ORIGINAL ARTICLE

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Rec. Nat. Prod. 15:3 (2021) 194-201

records of natural products

Trimacoside A, a High Molecular Weight Antioxidant Phenylpropanoid Glycoside from *Tricyrtis maculata*

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(Received September 16, 2020; Revised October 22, 2020; Accepted October 24, 2020)

Abstract: Trimacoside A (**1**), a new phenylpropanoid glycoside, together with nine known compounds (**2**−**10**) was isolated from *Tricyrtis maculata*. All compounds, except for **8**, were firstly isolated from this plant. The structure elucidation of the new compound was carried out by the analysis of spectroscopic data, including 1D, 2D NMR, and HRESIMS. Compounds **1**, **4**, **5**, **7**, **8**, and **10** showed significant antioxidant activities by DPPH and ABTS assays.

Keywords: Liliaceae; *Tricyrtis maculata*; phenylpropanoid glycoside; antioxidant. 2020 ACG Publications. All rights reserved.

1. Introduction

Tricyrtis maculata (Liliaceae), an endemic genus of north-east Asia, mainly distributed in China (Yunnan, Gansu, Shaanxi, and Sichuan provinces), India, and Nepal [1]. The whole plant of *T. maculata* have been extensively used as a folk medicine for the treatment of migraine, fever, cough, stomachache, tuberculosis, and ischemic cerebrovascular disease [2,3]. Previous phytochemical investigations showed that *T. maculata* contains diverse chemical constituents, such as flavonoids, phenolic acids, terpenoids, and steroids [4,5]. Those constituents possessed promising biological activities including anti-inflammatory, antibacterial, antiviral, analgesic, antioxidant, and neuroprotective effects [4,6]. In our recent research, triculata A, a novel compound featuring a rare naphtho[*b*,*c*]pyran carbon skeleton was isolated from this plant [7]. Further investigation on *T. maculata* led to the isolation of a new compound, trimacoside A, together with nine known compounds (**2**−**10**) (Figure 1). Herein, the isolation, structural elucidation, and antioxidant capacities of these isolates are described.

2. Materials and Methods

2.1. Plant Material

T. maculata, the family of Liliaceae, were collected in October 2018 from Zhashui county, Shaanxi Province of China, and was identified by Senior Experimentalist Ji-Tao Wang (Shaanxi University of Chinese Medicine, China). A voucher specimen (No. 20180928) was deposited at

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The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products May-June 2021 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.209.20.09.1810

Natural Product Laboratory of Shaanxi Collaborative Innovation Centre of Chinese Medicinal Resources Industrialization, Shaanxi University of Chinese Medicine.

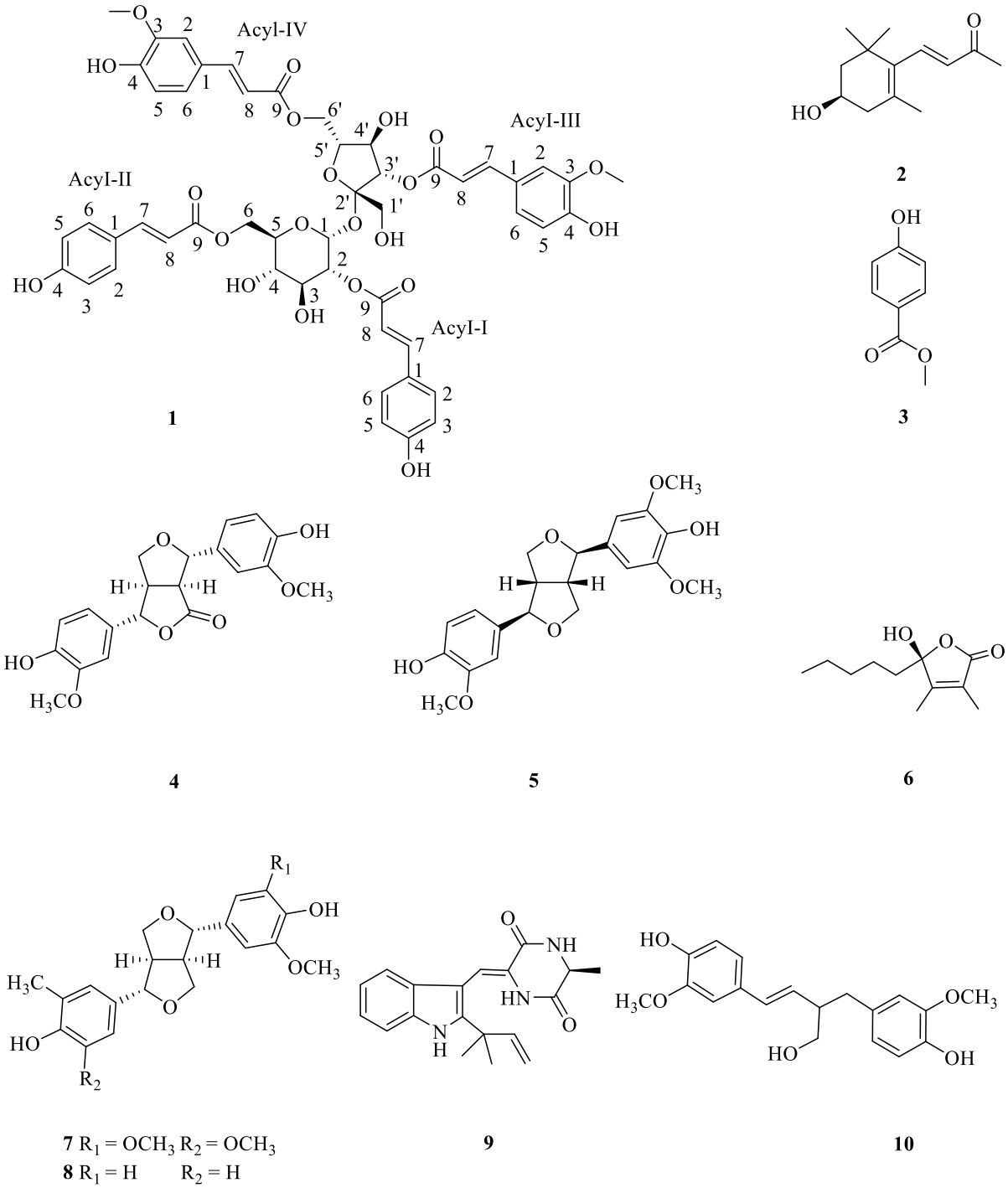

Figure 1. Structures of **1**−**10**

2.2. General Experimental Procedures

IR spectrum was tested on a Nicolet NEXUS 670 FT-IR infrared spectrophotometer with KBr pellets. The semipreparative HPLC system was equipped with a Waters-X Bridge BEH C_{18} (250 × 10 mm, 5 μm) column, a Waters 1525 binary pump system, and a Waters 2489 detector. 1D and 2D

NMR data were performed on a Bruker Advance 500 MHz or 600 MHz spectrometers with TMS as the internal standard. Chemical shifts were given in δ (ppm) scale with relative to the solvent signal. The HREIMS spectrum was tested with a Bruker APEX-II mass spectrometer. Optical rotation was measured on an SGW-533 polarimeter. Column chromatographies (CC) were performed on silica gel (300-400 mesh, Qingdao Haiyang Chemical Co., Ltd, Qingdao, China), Diaion HP-20 (Mitsubishi Chemical Co, Tokyo, Japan), MCI GEL CHP20P (Mitsubishi Chemical Industries, Tokyo, Japan), and Sephadex LH-20 (St. Louis, MO, USA). Fractions were monitored by TLC (GF254, Merck, Germany) detection. Spots were visualized by sprayed with 10% H_2SO_4 in ethanol followed by heating. The absorbance was measured and recorded using a microplate reader (1510 Multiskan GO, Thermo Scientific, USA).

2.3. Extraction and Isolation

T. maculata (whole plant) (10.0 kg) was cut, crushed, and extracted with MeOH (4 × 45 L, 7 days each) at room temperature. The percolates were combined and evaporated under reduced pressure to afford a crude extract (495.0 g) The extract was suspended in water and then successively extracted with EtOAc (5 × 1 L) and n-BuOH (5 × 1 L). The EtOAc extract (160.0 g) was subjected to Diaion HP-20 column chromatography with MeOH (50%, 75%, 85%, 100%) to obtain 4 fractions (1−4). Fraction 2 (24.0 g) was chromatographed over an MCI column (6 × 20 cm, 6 × 180 mL), eluted with aqueous MeOH (50%, 75% and 85%), to give three subfractions (2A−2C). Subfraction 2C (1.8 g) was subject to a silica gel column (300−400 mesh, 5 × 6 L) and eluted with a gradient system of $CHCl_3$-MeOH (v/v, 40:1→2:1) to yield 5 subfractions (2C1−2C5). Subfraction 2C5 (83.0 mg) was separated by silica gel CC (300−400 mesh, 3 × 160 mL) with PE-acetone (v/v, 20:1 to 1:1.5) to obtain seven subfractions (2C5a−2C5g). Subfraction 2C5g (28.5 mg) was purified by semipreparative HPLC with ACN-H_2O (35%) to yield **1** (12.3 mg, t_R = 19.5 min, flow rate = 2.5 mL/min). Subfraction 2C2 (73.2 mg) was purified by semipreparative HPLC with MeOH-H_2O (40%) to provide 10 subfractions (2C2a−2C2j). Subfraction 2C2j (4.9 mg) was further purified by semipreparative HPLC with ACN-H_2O (32%) to give **2** (3.6 mg, t_R = 14.2 min). Subfraction 2C1 (28.2 mg) was also purified via semipreparative HPLC with MeOH-H_2O (48%) to obtain **3** (4.2 mg, t_R =18.0 min). (0.6 g) was separated by silica gel CC (PE-EtOAc, 4:1→1:1, v/v, 300−400 mesh, 3 × 100 mL) to obtain 11 subfractions (2C3a−2C3k). Subfraction 2C3i (62.8 mg) was purified by semipreparative HPLC with MeOH-H_2O (48%) to afford **4** (6.6 mg, t_R = 23 min) and **5** (10.4 mg, t_R = 40 min). Subfraction 2C3j was purified via semipreparative HPLC with MeOH-H_2O (40%) to afford **6** (7.0 mg, t_R = 20.9 min). Compound **7** (9.2 mg, t_R = 22 min) was purified from subfraction 2C3k (27.8 mg) via semipreparative HPLC, eluting with ACN-H_2O (36%). Subfraction 2C3g (12.7 mg) was also purified via semipreparative HPLC, eluting with MeOH-H_2O (60%), to afford **8** (5.4 mg, t_R = 16 min). Subfraction 2C4 (0.8 g) was subjected to a Sephadex LH-20 with $CHCl_3$-MeOH (1:2) to obtain 6 fractions (2C4a−2C4f). Compounds **9** (5.8 mg, t_R = 37.4 min) and **10** (5.5 mg, t_R = 26.1 min) were obtained from subfraction 2C4e (22.5 mg) and 2C4d (37.0 mg) via semipreparative HPLC with ACN-H_2O (24% and 30%), respectively.

2.4. Spectroscopic Data

*Trimacoside A (**1**):* Yellowish amorphous powder; $[\alpha]_D^{26}$ = +16.0 (*c* 0.2, MeOH); IR (KBr) ν_{max}: 3340, 2929, 1683, 1628, 1600, 1587, 1512, 1446, 1429, 1328, 1255, 1154, 1055, 1025, 828, 515 cm^{-1}; HRESIMS *m/z* 1009.2730 $[M + Na]^+$ (calc. for $C_{50}H_{50}O_{21}Na$, 1009.2737). ^1H (600 MHz) and ^{13}C NMR (150 MHz) data, see Table 1.

2.5. DPPH Radical Scavenging Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was performed using a previously described method [7]. Briefly, stock solutions of DPPH (100 μM) in MeOH was prepared first, and then 100 μL of stock solution was mixed with 50 μL of the sample solution into the 96-well plate. The reaction plates were wrapped in aluminum foil and kept at 30 ℃ for 30 min in darkness. All measurements were done under dim light. The absorbance was measured at 517 nm with microplate

reader. Trolox was used as the positive control. IC_{50} was determined as the concentration of sample required to inhibit the formation of the DPPH radical by 50%. The results are expressed as the means with SD for experiments conducted in triplicates. All statistical analyses were executed using GraphPad Prism version 5.0 (San Diego, CA, USA).

2.6. ABTS Radical Scavenging Assay

For 2,2'-azino-*bis*-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) radical scavenging assay, $ABTS^{\bullet+}$ was produced directly by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate and following the mixture to stand for 12−16 minutes at room temperature. The sample solution (50 μL) was mixed with pre-formed $ABTS^{\bullet+}$ solution (100 μL). After 30 minutes, the absorbances of the samples were measured at 734 nm in the dark [8]. All sample were tested in three times. Trolox was used as the positive control. The IC_{50} values were calculated in the same way as described in the DPPH assay.

3. Results and Discussion

3.1. Structure Elucidation

Compound **1**: obtained as yellowish amorphous powder, possessed a molecular formula of $C_{50}H_{50}O_{21}$ by the positive HRESIMS (*m/z* 1009.2730 [*M* + Na]$^+$, calc. 1009.2743), requiring 26 indices of hydrogen deficiency. The ^{1}H NMR spectrum (Table 1) in CD_3OD of **1** showed four pairs of *trans*-olefinic protons at δ_H 7.71, 7.633, 7.627, 7.61, and 6.48, 6.352, 6.347, 6.33 with the coupling constants of 15.9 Hz, two pairs of almost overlapping *p*-substituted benzene ring proton signals at δ_H 7.38 (4H, d, *J* = 8.7 Hz) and 6.75 (4H, d, *J* = 8.7 Hz), two sets of characteristic 1,2,4-trisubstituted benzene ring protons at δ_H 7.23 (1H, d, *J* = 1.8 Hz), 7.11 (1H, dd, *J* = 8.2, 1.8 Hz), 6.81 (1H, d, *J* = 8.2 Hz), and 7.14 (1H, d, *J* = 1.8 Hz), 7.04 (1H, dd, *J* = 8.2, 1.8 Hz), 6.80 (1H, d, *J* = 8.2 Hz), as well as two methoxy signals at δ_H 3.86 and 3.89, which allowed to draw an inference that this structure contained two *p*-coumaroyl and two feruloyl moieties, consuming 24 degrees of unsaturation and 38 carbon atoms. In addition, the remaining ^{1}H NMR signals came from the region of oxygenated methines or methylenes, suggesting the existence of a disaccharide. The ^{13}C NMR spectrum (Table 1) showed the carbon signals due to two *p*-coumaroyl and two feruloyl moieties, and the remaining 12 oxygenated carbon signals included two anomeric carbons at δ_C 105.5 (s) and 90.5 (d), and three oxygenated methylenes at δ_C 65.3, 65.1, and 64.9. The above NMR features were similar to those of vanicoside A [9], a polyacylated sucrose ester. Comparison of the NMR data of **1** with those of vanicoside A suggested that the sucrose core in **1** should be acylated by two *p*-coumaroyl and two feruloyl groups. Careful analysis of the HMBC correlations (Figure 2) could accurately assign the ester carbonyl carbon signals of two *p*-coumaroyl and two feruloyl groups as 168.8, 169.4, and 168.6, 169.1, respectively. Further, the HMBC correlations from H-2 [δ_H 4.73 (1H, dd, *J* = 10.0, 3.8 Hz)] and H_2-6 [δ_H 4.29, 4.69 (each 1H, m)] to the two *p*-coumaroyl ester carbonyl carbons at δ_C 168.8, 169.4, and from H-3′ [δ_H 5.59 (1H, d, *J* = 8.5 Hz)] and H_2-6′ [δ_H 4.48, 4.51 (each 1H, m)] to the two feruloyl ester carbonyl carbons at δ_C 168.6, 169.1, confirmed that the positions of C-2, C-6, and C-3′, C-6′ of sucrose core were acylated by *p*-coumaroyl and feruloyl groups, respectively. The ^{1}H,^{1}H-COSY correlations (Figure 2) between those oxygenated proton signals further supported the coupling relationship of sucrose core. The relative configuration of **1** could be verified by the NOESY correlations of H-2↔H-4, H-3↔H-5, H-4↔H_2-6, and H-3′↔H-1′a/H-5′, in combination with the characteristic coupling constants. The absolute configuration of **1** was determined by alkaline hydrolysis and comparing the optical rotation of the product ($[\alpha]_D^{23}$ of the product +60.1) with that of the authentic standard ($[\alpha]_D^{23}$ of the standard +66.0) [10-13]. Therefore, the structure of **1** was undoubtedly established as shown in Figure 1 and named as trimacoside A.

The known compounds (-)-3-hydroxy-*β*-ionone (**2**) [14], methyl-4-hydroxybenzoate (**3**) [15], balanophonin B (**4**) [16], (-)-medioresinol (**5**) [17], (*S*)-5-hydroxy-3,4-dimethyl-5-pentylfuran-2(*5H*)-one (**6**) [18], (+)-syringaresinol (**7**) [19], (+)-pinoresinol (**8**) [19], neoechinulin A (**9**) [20], and *S*-(+)-

imperanene (**10**) [21] were authenticated by comparing their NMR data with those reported in the literatures. Compounds (**1**−**7**, **9**, and **10**) were firstly isolated from this plant.

Table 1. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of **1** (δ in ppm, J in Hz) in CD_3OD

No.	^{1}H NMR	^{13}C NMR	No.	^{1}H NMR	^{13}C NMR
1	5.79 (1H, d, 3.8)	90.5 (d)	7	7.61 (1H, d, 15.9)	147.1 (d)
2	4.73 (1H, dd, 10.0, 3.8)	74.6 (d)	8	6.35 (1H, d, 15.9)	115.0 (d)
3	3.93 (1H, dd, 10.0, 9.0)	72.3 (d)	9	—	169.4 (s)
4	3.45 (1H, t, 9.0)	72.2 (d)	Acyl-III-1	—	127.9 (s)
5	4.29 (1H, m)	72.2 (d)	2	7.23 (1H, d, 1.8)	112.0 (d)
6	4.29, 4.69 (each 1H, m)	65.3 (t)	3	—	149.5 (s)
1′	3.45 (1H, d, 11.9) 3.63 (1H, d, 11.9)	64.9 (t)	4	—	150.7 (s)
2′	—	105.5 (s)	5	6.81 (1H, d, 8.2)	116.6 (d)
3′	5.59 (1H, d, 8.5)	78.3 (d)	6	7.11(1H, dd, 8.2, 1.8)	124.6 (d)
4′	4.60 (1H, t, 8.5)	74.1 (d)	7	7.71 (1H, d, 15.9)	148.1 (d)
5′	4.11 (1H, m)	81.2 (d)	8	6.48 (1H, d, 15.9)	114.8 (d)
6′	4.48, 4.51 (each 1H, m)	65.1 (t)	9	—	168.6 (s)
Acyl-I-1	—	127.2 (s)	OCH_3	3.89 (3H, s)	56.6 (q)
2,6	7.38 (2H, d, 8.7)	131.4 (d)	Acyl-IV-1	—	127.9 (s)
3,5	6.75 (2H, d, 8.7)	116.9(d)[a]	2	7.14 (1H, d, 1.8)	111.8 (d)
4	—	161.5 (s)	3	—	149.4 (s)
7	7.63 (1H, d, 15.9)	147.4 (d)	4	—	150.7 (s)
8	6.33 (1H, d, 15.9)	114.8 (d)	5	6.80 (1H, d, 8.2)	116.6 (d)
9	—	168.8 (s)	6	7.04 (1H, dd, 8.2, 1.8)	124.3 (d)
Acyl-II-1	—	127.3 (s)	7	7.63 (1H, d,15.9)	147.1 (d)
2,6	7.38 (2H, d, 8.7)	131.4 (d)	8	6.35 (1H, d, 15.9)	115.4 (d)
3,5	6.75 (2H, d, 8.7)	116.9 (d)[a]	9	—	169.1 (s)
4	—	161.5 (s)	OCH_3	3.86 (3H, s)	56.6 (q)

Assignments were based on 1D and 2D NMR experiments. [a] Interchangeable.

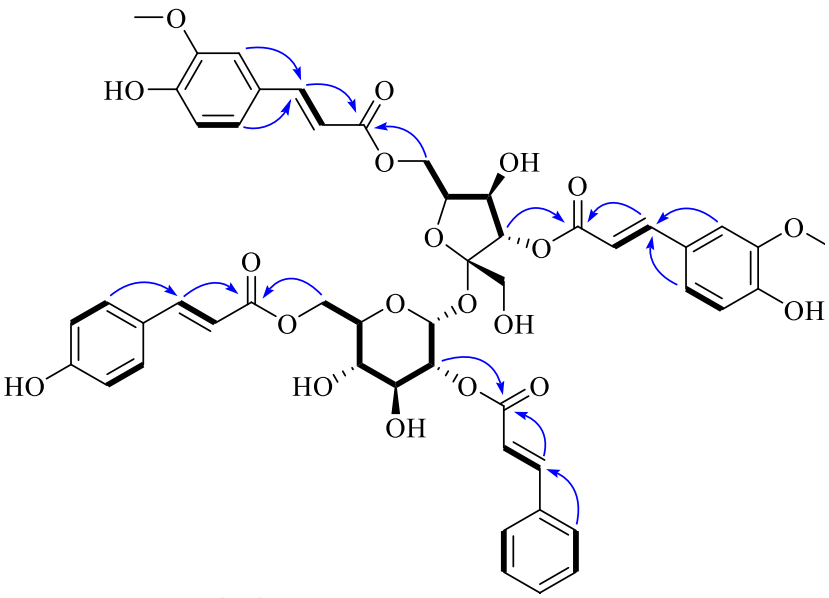

Figure 2. The ^{1}H,^{1}H-COSY and key HMBC correlations of **1**

3.2. Antioxidant Capacity

The antioxidant capacities of all compounds (**1**−**10**) were determined by DPPH and ABTS assays [22,23], with results given in Table 2. Trolox was used as the positive control. Consistent with previous research [24], compound **7** exhibited the most antiradical activity for DPPH with IC_{50} value of 0.028 mM, which was nearly 3-fold higher than that of Trolox (IC_{50} = 0.073 mM). The IC_{50} values of compounds **1**, **4**, **5**, **8**, and **10** were respectively calculated to be 0.095, 0.074, 0.080, 0.056, and 0.068 mM, which showed nearly the equal effects as the positive control regarding to DPPH radical scavenging activity. Furthermore, the ABTS assay was also used to determine the antioxidant activities of all compounds. The results showed that the ABTS radical scavenging activities decreased in the order **7** > **8** > **10** > Trolox > **4** > **5** > **1** > **9** > **2**. To the best of our knowledge, the antioxidant activities of compounds **1**, **2**, **4**, **6**, and **10** were firstly reported.

Table 2. The antioxidant activities of compounds **1**−**10**.

Compounds	IC_{50} (mM) ± SD	
	DPPH	ABTS
1	0.095 ± 0.007	0.083 ± 0.016
2	0.308 ± 0.001	0.269 ± 0.005
3	_ [a]	_ [a]
4	0.074 ± 0.005	0.046 ± 0.003
5	0.080 ± 0.004	0.061 ± 0.002
6	_ [a]	_ [a]
7	0.028 ± 0.001	0.011 ± 0.001
8	0.056 ± 0.004	0.035 ± 0.003
9	0.300 ± 0.007	0.128 ± 0.002
10	0.068 ± 0.016	0.036 ± 0.006
Trolox [b]	0.073 ± 0.001	0.044 ± 0.004

Values are expressed as mean ± SD (n = 3). [a] No activity. [b] Positive control.

Acknowledgments

This work was supported by the Program of Administration of Traditional Chinese Medicine of Shaanxi Province, China (15-ZY001).

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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