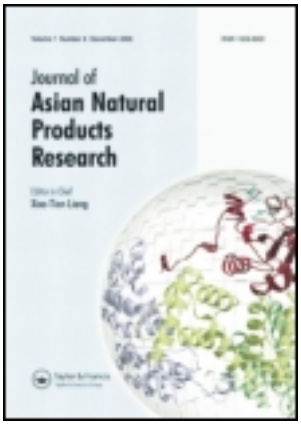

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Phenolic compounds from the aqueous extract of *Acacia catechu*

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Two new phenolic compounds, 5-hydroxy-2-[2-(4-hydroxyphenyl) acetyl]-3-methoxylbenzoic acid (**1**) and (2*S*,3*S*)-3,7,8,3′,4′-pentahydroxyflavane (**2**), were obtained from the aqueous extract of *Acacia catechu*, along with four known compounds identified as rhamnetin (**3**), 4-hydroxyphenyl ethanol (**4**), 3,3′,5,5′,7-pentahydroxyflavane (**5**), and fisetinidol (**6**). Their structures were determined on the basis of spectroscopic analysis. Free radical-scavenging activities of the new compounds were evaluated.

Keywords: Leguminosae; *Acacia catechu*; phenolic compounds; free radical-scavenging activity

1. Introduction

Acacia is the second largest genus in the Leguminosae family, comprising more than 1200 species worldwide, which are found in almost all habitats. The extract of *Acacia catechu* has been used in traditional Chinese medicine as antipyretic, anthelmintic, antipruritic, digestive, and antivenom agent. Preliminary pharmacological studies demonstrated antianemic, anti-inflammatory, antimicrobial, anticancer, antioxidant, and antidiabetic activities [1,2]. Previous studies on this plant revealed the presence of flavanes [3,4], flavonoids [4], fatty acids [5], and gums [5]. In the course of our investigation on the bioactive constituents of *A. catechu*, two new phenolic compounds were obtained from *A. catechu* (Figure 1). In this paper, we present their isolation, structure elucidation, and biological evaluation of free radical-scavenging activities.

2. Results and discussion

Compound **1**, obtained as green oily material, was assigned the molecular formula of $C_{16}H_{14}O_6$ by the pseudo-molecular ion in HR-ESI-MS at *m/z* 301.0697 $[M - H]^-$, with 10 degrees of unsaturation. The UV spectrum of **1** exhibited absorption maxima at 291 (3.99) and 202 (4.28) nm. Its IR spectrum showed the absorption bands for hydroxyl (3201 cm^{-1}), conjugated carbonyl (1685 cm^{-1}), and aromatic ring (1600 and 1515 cm^{-1}). In the ^1H NMR spectrum (Table 1), the AA′BB′ system at δ 7.02 (2H, d, $J = 8.4$ Hz, H-2′, 6′) and 6.62 (2H, d, $J = 8.4$ Hz, H-3′, 5′) indicated the presence of a 1,4-disubstituted benzene ring. Two *meta*-coupled protons at δ 5.93 (1H, d, $J = 1.2$ Hz, H-6) and 5.92 (1H, d, $J = 1.2$ Hz, H-4) implicated the existence of a 1,2,3,5-tetrasubstituted benzene ring. In addition, one methoxyl group

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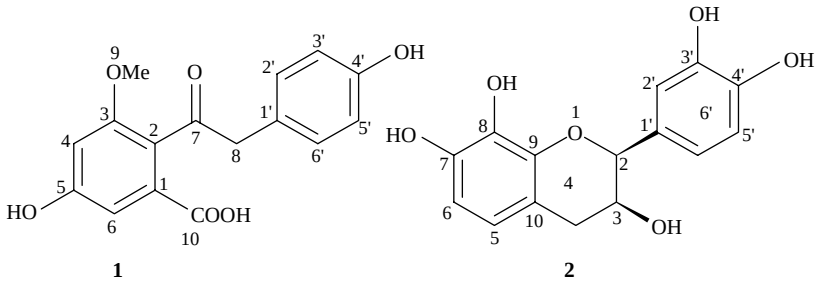


1 **2**

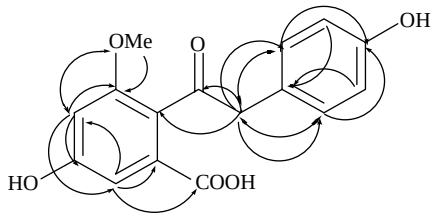
Figure 1. Structures of compounds **1** and **2**.

Table 1. ^{1}H and ^{13}C NMR spectral data of **1** and **2** in CD_3COCD_3 (J in Hz).

	1		**2**	
Compound no.	δ_H	δ_C	δ_H	δ_C
1		103.3		
2		106.3	4.93 (m)	79.9
3		160.6	4.15 (m)	67.2
4	5.92 (d, $J = 1.2$)	93.3	3.11 (dd, $J = 16.5, 4.5$) 2.72 (dd, $J = 16.5, 3.3$)	34.0
5		169.0	6.41 (d, $J = 8.4$)	115.4
6	5.93 (d, $J = 1.2$)	91.7	6.38 (d, $J = 8.4$)	108.9
7		193.1		143.8
8	3.06 (s)	41.6		133.5
9	3.75 (s)	56.0		144.7
10		173.7		112.3
1′		125.7		132.0
2′	7.02 (d, $J = 8.4$)	132.4	7.04 (d, $J = 1.5$)	120.3
3′	6.62 (d, $J = 8.4$)	115.4		145.0
4′		156.9		145.4
5′	6.62 (d, $J = 8.4$)	115.4	6.77 (d, $J = 8.1$)	115.5
6′	7.02 (d, $J = 8.4$)	132.4	6.83 (d, $J = 8.1, 1.5$)	119.5

resonating at δ 3.75 and one methylene at δ 3.06 (2H, H-8) were also displayed. In NOE difference spectrum, only the signal at δ 5.92 (H-4) was enhanced when irradiating the methoxyl, suggesting that the methoxyl group was located at C-3 (δ 160.6). The attachment of carboxyl group to C-1 was established by the HMBC correlations of H-4 (δ 5.92)/C-3, C-5 (δ 169.0) and C-6 (δ 91.7), H-6 (δ 5.93)/C-1 (δ 103.3), C-4 (δ 93.3), COOH (δ 173.7). The HMBC correlations of H-2′ (δ 7.02)/C-8 (δ 41.6) and H-6′ (δ 7.02)/C-8 indicated that B-ring was connected at C-8. The HMBC cross-peaks of H-8 (δ 3.06)/C-2 (δ 106.3) and H-8/C-7 (δ 193.1) indicated that 2-(4-hydroxyphenyl) acetyl was linked to C-2. Thus, compound **1** was elucidated as 5-hydroxy-2-[2-(4-hydroxyphenyl)acetyl]-3-methoxybenzoic acid (Figure 2).

Compound **2** was isolated as colloid with $[\alpha]_D^{20}$ +6.2 ($c = 0.5$, MeOH). The molecular formula of $C_{15}H_{14}O_6$ was determined based on the quasi-molecular ion peak at m/z 289.0714 $[M - H]^-$ by negative HR-ESI-MS, with nine degrees of unsaturation. The UV spectrum of **2** exhibited absorption maxima at 281 (3.5) and 205 (4.6) nm. The IR spectrum of **2** displayed absorption bands at 3202 (hydroxyl), 1609, and 1515 (aromatic



1

Figure 2. Key HMBC (H → C) and NOESY (↔) correlations of **1**.

ring) cm^{-1}. The ^1H NMR spectrum of **2** (Table 1) displayed ABX-type aromatic protons at δ 7.04 (1H, d, $J = 1.5$ Hz, H-2′), 6.83 (1H, dd, $J = 8.1$, 1.5 Hz, H-6′), and 6.77 (1H, d, $J = 8.1$ Hz, H-5′), two *ortho*-coupled aromatic protons at δ 6.41 (1H, d, $J = 8.4$ Hz, H-5) and 6.38 (1H, d, $J = 8.4$ Hz, H-6), two oxygenated methine protons at δ 4.93 (1H, m, H-2) and 4.15 (1H, m, H-3), one methylene at δ 3.11 (1H, dd, $J = 16.5$, 4.5 Hz, H-4a) and 2.72 (1H, dd, $J = 16.5$, 3.3 Hz, H-4b). The ^{13}C NMR spectral data (Table 1) showed 15 carbons. The above NMR spectra of compound **2** were very similar to those of mesquitol [6,7], except that the two oxygenated methine protons were at δ 4.61 (1H, d, $J = 7.2$ Hz, H-2) and 3.99 (1H, m, H-3) and the chemical shifts of C-2 and C-3 were at δ 83.1 and 68.3, respectively, in mesquitol. The absolute configuration at C-3 was determined as *S* by comparing its CD spectral data with that in Ref. [8]. In the CD spectrum of **2**, a positive Cotton effect at 350 nm was shown. Owing to the *cis*-configuration between H-2 and H-3, the absolute configuration of C-2 was also assigned to be *S*. Thus, the final structure of **2** was determined to be (2*S*,3*S*)-3,3′,4′,7,8-pentahydroxyflavane.

In addition, four known compounds were identified by comparing their spectroscopic data with those reported in the literature as rhamnetin (**3**) [9], 4-hydroxyphenylethanol (**4**) [10], 3,3′,5,5′,7-pentahydroxyflavane (**5**) [11], and fisetinidol (**6**) [12].

New compounds **1** and **2** were evaluated *in vitro* for scavenging effects on ROS (HO, O_2, ROO, and H_2O_2), RNS ($ONOO^-$), and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). There were significant differences between sample groups and model group. As far as the scavenging effect of stable DPPH was concerned, compound **2** exhibited the highest DPPH scavenging activity with absorbance values of 1.2966 ± 0.1088 by comparing with the model group with absorbance values of 1.5769 ± 0.054.

3. Experimental

3.1 General experimental procedures

The optical rotation values were determined with a JASCO P-2000 polarimeter. UV spectra were obtained with a JASCO V-650 spectrophotometer. IR spectra were obtained on an IMPACT 400 spectrometer. The ^1H NMR (300 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Mercury-300 and Mercury-400 spectrometers with TMS as an internal standard. NOE difference, HSQC, and HMBC spectra were run on Mercury-400 spectrometer. ESI-MS and HR-ESI-MS data were obtained from an Agilent 1100 LC/MSD Trap-SL mass spectrometer. CD spectra were measured on a JASCO J-810 spectropolarimeter. Silica gel GF_{254} prepared for TLC and silica gel (160–200 mesh) for column chromatography (CC) were obtained from Qingdao Marine Chemical Factory (Qingdao, China). RP-C_{18} silica gel was purposed from Merck Chemical Company Ltd. (Shanghai, China). HPLC separations were performed on a preparation YMC-Pack ODS-A column (10 μm, 250 × 20 mm ID) equipped with a Shimadzu SPD-6A UV spectrophotometric detector and a Thermo constametric pumping system. All the reagents were HPLC grade purchased from Beijing Chemical Company (Beijing, China).

3.2 Plant material

The aqueous extract of *A. catechu* was purchased from Beijing Pharmacy in July 2004 (Production Lot 20040309), and identified by Professor Lin Ma of the Institute of Materia Medica, Peking Union Medical College. A voucher specimen (ID-S-2285) has been deposited at the Herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, China.

3.3 Extraction and isolation

The medicine of *A. catechu* (1.0 kg) was extracted with water (5 L) using ultrasound for 45 min. The mixture was filtered and partitioned with EtOAc (5 L × 8) and H_2O-saturated *n*-BuOH (5 L × 8) successively. The EtOAc extract (200 g) was separated on a silica gel with $CHCl_3$–MeOH gradient solvent system (1:0 to 0:1) to obtain eight fractions (E1–E8), which were combined according to TLC analysis. Purification of E1 (180 mg) was performed by C-18 open CC eluting with a gradient system (MeOH–H_2O, 5:95 to 100:0) and led to the isolation of compound **4** (3 mg). Fraction E2 (5 g) was subjected to silica gel CC eluting with petroleum ether–acetone (9:1 to 1:1) to yield fractions E21–E28. The fraction of E22 (84 mg) was further purified by silica gel CC eluted with petroleum ether–acetone (9:1 to 1:1) to give compound **1** (4 mg). E26 (150 mg) was purified by C-18 CC eluted with MeOH–H_2O (9:1 to 10:0), and was separated by RP-HPLC with MeOH–H_2O (1:1) to afford compound **3** (13 mg, t_R 18 min). Fraction E3 (50 g) was separated by C-18 open CC eluted with a gradient system (MeOH–H_2O, 5:95 to 100:0) to get fractions E31–E40. E32 (50 mg) was purified by reversed-phase HPLC eluting with MeOH–H_2O (30:70) to afford compounds **5** (3 mg, t_R 5 min) and **6** (6 mg, t_R 10 min). E36 (60 mg) was subjected to RP-HPLC eluted with MeOH–H_2O (30:70) to afford compound **2** (15 mg, t_R 10 min).

3.3.1 5-Hydroxy-2-[2-(4-hydroxyphenyl) acetyl]-3-methoxybenzoic acid (*1*)

Green oil, UV (MeOH) (nm) λ_{max} (log ε): 202 (4.28), 291 (3.99); IR (neat) ν_{max}^{KBr} (cm^{-1}): 3201, 1685, 1600, 1512; ^1H and ^{13}C NMR spectral data, see Table 1; HR-ESI-MS *m/z*: 301.0696 $[M - H]^-$ (calcd for $C_{16}H_{13}O_6$, 301.0712).

3.3.2 (2S,3S)-3,7,8,3′,4′-Pentahydroxyflavane (*2*)

Colloid, $[\alpha]_D^{20}$ +6.2 (*c* 0.50, MeOH); UV (MeOH) (nm) λ_{max} (log ε): 205 (4.58), 281 (3.47); IR (neat) ν_{max}^{KBr} (cm^{-1}): 3202, 1609, 1600, 1515; CD (CH_2Cl_2:MeOH = 3:1): $\Delta\varepsilon_{350nm}$ +0.42; ^1H and ^{13}C NMR spectral data, see Table 1; HR-ESI-MS *m/z* 289.0714 $[M - H]^-$ (calcd for $C_{15}H_{13}O_6$, 289.0718).

3.4 Radical-scavenging activity assay

The antiradical activities of new compounds **1** and **2** were determined by the radical scavenging method described by Almeida et al. [13].

Acknowledgements

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