Cytotoxic Constituents of *Viscum coloratum*

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Phytochemical studies on *Viscum coloratum* have resulted in the isolation of nineteen compounds. The structures of the isolated compounds were identified on the basis of 1D, 2D NMR and HR-ESI-Q-TOF-MS. Pachypodol (**4**) and ombuine (**6**) were characterized in the family Loranthaceae for the first time. 1,7-Bis(4-hydroxyphenyl)-1,4-heptadien-3-one (**8**) and 5-hydroxy-3,7,3'-trimethoxyflavone-4'-*O*-*β*-D-glucoside (**13**) were two new natural compounds, which exhibited cytotoxic activities against four human tumour cell lines (HeLa, SGC-7901, MCF-7, and U251).

Key words: Viscum coloratum, Diarylheptanoids, Cytotoxic Activities

Introduction

Viscum coloratum (Kom.) Nakai is a perennial, evergreen, semi-parasitic plant which grows on the branches or stems of deciduous trees. It is known as Hujisheng in China (China Pharmacopoeia Committee, 2010). *V. coloratum* is an important medicinal herb, suitable for commercial production, which has been used for the treatment of various conditions including cancer, cardiovascular diseases, hepatitis, and hemorrhage (Wang *et al.*, 2005). It has been reported that the total alkaloids of *V. coloratum* exhibited cytotoxic activities against A-549 non-small cell lung, MCF-7 breast, and Eca-109 esophageal cancer cells, respectively (Chen *et al.*, 2005). Phytochemical investigation of *V. coloratum* also showed the presence of many other types of compounds including flavonoids, triterpenoids, lignans, and diarylheptanoids (Wang *et al.*, 2005; Leu *et al.*, 2006). Some of them exhibited inhibitory activity on cancer cell growth. For example, homoeriodictyol markedly inhibited the growth of HeLa human uterine carcinoma cells (Akihisa *et al.*, 1988). Oleanolic acid exhibited cytotoxic activity against A-549 non-small cell lung, SK-OV-3 ovary, SK-MEL-2 melanoma, and HCT-15 colon cancer cell lines, respectively (Kim *et al.*, 2000), while syringaresinol inhibited the proliferation of human promyelocytic HL-60 cells (Park *et al.*, 2008). In addition, 1,7-di(3',4'-dihydroxyphenyl)-4-hepten-3-one exhibited cytotoxic activity against UACC-62 melanoma, TK-10 renal, and MCF-7 breast cancer cells, respectively (Martín-Cordero *et al.*, 2001).

As part of our program aimed at the isolation of bioactive components, we undertook a detailed chemical study of *V. coloratum*. In the present paper, the isolation and identification of nineteen compounds, including two new compounds, 1,7-bis(4-hydroxyphenyl)-1,4-heptadien-3-one (**8**) and 5-hydroxy-3,7,3'-trimethoxyflavone-4'-*O*-*β*-D-glucoside (**13**), are described. Pachypodol **4** and ombuine **6** were isolated from the family Loranthaceae for the first time. The *in vitro* antiproliferative activities of compounds **8** and **13** against four human cancer cell lines were also evaluated.

Material and Methods

General

The NMR spectra were recorded on a Bruker-ARX 300 or a Bruker Avance-600 spectrometer (Fällanden, Switzerland) operating at 300 or 600 MHz for ^{1}H and 75 or 150 MHz for ^{13}C NMR spectroscopy, respectively. Chemical shifts were reported in ppm on the δ scale with tetramethyl-

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silane (TMS) as internal standard. Electrospray-ionization (ESI) mass spectra were recorded on a Shimadzu 2010 liquid chromatograph-mass spectrometer (Kyoto, Japan). High-resolution electrospray-ionization mass spectroscopy (HR-ESI-MS/MS) was performed on a Bruker ESI-Q-TOF-MS/MS spectrometer (Bremen, Germany). The melting points were obtained from a thermal values analysis with a microscope and are uncorrected (Beijing Taike Chemical Apparatus Co., Ltd., Beijing, China). 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 *V. coloratum* were collected in Liaoning province of China (host tree: *Populus ussuriensis* KOM.). They were authenticated by Professor Qi-Shi Sun, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, China, where a voucher specimen (No. 2008001) was deposited.

Extraction and isolation

Air-dried stems and leaves of *Viscum coloratum* (10 kg) were extracted with 95% EtOH under reflux. The extract was concentrated under reduced pressure to give a brown syrup (1.2 kg). The syrup was suspended in H_2O and partitioned with petroleum ether, EtOAc, and *n*-BuOH, successively. The EtOAc extract (200 g) was chromatographed on a silica gel column eluted with a gradient mixture of $CHCl_3$/MeOH (100:0 to 0:100) to provide 9 fractions (F1 to F9). F1, F2, and F3 yielded compounds **1** (15 mg), **2** (20 mg), and **3** (150 mg) after recrystallization from MeOH, respectively. F4 was subjected to chromatography on a silica gel column using a gradient mixture of petroleum ether and Et_2O (50:1 to 1:1). Subfractions from F4 were further purified using a Sephadex LH-20 column to yield compounds **4** (17 mg) and **5** (20 mg). F5 was chromatographed on a silica gel column using a gradient mixture of petroleum ether and Et_2O (20:1 to 1:1), and then further purified by passage through a Sephadex LH-20 column and recrystallization to yield compounds **6** (25 mg) and **7** (15 mg). Separation of F6 on a silica gel column using a gradient mixture of petroleum ether and Et_2O (10:1 to 1:1) and on an ODS column by preparative HPLC (60% MeOH) afforded compound **8** (30 mg). F7, F8, and F9 were applied to a silica gel column eluted with a gradient of $CHCl_3$ and MeOH to give compounds **9** (45 mg), **10** (50 mg), and **11** (10 mg).

The *n*-BuOH extract (79 g) was loaded onto a polyamide column eluted sequentially with H_2O, 25%, 50%, and 95% EtOH (v/v). The eluates were concentrated to give 4 fractions: F-I (H_2O), F-II (25% EtOH), F-III (50% EtOH), and F-IV (95% EtOH). F-II was rechromatographed on a polyamide column eluted with a gradient mixture of $CHCl_3$ and MeOH (100:1 to 0:100) to afford 6 subfractions (sub-1 to sub-6). F-II sub-2 was further purified by passage through a Sephadex LH-20 column and by preparative HPLC (ODS, 55% MeOH) to obtain compounds **12** (120 mg) and **13** (30 mg). F-II sub-3 was purified using preparative HPLC to afford compounds **14** (1.1 g) and **15** (40 mg). F-III was rechromatographed on a polyamide column eluted with a gradient mixture of $CHCl_3$ and MeOH (100:0 to 0:100) to give 5 subfractions (sub-1 to sub-5). Further purification of F-III sub-2 using a Sephadex LH-20 column gave compounds **16** (6 mg) and **17** (21 mg). Compound **18** (25 mg) was purified using preparative HPLC of F-III sub-3. Further chromatography of F-IV on a polyamide column eluting with a gradient mixture of $CHCl_3$ and MeOH (100:0 to 0:100) produced five subfractions (sub-1 to sub-5). Compound **19** (24 mg) was purified using preparative HPLC of F-IV sub-2.

1,7-Bis(4-hydroxyphenyl)-1,4-heptadien-3-one (**8**): Pale yellow amorphous powder (MeOH). – M.p. 145~147 °C. – ESI-MS (positive ion mode): m/z = 295.1 $[M+H]^+$, 589.2 $[2M+H]^+$. – ESI-MS (negative ion mode): m/z = 293.1 $[M–H]^-$, 587.3 $[2M–H]^-$. – HR-ESI-MS: m/z = 295.1328 $[M+H]^+$ (calcd. for $C_{19}H_{19}O_3$: 295.1329). – ^1H, ^{13}C NMR, and HMBC: see Table I.

5-Hydroxy-3,7,3'-trimethoxyflavone-4'-O-β-D-glucoside (**13**): Yellow cluster crystals (MeOH). – M.p. 288~289 °C. – ESI-MS: m/z = 505.2 $[M–H]^-$. – ^1H NMR (300 MHz, DMSO-d_6): δ = 3.88 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 5.07 (1H, d, J = 7.2 Hz, H-1″), 6.40 (1H, d, J = 1.7 Hz, H-6), 6.83 (1H, d, J = 1.7 Hz, H-8), 7.71 (1H, d, J = 1.1 Hz, H-2′), 7.28 (1H, d, J = 8.4 Hz, H-5′), 7.68 (1H, dd, J = 8.4 Hz, H-6′), 12.6 (1H, s,

OH-5). – ^{13}C NMR (75 MHz, DMSO-d_6): δ = 60.0 (OCH_3), 55.9 (OCH_3), 56.2 (OCH_3), 155.4 (C-2), 138.5 (C-3), 178.3 (C-4), 161.5 (C-5), 98.0 (C-6), 165.4 (C-7), 92.7 (C-8), 156.5 (C-9), 105.4 (C-10), 123.3 (C-1′), 121.9 (C-2′), 148.7 (C-3′), 149.1 (C-4′), 115.0 (C-5′), 112.2 (C-6′), 99.6 (C-1″), 73.2 (C-2″), 77.2 (C-3″), 69.7 (C-4″), 76.9 (C-5″), 60.9 (C-6″).

Pachypodol (**4**): Yellow needle crystals ($CHCl_3$/MeOH). – M.p. 171~173 °C. – ESI-MS: *m/z* = 343.1 $[M–H]^-$. – ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.81 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 6.37 (1H, d, J = 2.0 Hz, H-6), 6.78 (1H, d, J = 2.0 Hz, H-8), 6.96 (1H, d, J = 8.0 Hz, H-5′), 7.62 (1H, d, J = 8.4 Hz, H-6′), 7.66 (1H, d, J = 2.0 Hz, H-2′), 9.97 (H, s, 4′-OH), 12.65 (1H, s, 5-OH). – ^{13}C NMR (75 MHz, DMSO-d_6): δ = 55.9 (OCH_3), 56.3 (OCH_3), 59.9 (OCH_3), 92.6 (C-8), 98.0 (C-6), 105.3 (C-10), 112.1 (C-2′), 115.8 (C-5′), 120.8 (C-1′), 122.5 (C-6′), 138.1 (C-3), 147.6 (C-3′), 150.0 (C-4′), 155.9 (C-2), 156.4 (C-5), 161.0 (C-9), 165.2 (C-7), 178.2 (C=O).

Ombuine (**6**): Lemon yellow needle crystals (MeOH). – M.p. 221~223 °C. – ESI-MS: *m/z* = 329.2 $[M–H]^-$. – ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.88 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 6.36 (1H, d, J = 2.1 Hz, H-6), 6.79 (1H, d, J = 2.1 Hz, H-8), 6.95 (1H, d, J = 8.4 Hz, H-5′), 7.74 (1H, d, J = 8.4 Hz, H-6′), 7.78 (1H, d, J = 5.2 Hz, H-2′). – ^{13}C NMR (75 MHz, DMSO-d_6): δ = 56.1 (OCH_3), 56.3 (OCH_3), 92.4 (C-8), 97.8 (C-6), 104.2 (C-4a), 111.9 (C-5′), 115.8 (C-2′), 122.1 (C-6′), 122.1 (C-1′), 136.4 (C-3), 147.3 (C-3), 147.7 (C-2), 149.4 (C-4′), 156.4 (C-8a), 160.6 (C-5), 165.2 (C-7), 176.2 (C-4).

Cell culture

HeLa (human uterine carcinoma), SGC-7901 (human gastric cancer), MCF-7 (human breast cancer), and U251 (human glioma) cell lines were obtained from American Type Culture Collection (#CRL, 1872; ATCC, Manassas, VA, USA) and cultured in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) including 10% fetal bovine serum. All cells were maintained in an incubator at 37 °C, in a humidified 5% CO_2 atmosphere. The confluent cells were used for the cytotoxicity assay.

Cytotoxic assay

Inhibition of cellular growth was estimated using 3-(dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, Milwaukee, WI, USA) as described by Mosmann (1983). *cis*-Diamminedichloroplatinum (DDP) (Qilu Pharmaceutical Co., Ltd., Jinnan, Shandong, China) was the reference drug.

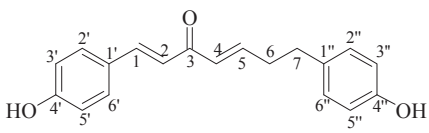
Results and Discussion

The EtOAc and *n*-BuOH extracts of *Viscum coloratum* yielded nineteen compounds. Compound **8** is a new compound, 1,7-bis(4-hydroxyphenyl)-1,4-heptadien-3-one. Compound **13** 5-hydroxy-3,7,3'-trimethoxyflavone-4'-*O*-*β*-D-glucoside, had been synthetized previously (Ishitsuka *et al.*, 1980); however, this is the first time it has been found as a natural product. The known compounds, lupeol acetate (**1**) (Wang *et al.*, 1995), *β*-sitosterol (**2**) (Li *et al.*, 2001), oleanolic acid (**3**) (Dai *et al.*, 2006), pachypodol (**4**) (Itokawa *et al.*, 1981), syringaresinol (**5**) (Nawwar *et al.*, 1982), ombuine (**6**) (Itokawa *et al.*, 1981), quercetin-3,3'-dimethyl ether (**7**) (Kumari *et al.*, 1986), homoeriodictyol-7-*O*-*β*-D-apiosyl-(1→5)-*β*-D-apiosyl-(1→2)-*β*-D-glucoside (**9**) (Kong *et al.*, 1988a), syringin (**10**) (Sun *et al.*, 2000), protocatechuic acid (**11**) (Gutzeit *et al.*, 2007), rhamnazin-3-*O*-*β*-D-glucoside (**12**) (Kong *et al.*, 1987), homoeriodictyol-7-*O*-*β*-D-glucoside (**14**) (Fukunaga *et al.*, 1988), homoeriodictyol-7-*O*-*β*-D-apiosyl-(1→2)-*O*-*β*-D-glucoside (**15**) (Kong *et al.*, 1988a), rhamnazin-3-*O*-*β*-D-6''-acetylglucoside (**16**) (Kong *et al.*, 1987), rhamnazin-3-*O*-*β*-D-(6''-*β*-hydroxy-*β*-methylglutaryl)-glucoside (**17**) (Kong *et al.*, 1988b), isorhamnetin-3-*O*-*β*-D-glucoside (**18**) (Kong *et al.*, 1988a), and homoeriodictyol (**19**) (Wagner *et al.*, 1976), were identified by comparison of their spectral data with corresponding literature values. Among them, pachypodol (**4**) and ombuine (**6**) were isolated from the family Loranthaceae for the first time (Fig. 1).

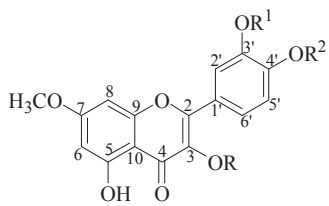
Compound **8** was obtained as a pale yellow powder (MeOH). Its structure was elucidated by examination of the MS and NMR data. Compound **8** exhibited an $[M+H]^+$ pseudomolecular ion at *m/z* 295.1328 (calcd. for $C_{19}H_{19}O_3$: 295.1329) using positive HR-ESI-MS, consistent with the molecular formula $C_{19}H_{18}O_3$. The ^{1}H NMR spectrum (Table I) of **8** showed the presence of two pairs of doublet signals for two protons, δ_H 6.66 ppm (H-3″/5″, J = 7.8 Hz), 7.01 ppm (H-2″/6″, J = 7.8 Hz), 6.80 ppm (H-3′/5′, J = 8.4 Hz), 7.58 ppm (H-2′/6′, J = 8.4 Hz), due to two *para*-substituted benzene rings, two pairs of *trans*-olefinic doublet protons at

δ_H 6.48 ppm (H-4, d, J = 15.8 Hz), 6.95 ppm (H-5, d, J = 15.8 Hz), 6.97 ppm (H-2, d, J = 15.8 Hz), 7.54 ppm (H-1, d, J = 15.8 Hz), two methylene protons with chemical shifts of δ_H 2.50 ppm (H-6, t) and 2.67 ppm (H-7, t), and two phenolic hydroxy protons at δ_H 9.17 ppm (4″-OH, brs), 10.1 ppm (4′-OH, brs). The ^{13}C NMR spectrum (Table I) of **8** consisted of 15 signals, including characteristic signals of two methylene carbon atoms (C-6, δ_C 33.1 ppm, and C-7, δ_C 34.2 ppm), a carbonyl group (C-3, δ_C 188.4 ppm), and two phenolic carbon atoms (C-4'', δ_C 155.6 ppm, and C-4', δ_C 160.1 ppm). The structure of **8**, including the locations of the substituents, was determined from the HMBC spectrum (Fig. 2). The long-range correlations H-1/C-2′6′, H-2′,6′/C-1, H-7/C-2″,6″, and H-2″,6″/C-7 indicated that two *para*-substituted benzene rings were located at C-1 and C-7, respectively, while the correlations between the carbonyl carbon atom and H-1, H-2, H-4, or H-5 indicated the position of the carbonyl group at C-3. Thus, the structure of compound **8** was identified as 1,7-bis(4-hydroxyphenyl)-1,4-heptadien-3-one (Fig. 1).

Compound **13** was obtained as yellow cluster crystals. The ^{13}C NMR spectrum consisted of 24 signals. The ^{1}H NMR signals showed a typical β-D-glucoside pattern with chemical shifts of δ_H 5.07 ppm (1H, d, J = 7.2 Hz). Furthermore, it was found that there was one phenolic hydroxy proton at δ_H 12.6 ppm, three methoxy signals at δ_H 3.84 ppm (3-OMe) and 3.88 ppm (7-OMe, 3′-OMe), and five aromatic proton signals at δ_H 6.40 ppm (1H, d, J = 1.7 Hz), 6.83 ppm (1H, d, J = 1.7 Hz), 7.28 ppm (1H, d, J = 8.4 Hz), 7.68 ppm (1H, dd, J = 8.4 Hz), and 7.71 ppm (1H, d, J = 1.1 Hz), suggesting that **13** has a flavonoid skeleton with one substituted hydroxy group, three methoxy groups, and one glucose moiety. The cross-peaks of C-3 (138.5 ppm)/OMe (3.84 ppm) and C-7 (165.4 ppm), C-3′ (148.7 ppm)/OMe (3.88 ppm) in the HMBC spectrum demonstrated that these methoxy groups are linked to C-3, C-7, and C-3′, respectively. The position of the glycosidation was deduced to be C-4' (O) from an HMBC experiment which provided a key long-range correlation between the anomeric proton signal at δ_H 5.07 ppm and the carbon resonance at δ_C 149.1 ppm (C-4'). Thus, compound **13** was assigned the structure 5-hydroxy-3,7,3'-trimethoxyflavone-4'-*O*-β-D-glucoside (Fig. 1). As



8



	R	R^1	R^2
4	CH_3	CH_3	H
6	H	H	CH_3
13	CH_3	CH_3	Glc

Fig. 1. Chemical structures of compounds **4**, **6**, **8**, and **13**.

Table I. Chemical shifts (δ in ppm, J in Hz) and correlations of **8** in DMSO-d_6 (600 MHz for ^{1}H and 150 MHz for ^{13}C NMR).

Position	^{1}H	^{13}C	HMBC
1	7.54 (d,15.8)	143.1	C-2, 3, 1′, 2′, 6′
2	6.97 (d,15.8)	121.9	C-3, 4, 1′
3	-	188.4	
4	6.48 (d,15.8)	129.6	C-3, 6
5	6.95 (d,15.8)	146.7	C-3, 6, 7
6	2.50 (t)	33.1	C-5, 7, 1″, 2″, 6″
7	2.67 (t, 7.8)	34.2	C-5, 6, 1″, 2″, 6″
1′	-	125.8	-
2′, 6′	7.58 (d, 8.4)	130.7	C-1, 4′
3′, 5′	6.80 (d, 8.4)	116.0	C-1′, 4′
4′	-	160.1	-
1″	-	131.2	-
2″, 6″	7.01 (d, 7.8)	129.3	C-7, 4″
3″, 5″	6.66 (d, 7.8)	115.2	C-1″, 4″
4″	-	155.6	-
4′-OH	10.1 (brs)	-	C-3′, 5′, 4′
4″-OH	9.17 (brs)	-	C-3″, 5″, 4″

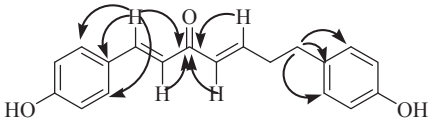


Fig. 2. Significant HMBC correlations of **8**.

the structure of **13** has already been reported as a synthetic product (Ishitsuka *et al*., 1980), it was isolated as a new natural product in the present study.

The cytotoxic activities of compounds **8** and **13** were determined using HeLa, SGC-7901, MCF-7, and U251 cells. The results are summarized in Table II. Compound **8** showed significant cytotoxic activity against HeLa, SGC-7901, and MCF-7 cells, respectively, while compound **13** exhibited moderated cytotoxic activity against HeLa, MCF-7, and U251 cells, respectively. This is the first time these cytotoxic activities have been reported.

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Table II. Cytotoxic activity (IC_{50}) of **8** and **13** against HeLa, SGC-7901, MCF-7, and U251 cell lines.

Compound	IC_{50} [μM][a]			
	HeLa	SGC-7901	MCF-7	U251
8	12.08 ± 0.16	44.69 ± 3.23	13.13 ± 0.41	> 100
13	75.74 ± 8.42	> 100	34.78 ± 1.77	23.08 ± 2.22
DDP[b]	17.65 ± 1.52	4.35 ± 0.12	19.12 ± 2.38	59.21 ± 5.36

[a] IC_{50} is defined as the concentration which resulted in a 50% decrease in the cell number. The values represent the mean of three independent experiments.
[b] Reference drug *cis*-diamminedichloroplatinum.

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