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Chinese Journal of Natural Medicines 2019, **17**(7): 0545–0550

Chinese Journal of Natural Medicines

Three new bioactive flavonoid glycosides from *Viscum album*

DAI Jia-Kun[1, 5Δ], CAO Duo[1, 2, 3Δ*], LI Cui-Hua[1], GAO Jing[1], LI Meng-Qing[1], FAN Na[1, 7], WEI Ya-Hui[1*], SUN Zheng-Liang[4*], HOU Meng-Yang[6]

[1] *The College of Life Science, Northwest University, Xi'an 710069, China;*
[2] *State Key Laboratory of New Drug and Pharmaceutical Process, Shanghai Institute of Pharmaceutical Industry, China State Institute of Pharmaceutical Industry, Shanghai 201203, China;*
[3] *The College of Life Science, Yan'an University, Yan'an 710000, China;*
[4] *Shanghai University of Medicine & Health Sciences Affiliated Sixth People's Hospital South Campus, Shanghai 201499, China;*
[5] *Bio-Agriculture Institute of Shaanxi, Xi'an 710043, China;*
[6] *School of Life Science and Biotechnology, Dalian University of Technology, Dalian 116024, China;*
[7] *College of Healthy Management, Shangluo University, Shangluo 726000, China*

[ABSTRACT] Two new flavonoid glycosides, named viscumneoside XII (**1**), and viscumneoside XIII (**2**); a new dihydrogen flavonoid glycoside product named viscumneoside XIV (**3**), were isolated from the aerial part of *Viscum album*, along with seven known compounds (**4**−**10**). Their structures were identified by analysis of spectroscopic data. In addition, cytotoxicity assay showed that **1**, **2** and **3** possessed significant inhibitory activities against C6, A549 and MDA-MB-231 (the inhibition rate arrived about 50%, 70% and 74% respectively with $IC_{50} \leq 60.00\ \mu mol \cdot L^{-1}$), while the inhibition of TF-1 and Hela was not significant.

[KEY WORDS] *Viscum album*; Flavonoid glycosides; Cytotoxic activities

[CLC Number] R284 **[Document code]** A **[Article ID]** 2095-6975(2019)07-0545-06

Introduction

As a traditional Chinese medicine, *Viscum album* belongs to *Loranthaceae* genus of the family *Viscum L.*. The recent studies showed that flavonoids, triterpenoids, and alkaloids are the main chemical constituents. Besides, pharmacological investigations revealed the activities of anti-tumor [1-6], anti-cardiovascular, anti-inflammation and antioxidant. The studies on the pharmacological activities of *Viscum album* were mainly concentrated in the extract, while the compounds activities were less reported. In order to elucidate the bioactive components of *Viscum album*, the chemical investigations of the dry stems and leaves were carried out.

Results and Discussion

Compound **1** was obtained as a yellow amorphous powder. The positive ESI-MS of **1** showed a $[M + Na]^+$ peak at *m/z* 724.03, a $[M + K]^+$ peak at *m/z* 740.04, a $[2M + Na]^+$ peak at *m/z* 1425.15 and a $[2M + K]^+$ peak at *m/z* 1446.99, pointing out the relative molecular mass of **1** was 701. The HR-ESI-MS showed a $[M + Na]^+$ peak at *m/z* 724.1877 (Calcd. 724.1854) and established a molecular formula of $C_{33}H_{35}NO_{16}$, implying 17 degrees of unsaturation. The ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) spectra show the presence of thirty-three carbons, including two glycosyl groups, one methoxy group and one flavonoid

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[Received on] 26-Mar.-2019

[Research funding] This work was supported by the National Science and Technology Development Fund (No. 2009ZX09301-007), the Scientific Research Projects of Shannxi Academy of Sciences (No. 2016K-17), the Projects of Shaanxi Provincial Science and Technology Department (No. 2016SF-264), Special Fund for Agro-scientific Research in The Public Interest (Grant 389, No. 201203062), Support Plan for Young 392 Talents of Science and Technology Association of Colleges and Universities in Shaanxi Province 393 (No. 20150105), Key Research and Development Plan Project of Shaanxi Province (No. 2018SF-034) and Scientific Research from Shaanxi Provincial Department of Education (Nos. 14JS100 and 17JK0243).

[*Corresponding authors] Tel/Fax: 86-21-20572000, E-mail: sunzl6@126.com (SUN Zheng-Liang); Tel: 86-13709120102, E-mail: caoduo2013@163.com (CAO Duo); Tel/Fax: 86-29-88302119, E-mail: weiyahui@nwu.edu.cn (WEI Ya-Hui)

ΔThese authors contributed equally to this work.

These authors have no conflict of interest to declare.

nucleus. The ^{1}H NMR spectropic data (Table 1) showed protons resonating at δ_H at 5.52 (1H, s, *β*-D-api C_1-H), 6.00 (1H, d, *J* = 8.0 Hz, *β*-D-glu C_1-H) and 3.0−4.5 (8H, m), with the eleven carbon signals at 60−100 ppm [including three secondary carbons at 66.7, 75.3 and 62.4, two quaternary carbons at 78.8 and 80.9, six tertiary carbons at 110.3 (C-1‴), 99.6 (C-1″). 71.4, 78.3, 78.1 and 78.8], suggested the presence of one glucose and one apiose group. Seven benzene protons at δ_H 6.82 (1H, d, *J* = 2.0 Hz, H-6), 7.00 (1H, d, *J* = 8.0 Hz, H-5'), 8.23 (1H, dd, *J* = 2.0, 8.0 Hz, H-6'), 8.66 (1H, d, *J* = 2.0 Hz, H-2'), 6.83(1H, d, 2.0 Hz), 6.16 (1H, d, 2.0 Hz), 6.88 (1H, d, 2.0 Hz) with the signals at δ_C 179.8 (C-4), 165.9 (C-2), 133.0 (C-3), 164.6 (C-5), 99.6 (C-6), 167.6 (C-7), 110.3 (C-8), 154.1 (C-9), 104.9 (C-10), 125.3 (C-1'), 116.3 (C-2'), 149.3 (C-3'), 148.3 (C-4'), 116.3 (C-5'), 120.7 (C-6'), confirmed the presence of the flavonoid nucleus [7]. The carbon signal resonating at δ_C 99.6, 164.6, 167.6 and104.9 has correlation with the proton signals at δ_H 6.00 (1H, d, *J* = 8.0 Hz, *β*-D-glu, C_1-H),

Table 1 ^{1}H and ^{13}C NMR data of compound 1−3^a

Position	1		3			2	
	δ_C (mult.)^b	δ_H (*J* in Hz)^c	δ_C (mult.)^b	δ_H (*J* in Hz)^c		δ_C (mult.)^b	δ_H (*J* in Hz)^c
2	165.9 (C)	——	79.3 (CH)	5.47 (1H, dd, 12.0, 2.8Hz)		158.6 (C)	——
3	133.0 (CH)	6.83 (s)	42.5 (CH$_2$)	2.75 (1H, dd, 17.2, 2.8Hz), 3.31 (1H, m)		134.8 (C)	——
4	179.8 (C)	——	197.5 (C)	——		179.5 (C)	——
5	164.6 (C)	——	163.2 (C)	——		166.5 (C)	——
6	99.6 (CH)	6.82 (1H, d, 2.5z)	96.9 (CH)	6.09 (1H, s)		99.9 (CH)	6.22 (1H, s)
7	167.6 (C)	——	165.3 (C)	——		167.8 (C)	——
8	110.3 (C)	——	95.8 (CH)	6.12 (1H, s)		95.0 (CH)	6.44 (1H, s)
9	154.1 (C)	——	163.1 (C)	——		158.8 (C)	——
10	104.9 (C)	——	103.8 (C)	——		104.2 (C)	——
1'	125.3 (C)	——	129.6 (C)	——		124.1 (C)	——
2'	116.3 (CH)	8.66 (1H, d, 2.5Hz)	111.7 (CH)	7.07 (1H, d, 1.6 Hz)		131.1 (CH)	7.62 (1H, dd, 2.0, 7.5Hz)
3'	149.3 (C)	——	148.0 (C)	——		116.0 (C)	6.91 (1H, d, 7.5Hz)
4'	148.3 (C)	——	147.4 (C)	——		158.5 (C)	——
5'	116.3 (CH)	7.00 (1H, d, 8.0 Hz)	115.7 (CH)	6.79 (1H, d, 8.4 Hz)		115.9 (CH)	6.91 (1H, d, 7.5Hz)
6'	120.7 (CH)	8.23 (1H, dd, 2.5, 8.0Hz)	120.1 (CH)	6.91 (1H, d, 8.4 Hz)		131.1 (CH)	7.62 (1H, dd, 2.0, 7.5Hz)
OCH$_3$	56.6 (CH$_3$)	3.85 (3H, s)	56.2 (CH$_3$)	3.77 (3H, s)		56.6 (CH$_3$)	3.97 (3H, s)
1"	99.6 (CH)	6.00 (1H, d, 8.0 Hz)	98.3 (CH)	5.04 (1H, d, 6.8 Hz)		100.1 (CH)	5.27 (1H, d, 5.5Hz)
2"	78.1 (CH)	——	76.6 (CH)	3.74 (1H, m)		78.0 (CH)	——
3"	78.3 (CH)	——	77.3 (CH)	3.45 (1H, m)		75.9 (CH)	——
4"	71.4 (CH)	——	70.2 (CH)	3.17 (1H, m)		71.59 (CH)	——
5"	78.8 (CH)	——	77.0 (CH)	3.45 (1H, m)		75.8 (CH)	——
6"	62.4 (CH$_2$)	3.52, 3.33	61.0 (CH$_2$)	3.41 (2H, m)		64.5 (CH$_2$)	3.51, 3.31
1‴	110.3 (CH)	——	109.2 (CH)	5.26 (1H, d, 12.0 Hz)	1‴	172.5 (C=O)	——
2‴	78.8 (CH)	——	76.6 (CH)	3.74 (1H, m)	2‴	47.5 (CH$_2$)	2.19 (2H, t)
3‴	80.9 (C)	——	79.6 (CH)	——	3‴-11‴	12−47 (CH2)	1.33 (18H, m)
4‴	75.3 (CH$_2$)	4.07, 3.62	74.2 (CH)	3.83 (2H, dd, 9.6, 2.4 Hz)	12‴	14.1 (CH$_3$)	0.89 (3H, t)
5‴	66.7 (CH$_2$)	3.78, 3.53	64.5 (CH$_2$)	3.32 (2H, m)			
1''''	127.6 (C)	-					
2''''	113.6 (CH)	6.83 (1H, d, 2.0Hz)					
3''''	151.3 (C)	-					
4''''	111.4 (CH)	6.16 (1H, d, 2.0Hz)					
5''''	154.1 (C)	-					
6''''	115.0 (CH)	6.88 (1H, d, 2.0Hz)					

^{a} ^{1}H and ^{13}C NMR data were measured at 500 and 125 MHz (MeOD), respectively; chemical shifts are in ppm;
^{b} *J* values (in Hz) in parentheses; ^{c} Attached protons determined by DEPT experiment

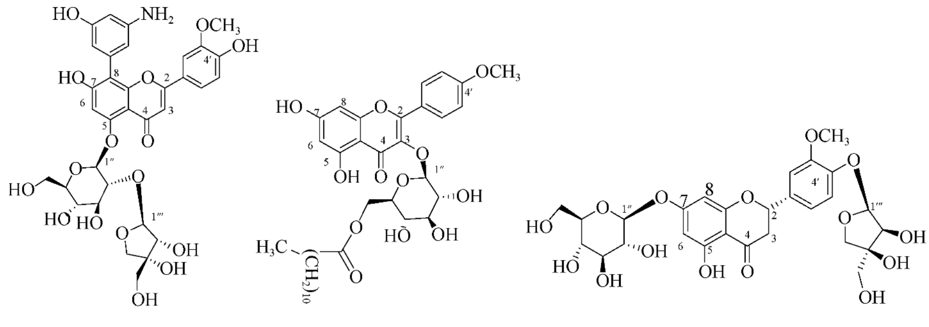
indicated that flavonoid nucleus with glucose to form glycosides at C-3. By comparing the glucose carbon signal with the compound **7** carbon nucleus signal, the C-2″ moved 4.9 to the low field, C-1' and C-3″ shifted 3.9 and 0.4 respectively to the high field, suggested the presence of api-(1→2)-*O*-*β*-D-glu. The ^{1}H NMR spectroscopic data showed proton signals resonating at δ_H 3.85 (3H, s, OCH_3), 4.62 (2H, s, br, NH_2), with the ^{13}C NMR carbon signal at 56.6 (CH_3), confimed the presence of one methoxy group, and one amine group. All protons and their associated carbons were futher assigned by the heteronuclear single quantum coherence (HSQC) spectroscopy correlations. The correlations found in correlation spectroscopy (^{1}H-^{1}HCOSY) spectrum revealed eight separated proton sequences, as depicted in Fig. 2. Three proton signals at δ_H 6.83 (1H, d, 2.0 Hz), 6.16 (1H, d, 2.0 Hz), 6.88 (1H, d, 2.0 Hz) with ^{13}C NMR data δ_C 127.6, 115.0, 151.3, 111.4, 154.1, 113.6, confimed the presence of one symmetric trisubstituted phenyl. The ^{1}H NMR spectroscopic data showed proton signals resonating at δ_H 6.83 (1H, d, 2.0 Hz), 6.88 (1H, d, 2.0 Hz) correlation with δ_C 167.6 (C-7) and 154.1 (C-9) respectively, suggested that trisubstituted phenyl with flavonoid nucleus at C-8. The HR-ESI-MS ($C_{33}H_{35}NO_{16}$) with ESI-MS (701) suggested trisubstituted phenyl with hydroxyland amine at C-3‴, C-5‴. The other HMBC correlations illustrated in Fig. 2 establisged the planar structure of **1**. Thus, the structure of **1** was established. Retrieved **1** in the Scifinder database, confirmed that the compound **1** was a new compound, 8-(3-amine-5-hydroxyphenyl)-rhamnazin-3-*O*-*β*-D-apiofuranosyl (1→2)-*O*-*β*-D-glucopyranoside, named as viscumneoside XII.

Compound **2**: Faint yellow amorphous powder. The ESI-MS of **2** showed a $[M + Na]^+$ peak at *m/z* 667.14, a $[M + H]^+$ peak at *m/z* 644.90, pointed out the relative molecular mass of **2** was 644. The HR-ESI-MS showed a $[M + Na]^+$ peak at *m/z* 667.2738 (Calcd. 667.2729) and established a molecular formula of $C_{34}H_{44}O_{12}$, impled 7 degrees of unsaturation. The ^{1}H NMR spectroscopic data showed proton signals resonating at δ_H: 0.89 (3H, t), 1.33 (18H, m), 2.19 (2H, t), 3.97 (3H, s, OCH_3), and confirmed the presence of a methoxy and a ethyl. All protons and their associated carbons were futher assigned by the heteronuclear single quantum coherence (HSQC) spectroscopy correlations. The correlations found in correlation spectroscopy (^{1}H-^{1}HCOSY) spectrum revealed eight separated proton sequences, as depicted in Fig. 2. The carbon signals at δ_C: 100.1 (C-1'), 78.0 (C-2'), 75.9 (C-3'), 71.5 (C-4'), 75.8 (C-5'), 64.5 (C-6') with the proton signal at δ_H 5.27 (1H, d, *J* = 5.5Hz, C1″-H), suggested that the presence of *α*-*D*-glu group. The ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) spectra show the presence of thirty-four carbons, including one glycosyl groups, one methoxy group, one carbonyl and one flavonoid nucleus. Six benzene protons at δ_H 6.22 (1H, s, H-6), 6.44 (1H, s, H-8), 7.62 (2H, dd, *J* = 2.0, 7.5 Hz, H-2', H-6'), 6.91 (2H, d, *J* = 7.5Hz, H-3', H-5') with the signals at δ_C 95.0 (C-8), 99.9 (C-6), 104.2 (C-10), 131.1 (C-2'), 115.9 (C-5'), 124.1 (C-1'), 131.1 (C-6'), 134.8 (C-3), 158.5 (C-4'), 116.0 (C-3'), 156.8 (C-9), 158.6 (C-2), 166.3 (C-5), 167.8 (C-7), 179.5 (C-4), confirmed the presence of the flavonoid nucleus [14] and the C ring by symmetrical replace. By comparing the glucose carbon signal with the compound **7** carbon nucleus signal, the $\delta_{C\text{-}2}$ moved 8.8 ppm to the low field, $\delta_{C\text{-}3}$ shifted 2.3 ppm, respectively, to the high field, suggested that flavonoid nucleus with glucose to form glycosides at C-3, which was also recognized by the heteronuclear multiple bond correlation (HMBC) spectroscopy correlation from 5.27 (1H, d, *J* = 5.5 Hz *α*-D-gluC$_{1''}$-H) to δ_C 134.8 (C-3). By comparing the glucose carbon signal with the compound **1** carbon signals, the δ (C-6″) moved 2.1 to the low field, δ (C-5″) shifted 3.0, respectively, to the high field, suggested that glucose is carbonylated at C-6″ and the ^{1}H NMR spectroscopic data showed proton signals resonating at δ_H H-6″ correlation with δ_C 172.5 (C=O), confirmed that glucose is carbonylated at C-6″. The DEPT) spectra show the presence of ten carbon (CH_2) signals at δ_C 12–47, in the HMBC spectra the proton at δ 2.19 (2H, t) correlated with the carbon signal at δ 172.5 (C), and with the ^{1}H NMR spectroscopic data showed proton signals resonating at δ 0.89 (3H, t), 1.33 (18H, m), 2.19 (2H, t), suggested that the glucose is esterified with dodecanoic acid at C$_{6''}$-OH. The other HMBC correlations illustrated in Fig. 2 establisged the planar structure of **2**. Thus, the structure of **2** was established. Retrieved by Scifinder database, confirmed compound **2** as a new flavonoid glucopyranoside, 4'-methoxyl-5, 7-dihydroxy-3-*O*-(twelve carbonyl)-*α*-*D*-flavonoid glucopyranoside, named viscumneoside XIII.

Compound **3** was isolated as ochre amorphous powder. The ESI-MS of **3** showed a $[M + H]^+$ peak at *m/z* 597.01, a $[M + H - 132]^+$ peak at *m/z* 465.03, $[M + H - 162]^+$ peak at *m/z* 435.04, point out the relative molecular mass of **3** was 596. The ^{13}C NMR and DEPT spectra show the presence of one methoxyl, two glycosyl groups and one dihydroflavone nucleus. All protons and their associated carbons were futher assigned by the heteronuclear single quantum coherence (HSQC) spectroscopy correlations. The correlations found in correlation spectroscopy (^{1}H-^{1}HCOSY) spectrum revealed eight separated proton sequences, as depicted in Fig. 2. The ^{1}H NMR spectroscopic data showed proton signals resonating at δ_H: 3.80 (3H, s, OCH_3), 12.03 (1H, s, heavy water exchange signal disappears, OH-5) suggested the presence of a methoxyl and a hydyoxyl on the dihydroflavone nucleus [8]. Eight protons at δ_H 2.75 (2H, dd, *J* = 2.4, *J* = 18.0 Hz, H-3), 5.48 (1H, dd, *J* = 4.0, 12.0 Hz, H-2), 6.13, 6.15 (2H, *J* = 2.5 Hz, H-6, 8), 6.80 (1H, d, *J* = 8.0 Hz, H-5'), 7.02 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'), 7.10 (1H, d, *J* = 2.0 Hz, H-2') with the signals at δ_C 79.3 (C-2), 42.6 (C-3), 197.6 (C-4), 163.3 (C-5), 97.0 (C-6), 165.5 (C-7), 95.8 (C-8), 163.3 (C-9), 103.9 (C-10), 129.7 (C-1'), 111.7 (C-2'), 148.0 (C-3'), 147.4 (C-4'), 115.7 (C-5'), 120.1 (C-6'), suggested the presence of the flavonoid nucleus. The carbon signals at 56.2 (OCH_3), 98.3 (C-1″), 76.6

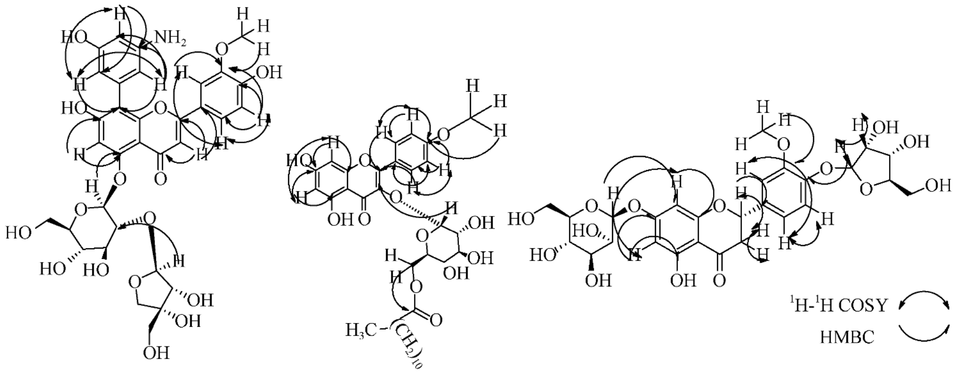
(C-2″), 77.3 (C-3″), 70.2 (C-4″), 77.0 (C-5″), 61.0 (C-6″) and 109.2 (C-1‴), 76.6 (C-2‴), 79.6 (C-3‴), 74.2 (C-4‴), 64.5 (C-5‴), indicated the presence of one methoxyl, one glucose and one apiose group which was also recognized by the proton signals at 5.05 (1H, d, *J* = 8.0 Hz, $C_{1''\text{-H}}$), 5.32(1H, 12.0 Hz, $C_{1'''\text{-H}}$), and confirmed the presence of the *β*-D-glu and *α*-D-api. The ^{1}H NMR spectroscopic data showed proton signals resonating at δ_H 5.05 (1H, d, *J* = 8.0 Hz, $C_{1''\text{-H}}$), 5.32 (1H, *J* = 12.0 Hz, $C_{1'''\text{-H}}$) correlated with C-7, C-4' respectively, confirmed that the glucose and apiose group with the dihydroflavone nucleus at C-7 and C-4'. The other HMBC correlations illustrated in Fig. 2 establisged the planar structure of **3**. Compound **3** shows negative cotton effect at 301.8 nm, and positive Cotton effect at 330 nm in CD spectrum (Fig. 3), confirmed the absolute configuration of C-2 as *S* [9]. Thus, the structure of **3** was established. Retrieved by Scifinder database, confirmed **3** a as new flavonoid glucopyranoside, (2*S*)-homeriodictyol-7-*O*-*β*-D-glucoside-4′-*O*-*α*-D-apiofuranosyl, named as named viscumneoside XIV.

According to the reported data, the structures of the seven known compounds were identified as rhamnazin-3-*O*-*β*-D-api-(1→2)-[6"-(3-hydroxy-3-methylglutaryl)]-*O*-*β*-D-glucopyranoside (**4**) [10], rhamnazin-3-*O*-*β*-D-[6"-(3-hydroxy-3-methylglutaryl)]-*O*-*β*-D-glucopyranoside (**5**) [11], rhamnazine (**6**) [10-11], rhamnazin- 3-*O*-*β*-*D*-glucopyranoside (**7**) [12], rhamnazin-4'-*O*-*β*-D-glucopyranoside (**8**) [13], rhamnazin-3- *O*-*β*-D-6"-acetyl-*O*-*β*-D-glucopyranoside (**9**) [14], 3', 4', 3, 5-tetrahydroxyl-7-methoxyl flavonoid (**10**) [15]. Among them, compounds **1**, **2** and **3** were new natural productes, their NMR data were not reported previously.



1 **2** **3**

Fig. 1 Structures of compounds 1−3



1 **2** **3**

Fig. 2 Selected ^{1}H-^{1}HCOSY and HMBC correlation of planar structres of compounds 1−3

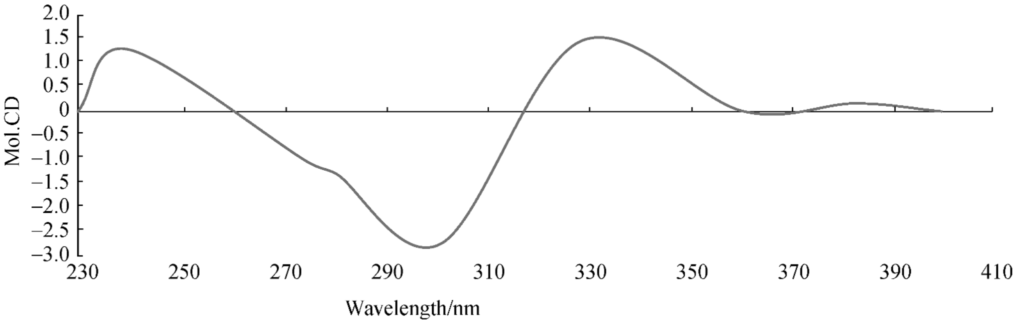


Fig. 3 CD spectrum of compound 3

Compounds **1−10** were evaluated for cytotoxic activity against C6, A549, TF-1, Hela and MDA-MB-231 cell lines using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide].

Compounds **1−10** demonstrated significant inhibitory activities against C6, A549 and MDA-MB-231, and the inhibition rate arrived about 50%, 70% and 74% respectively with $IC_{50} \leq 60.00$ μmol·L^{-1}, however the inhibition of TF-1 and Hela only about 10%. A549, TF-1, Hela, MDA-MD-231 and C6 cell lines were obtained from the Shanghai Cell Bank, Novartis Pharmaceuticals. All assays were performed in duplicate. Doxorubicin hydrochloride (Sigma; Shanghai; China; CAS No. 25316-40-9; purity ≥ 95%) was used as a positive control.

The acid hydrolysis and detection of the sugar component in compound **1**, **2** and **3** was carried out according to the method described in the literature [16]. Compound **1**, **2** and **3** (about 1.5 mg) was refluxed respectively in 2 mL of dioxane (1 : 1) for 2 h, evaporated to dryness, dissolved with 100 μL of anhydrous pyridine and 200 μL (0.1 mol·L^{-1}) of cysteine Methyl ester hydrochloride. The mixture was heated at 60 °C for 1 h and 2.6 mL of trimethyl silicide HMDS-TMCS (hexamethyldisilazane : trimethylchlorosilane : pyridine, 2 : 1 : 10) was added and continued at 60 °C After heating for 30 min and the reaction wascomplete, the mixture was extracted with 2 mL of cyclohexane and the extract was subjected to GC-MS analysis to identify the sugar configuration.

The reference substance monosaccharide (*α* and *β*-D-glucopyranose, *α*-D-apiofuranose) was also treated by the above-mentioned method, and its cyclohexane extract was analyzed by GC-MS. The test results $t_{R(\alpha\text{-D-glu})}$ = 5.6 min, $t_{R(\beta\text{-D-glu})}$ = 6.2 min and $t_{R(\alpha\text{-D-api})}$ were taken as control.

Table 2 Cytotoxic activities of compounds 1−10 against five tumor cell lines

Compound IC_{50} (μmol·L^{-1})	1	2	3	4	5	6	7	8	9	10	DOX[a]
C6	22.41	22.11	12.66	24.05	53.69	33.75	53.76	48.00	37.67	47.56	6.58
MDA-MB-231	30.70	30.86	30.50	59.70	40.05	29.80	50.00	30.30	49.76	39.43	5.32
A549	39.84	40.73	29.49	50.21	10.75	39.69	50.63	9.61	40.32	40.22	4.21

[a] DOX: Doxorubicin hydrochloride, positive control

Experimental

General

NMR spectra were measured on a Bruker AV-600 spectrometer (Bruker Beijing Co., Ltd., Beijing, China) with TMS as the internal standard. UV spectra were recorded on a Shimadzu UV-2550 spectrophotometer (Shimadzu Guangdong Co., Ltd., Guangdong, China). ESI-MS were acquired on an Agilent LC-MSD Trap XCT mass spectrometer (Agilent Beijing Co., Ltd., Beijing, China), whereas HR-ESI-MS were measured using a Waters Q-TOF micro mass spectrometer (Waters, Shanghai Co., Ltd., Shanghai, China). Analytical HPLC was carried out with a Waters 515/2487 instrument and a Chiralpak Semi-preparative HPLC was conducted on a Waters 510/484 instrument (Waters, Shanghai Co., Ltd., Shanghai, China) with a YMC-Pack ODS-A column (5 μm, 10.0 mm × 250 mm). Materials for column chromatography were silica gel (200−300 and 300−400 mesh; Huiyou Silical Gel Development Co.), Sephadex LH-20 (40−7 μm; Amersham Pharmacia Biotech), and RP-18 silica gel (Greenherbs Sci & Tech Development Co.). All other chemicals used in the study were of analytical grade.

Plant material

The dry stem and leaves of *Viscum album* were collected in Anhui Province, China, in June 2014, and were identified by WU Tong, Ph.D., Department of Chinese Traditional Medicine, Shanghai Institute of Pharmaceutical Industry. A voucher specimen has been deposited in the Department of Chinese Traditional Medicine, Shanghai Institute of Pharmaceutical Industry (No. 14031901).

Extraction and Isolation

The dry stem and leaves of *Viscum album* were extracted with 95% Ethanol under refluxing four times each for 2 h. After evaporation of the solvent, the extract was diluted by water (4.0 L) then partitioned three times with $CHCl_3$ (1.8 L). The fluid extract of water diluted by 3.8 L water, was subjected to column chromatography (CC) over macroporous resin (D101) and eluted with a mixture of water−Ethanol (0% ethanol, 10%, 30%, 50%, 70%, 95% Ethanol) to give 6 major fractions on the basis of TLC (water, 10%, 30%, 50%, 70%, 95% Ethanol). 50% extract was subjected to column chromatography (CC) over silica gel (200−300 mesh, 2.0 kg, 10 cm × 120 cm) and eluted with a mixture of ethyl acetate−MeOH (100 : 0→0 : 100) gained 5 subfractions on the basis of TLC (Fr. B1−B5). Fr. B3 (23.0 g) was rechromatographed on silica gel CC (200−300 mesh, 600 g, 5 cm × 80 cm) eluted with a mixture of ethyl Acetate−MeOH (50 : 1→1 : 1) to afford 7 subfractions on the basis of TLC (Fr. B3.1−B3.7). Fr. B3.4 (7 g) was futher separated by repeated silica gel (300−400 mesh, 120 g, 2.5 cm × 60 cm) chromatogry ($CHCl_3$−MeOH 18 : 1→1 : 1), one fraction has the crystal precipitation in the process of placing then after recrystallized from absolute ethanol to give ochre yellow powder **3** about 1.5 g and other fractions purified by Sephadex LH-20 (100 g, 3.0 cm × 100 cm, MeOH, 800 cm) to give **5** (16.8 mg) and **6** (33.7 mg) and **8** (28.6 mg). Fr. B3.5 (5.8 g) was subjected to an RP-18 column (180 g, 5.0 cm × 60 cm) eluted with H_2O−MeOH (100%→30%, 1800 mL) to obtain **3** (9.9 mg, 200−300 mL),

4 (18.2 mg, 600−900 mL) and **7** (15.2 mg, 1200−1400 mL). Fr. B3.7 (8.2 g) was subjected to silica gel CC (300−400 mesh, 200 g, 2.5 cm × 120 cm) eluted with Acetate−MeOH (30 : 1→1 : 1), and finally purified by semi-preparative HPLC (MeOH : H_2O = 40 : 60, 3.0 mL·min^{-1}) to give **1** (8.50 mg, t_R = 11.5 min), **2** (7.9 mg, t_R = 15.5 min), **9** (10.2 mg, t_R = 27.7 min), and **10** (8.3 mg, t_R = 32.3 min).

Acknowledgements

We thank ZHONG Jia-Liang (Analysis and Test Center, Shanghai Institute of Pharmaceutical Industry) for IR and Prof. WU Jian-Jun (Shanghai Institute of Metrology and Testing, SIMT) for NMR spectral measurement.

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Cite this article as: DAI Jia-Kun, CAO Duo, LI Cui-Hua, GAO Jing, LI Meng-Qing, FAN Na, WEI Ya-Hui, SUN Zheng-Liang, HOU Meng-Yang. Three new bioactive flavonoid glycosides from *Viscum album* [J]. *Chin J Nat Med*, 2019, **17**(7): 545-550.