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Mistletonone, a Novel Antioxidative Diarylheptanoid from the Branches and Leaves of *Viscum coloratum*

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Abstract: Mistletonone, a novel diarylheptanoid, was isolated from the branches and leaves of *Viscum coloratum* (Kom.) Nakai (Loranthaceae). It was identified as 1,7-di-(*p*-hydroxyphenyl)-5-hydroxyl-*cis*-2,3-epoxy-1-one on the basis of spectral evidence. The compound showed significant scavenging effects on hydroxyl radicals and superoxide anion radicals in the direct assay using the electron spin resonance (ESR) technique.

Keywords: *Viscum coloratum*; diarylheptanoid; mistletonone; antioxidative activity

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Introduction

Viscum coloratum (Kom.) Nakai (Loranthaceae) is a semi-parasitic plant distributed throughout the southwestern provinces of China. The branches and leaves of this plant are used in traditional Chinese medicine mainly for the treatment of hypertension, artherosclerosis, rheumatism and neuralgia [1]. Previous phytochemical investigations revealed that flavonoids are the major secondary metabolites of

this species [2-6]. The ethanol extract of *V. coloratum* showed potent antioxidative properties in our screening program searching for antioxidative natural substances from traditional Chinese medicines, and further bioassay-guided analysis led to the isolation of a novel diarylheptanoid, mistletonone (**1**), from the active antioxidative extract. In this paper, we report the isolation, structure elucidation and antioxidative activities of this compound.

Results and Discussion

Mistletonone (**1**) was obtained as a pale yellow powder. Its molecular formula was established as $C_{19}H_{20}O_5$ by HR-ESI-MS at *m/z* 351.1203 $[M+Na]^+$ (calcd. 351.1208), and confirmed by the ^{13}C-NMR spectrum. Compound **1** displayed an absorption maximum at 286 nm in the UV spectrum and intense IR bands at 3448 and 1630 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectra data are summarized in Table 1.

Table 1. ^{1}H- and ^{13}C-NMR data of mistletonone*.

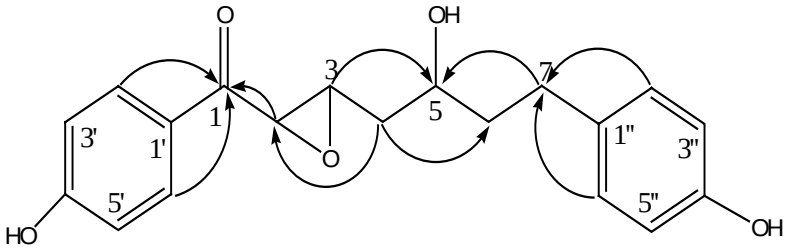
C/H	δ C	δ H	^{1}H-^{1}H COSY
1	191.8 (s)		
2	56.4 (d)	4.38 (d, 1H, *J* = 4.8 Hz)	H-3
3	55.8 (d)	3.48 (m, 1H)	H-2, H-4
4	35.2 (t)	1.47 (m, 2H)	H-3, H-5
5	67.7 (d)	3.48 (m, 1H)	H-4, H-6
6	40.2 (t)	1.47 (m, 2H)	H-5, H-7
7	30.8 (t)	2.35 (m, 2H)	H-6
1'	127.7 (s)		
2'	130.9 (d)	7.84 (d, 1H, *J* =8.3 Hz)	H-3’
3'	115.8 (d)	6.76 (d, 1H, *J* = 8.3 Hz)	H-2’
4'	163.3 (s)		
5'	115.8 (d)	6.76 (d, 1H, *J* = 8.3 Hz)	H-6’
6'	130.9 (d)	7.84 (d, 1H, *J* = 8.3 Hz)	H-5’
1"	132.5 (s)		
2"	129.2 (d)	6.84 (d, 1H, *J* = 7.9 Hz)	H-3”
3"	115.4 (d)	6.60 (d, 1H, *J* = 7.9 Hz)	H-2”
4"	155.5 (s)		
5"	115.4 (d)	6.60 (d, 1H, *J* =7.9 Hz)	H-6”
6"	129.2 (d)	6.84 (d, 1H, *J* =7.9 Hz)	H-5”
4’, 4"(OH)		3.30 (s, 2H)	

* 500 and 125 MHz, respectively, DMSO-d_6.

NMR resonances were assigned by means of DEPT, COSY, HSQC, and HMBC (Figure 1) experiments. The relative configuration of the 2,3-epoxy group was determined to be *cis*-, as judged by the coupling constants ($J_{2,3}$= 4.8 Hz) [7-8]. The stereochemistry of the hydroxyl group at C5 was not

determined due to the limited amount of sample available. The structure of compound **1** was therefore elucidated as 1,7-di-(*p*-hydroxyphenyl)-5-hydroxyl-*cis*-2,3-epoxy-1-one, which we have named mistletonone.

Figure 1. Structure and the key HMBC correlations of mistletonone.



The antioxidative activities of mistletonone (**1**) were directly detected by electron spin resonance (ESR). The compound showed scavenging effects on hydroxyl radicals and superoxide anion radicals with IC_{50} values of 0.485 mM and 0.273 mM, comparable with those of the positive control EGCG (0.538 mM, 0.131 mM, respectively). The ESR spectra of hydroxyl radicals and superoxide anion radicals, and the dose dependent scavenging effects of mistletonone are shown in Figures 2 and 3, respectively.

Figure 2. The ESR spectrum of hydroxyl radicals and the dose dependent scavenging effects of mistletonone. Hydroxyl radicals were generated by a Fenton reaction. ESR conditions were as described in the text. a ~ e were the scavenging effects of mistletonone in the concentration of 0.18, 0.36, 0.54, 0.72, and 0.90 mM, respectively; f: blank.

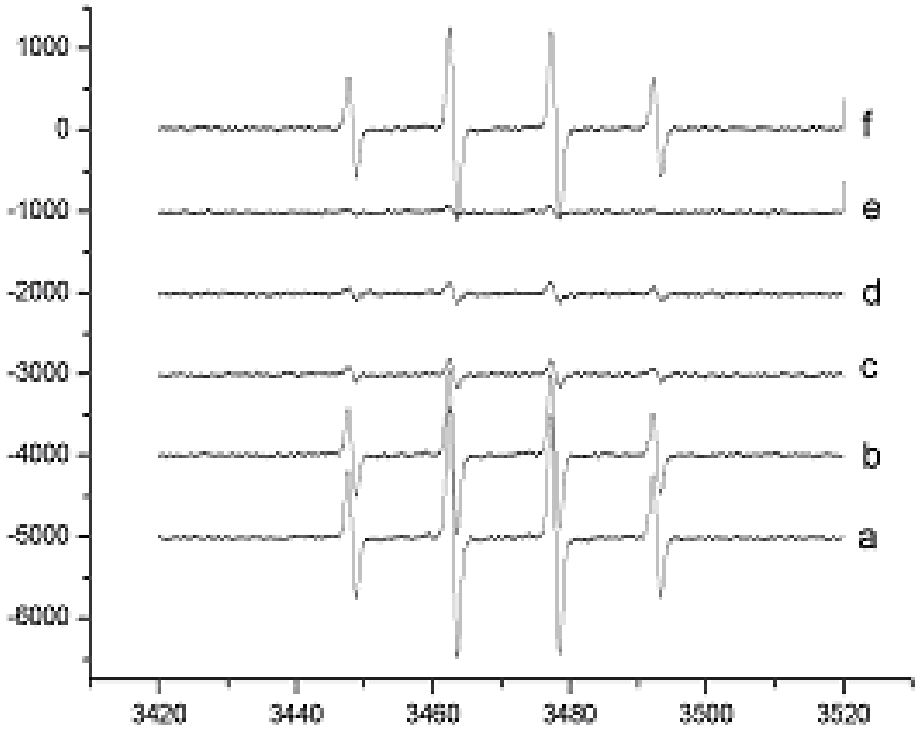
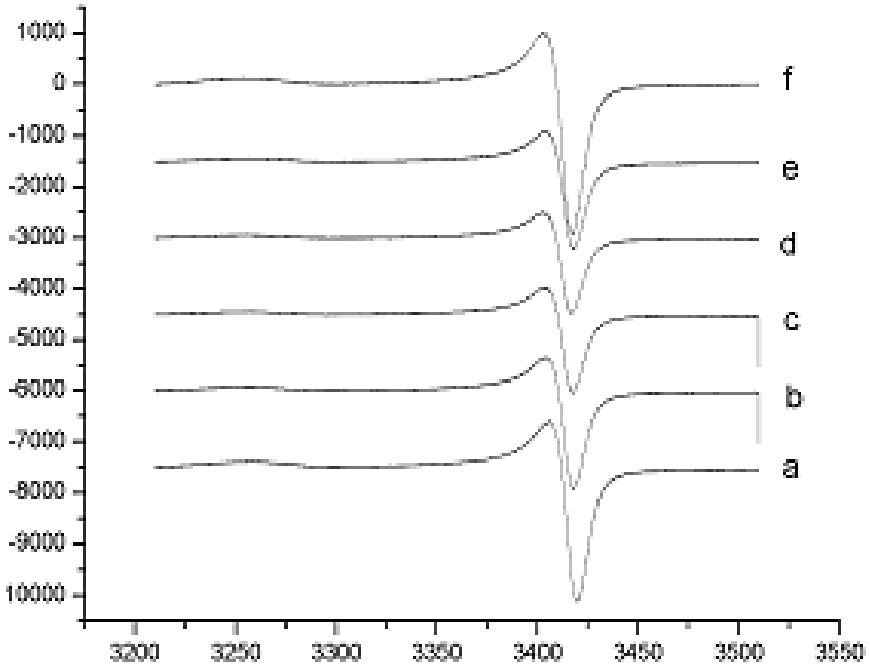


Figure 3. The ESR spectrum of superoxide anion radicals and the dose dependent scavenging effects of mistletonone. Superoxide anion radicals were generated using the NaOH/DMSO system. ESR conditions were as described in the text. a ~ e were the scavenging effects of mistletonone in the concentrations of 0.06, 0.12, 0.18, 0.24, and 0.30 mM, respectively; f: blank.



Conclusions

Diarylheptanoids have been isolated previously from Aceraceae, Betulaceae, Zingiberaceae, Leguminosae [9], *Dioscorea spongiosa* [10], and *Viscum cruciatum* [11]. These compounds exhibited wide range of biological activities [9], especially cytotoxic [11] and antiosteoporotic [10] actions. Except for diosponin A and B, two antiosteoporotic components from *Dioscorea spongiosa* [10], which displayed a 1-ketone structure, all other known diarylheptanoids belonged to the 3-ketone or 3,5-diketone types. Our finding should prompt interest in further studies on the phytochemistry and bioactivity of the 1-ketone type diarylheptanoids.

Experimental

General

IR (KBr discs): NEXUS47O FT-IR; UV: Lengguang Tech Spectrumlab 54; NMR: Bruker DMX-500 with TMS as an internal standard and DMSO-d_6 as solvent. 2D data were obtained using the standard XWIN-NMR 3.1 software package (Bruker); MS: Bruker APEXIII 7.0 TESLA FTMS; ESR: ER200D-SRC with DMPO as spin trap. 5,5-Dimethyl-1-pyrroline-N-oxide (DMPO), xanthine (X), xanthine oxidase (XO) and (-)-epigallocatechin gallate (EGCG) were purchased from Sigma Chemical Company (St. Louis, MO, USA).

Plant material

Branches and leaves of *Viscum coloratum* (Kom.) Nakai were collected in July 2002 from Yunnan Province of China, and authenticated by Dr. Tong-Shui Zhou, School of Life Sciences, Fudan University, Shanghai, China. A voucher specimen (No. Z200298) was deposited in the Herbarium of Fudan University, Shanghai, China.

Extraction and Isolation

Dried branches and leaves (2 kg) were refluxed twice with 90% EtOH for 2 h. The solvent was removed from the liquid extracts under vacuum to afford a residue (82 g), which was re-suspended in H_2O (10 L) and extracted with petroleum (2 L×5). The H_2O phase was filtered and the filtrate was loaded onto a column containing D_{101} resin (1 Kg), and then eluted sequentially with H_2O, followed successively by 20%, 40%, 60%, 80% and 95% aqueous EtOH (2.5 L each). The second fraction, that was eluted with 40% EtOH (24 g), was rechromatographed on a Sephadex LH-20 column (5×45 cm) which was eluted with aqueous MeOH (50%→80% gradient) to give four fractions. Fraction 2c (60 mg) was further separated on an ODS column (2×25 cm) eluted with 40% MeOH to yield *mistletonone* (**1**, 11.5 mg) as a pale yellow amorphous solid; UV (MeOH) λ_{max} (log ε): 286, 222 (sh) nm; IR υ_{max} (KBr) cm^{-1}: 3448, 2924, 2852, 1630, 1514, 1439, 1385, 1163, 1078, 966, 835, 789, 700, 617, 467; ^{1}H-NMR and ^{13}C- NMR, see Table 1; EIMS *m/z* (rel. int.): 207 (9.42), 121 (76.6), 108 (10.26), 107 (100), 93 (11.31), 77 (17.86), 65 (15.54), 39 (10.23); ESIMS *m/z*: 329.2 $[M+H]^+$; HR-ESIMS *m/z* 351.1203 $[M+Na]^+$ (calcd. for $C_{19}H_{20}O_5Na$, 351.1208).

Hydroxyl Radical Scavenging Assay using ESR

Hydroxyl radicals were obtained by the Fenton reaction in a system consisting of 10 mM H_2O_2 (10 μL), 3 mM $FeCl_2 \cdot 4H_2O$ (10 μL), 0.3 M DMPO (10 μL) as spin trap (blank), and sample or control in 0.1% DMSO (60 μL). ESR spectra were recorded after the system had reacted for 5 min, with the following spectrometer settings: field modulation 100 GHz, modulation amplitude 1.0 GHz, receiver gain 1×10^5, time constant 10 ms, conversion time 100 ms, center field 3470.00 GHz, sweep width 100.00 GHz, x-band frequency 9.6 GHz, power 20.00 mW, temperature 130 K. The scavenging effect (*E*) of the sample was defined as: $E = 100 \cdot (h_0 - h_x)/h_0$ (%), where h_0 and h_x were the ESR signal intensities of the blank and the probe, respectively.

Superoxide Anion Radicals Scavenging Assay using ESR

Superoxide anion radicals were obtained by the NaOH/DMSO reaction in a system consisting of DMSO (855 μL), 0.5 M KO_2 in DMSO (100 μL), sample or control in 0.1% DMSO (25 μL), 0.3 M DMPO (10 μL) as spin trap (blank), and 0.5 M NaOH (10 μL). ESR spectra were recorded immediately after the addition of NaOH. ESR measurement conditions and the calculation method for the scavenging effects of sample were the same as those described above.

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Sample Availability: Available from the authors.