Three new bioactive flavonoid glycosides from *Viscum album*

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**[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\textsubscript{50} \leq 60.00 \mu mol·L\textsuperscript{-1}), while the inhibition of TF-1 and Hela was not significant.

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

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**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**

Compounds 1 was obtained as a yellow amorphous powder. The positive ESI-MS of 1 showed a [M + Na\textsuperscript{+}] peak at m/z 724.03, a [M + K\textsuperscript{+}] peak at m/z 740.04, a [2M + Na\textsuperscript{+}] peak at m/z 1425.15 and a [2M + K\textsuperscript{+}] peak at m/z 1446.99, pointing out the relative molecular mass of 1 was 701. The HR-ESI-MS showed a [M + Na\textsuperscript{+}] peak at m/z 724.1877 (Calcd. 724.1854) and established a molecular formula of C\textsubscript{33}H\textsubscript{35}NO\textsubscript{16}, implying 17 degrees of unsaturation. The 13C 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...
nucleus. The $^1$H NMR spectropic data (Table 1) showed protons resonating at δ_H at 5.52 (1H, s, β-D-api C1-H), 6.00 (1H, d, J = 8.0 Hz, β-D-glu C1-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), 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).

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<td>5″</td>
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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$_3$), confirmed the presence of one methoxy group, and one amine group. All protons and their associated carbons were further 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, 6.0 Hz), 6.88 (1H, d, 2.0 Hz) with $^{13}$C NMR data δ$_C$ 127.6, 115.0, 151.3, 144.1, 113.6, confirmed the presence of one symmetric trisubstituted phenyl. The $^1$H NMR spectroscopic data showed proton signals resonating at δ$_H$ 6.93 (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 of (C$_3$H$_7$NO$_3$) with ESI-MS (701) suggested trisubstituted phenyl with hydroxyland amine at 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$_H$ 6.6-6.8 ppm to the low field, δ$_C$-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 7 carbon nucleus signal, the δ(C-6") moved 8.8 ppm to the low field, δ(C-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 δ(C-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). 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(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″″-H), 5.32 (1H, 12.0 Hz, C-1″″-H), and confirmed the presence of the β-D-glu and α-D-api. The 1H NMR spectroscopic data showed proton signals resonating at δH 5.05 (1H, d, J = 8.0 Hz, C-1″″-H), 5.32 (1H, J = 12.0 Hz, C-1″″-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 established 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 as a new flavonoid glucopyranoside, (2S)-homericidictyol-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-tetrahydroxy-7-methoxyl flavonoid (10) [15]. Among them, compounds 1, 2 and 3 were new natural products, their NMR data were not reported previously.

![Fig. 1 Structures of compounds 1–3](image1.png)

![Fig. 2 Selected 1H-1HCOSY and HMBC correlation of planar structures of compounds 1–3](image2.png)

![Fig. 3 CD spectrum of compound 3](image3.png)
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 IC50 ≤ 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 was complete, 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 \( t_{f(β-D-glu)} = 5.6 \text{ min, } t_{f(β-D-apio)} = 6.2 \text{ min and } t_{f(β-D-apio)} \) were taken as control.

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

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<th>4</th>
<th>5</th>
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<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>DOX*</th>
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<td>22.11</td>
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<td>53.76</td>
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*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 conducted 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 CHCl₃ (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 → 100 : 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) chromatography (CHCl₃–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 H₂O–MeOH (100%→30%, 1800 mL) to obtain 3 (9.9 mg, 200–300 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 : H₂O = 40 : 60, 3.0 mL·min⁻¹) to give 1 (8.50 mg, tᵣ = 11.5 min), 2 (7.9 mg, tᵣ = 15.5 min), 9 (10.2 mg, tᵣ = 27.7 min), and 10 (8.3 mg, tᵣ = 32.3 min).

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References