) of test samples were prepared. The reaction mixtures consisting of 100 μL different concentratons of test samples and 100 μL of 100 μg/mL DPPH in methanol were fixed in quartz cuvettes and incubated for 30 min. The absorbance (A) was measured at 515 nm. The antioxidant activity was obtained from the following equation: Antioxidant activity (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100\%$. The percent antioxidant activity was plotted against concentration. The antioxidant activity of the compounds was expressed as EC_{50}, which was defined as the concentration in μmol/mL. The measurement was performed in triplicate. Vitamin C was used as positive control.

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REFERENCES

1. H. Zwierzina, L. Bergmann, H. Fiebig, S. Aamdal, P. Schoffski, K. Witthohn, and H. Lentzen, *Eur. J. Cancer*, 2011, **47**, 1450.
2. P. O. Osadebe and E. O. Omeje, *J. Ethnopharm*., 2009, **126**, 287.
3. G. S. Kienle, A. Glockmann, M. Schink, and H. Kiene, *J. Exp. Clin. Cancer Res.*, 2009, **28**, 79.
4. X. M. Piao, W. F. Chu, and G. F. Qiao, *J. Harbin Med. Univ*., 2006, **40**, 20.
5. W. F. Chu, G. F. Qiao, Y. L. Bai, Z. W. Pan, G. Y. Li, X. M. Piao, L. W. Wu, Y. J. Lu, and B. F. Yang, *Phytother. Res.*, 2008, **22**, 134.
6. R. J. Li, G. E. Yang, H. J. Bai, Q. Zhang, J. K. Li, Q. S. Li, and Z. M. Zhang, *Chem. Nat. Comp.*, 2012,

5, 761.

7. X. Yao, G. S. Zhou, Y. P. Tang, S. Guo, C. Jin, Y. Qin, D. W. Qian, and J. A. Duan, *Nat. Prod. Res. Dev.*, 2012, **24**, 1377.
8. M. Díaz, A. González, I. Castro-Gamboa, D. Gonzalez, and C. Rossini, *Carbohydr. Res.*, 2008, **343**, 2699.
9. G. L. Boyer and S. S. Dougherty, *Phytochemistry*, 1988, **27**, 1521.
10. L. V. Alegrio, R. B. Filho, and O. R. Gottlieb, *Phytochemistry*, 1989, **28**, 2359.
11. J. B. He, G. M. Liu, and Y. X. Cheng, *Chin. Tradit. Herb. Drugs*, 2011, **42**, 1905.