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Three new flavanols from *Daphne giraldii*

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Three new flavanols, 5-*p*-hydroxybenzoxy-7-hydroxyl-8-ethoxycarbonyl-(−)-afzelechin (**1**), 5-*p*-hydroxybenzoxy-7-(2, 3, 5-trihydroxybenzoxy)-8-ethoxycarbonyl-(−)-afzelechin (**2**), and 5-*p*-hydroxybenzoxy-7-(2, 3, 5-trihydroxybenzoxy)-8-methoxycarbonyl-(−)-afzelechin (**3**), were isolated from *Daphne giraldii* Nitsche and their structures were elucidated by spectroscopic methods, especially by 2D-NMR and MS analyses.

Keywords: *Daphne giraldii*; Thymelaeaceae; flavanol; afzelechin

1. Introduction

Daphne giraldii Nitsche is mainly distributed in central and western China. The stems and roots of this plant (Chinese name 'Zu Shima') have been used in Chinese folk medicines to treat ache and rheumatism, especially for toothache, waistache, rheumatoid arthritis and quadriplegia.[1] Earlier chemical research on this plant was focused on diterpenoids,[2,3] coumarins,[4,5] and biflavonoids.[6] In our search for natural products with biological activities, three new flavanols were isolated from *D. giraldii*. In the present study, we report the isolation and characterization of three new flavan-3-ols.

2. Results and discussion

Compound **1** was isolated as yellow powder. The HRESIMS indicated its molecular formula $C_{25}H_{22}O_9$ at *m/z* 489.1159 $[M + Na]^+$. The UV spectrum showed the absorption maxima at 216 and 260 nm. The characteristic carbon resonances at δ_C 80.9, 65.2 and 27.2 together with the corresponding protons at δ_H 4.80, 3.99 and 2.47 suggested that compound **1** was a derivative of flavan-3-ol. The ^{1}H NMR spectrum exhibited an aromatic proton singlet at δ_H 6.28, together with three aromatic quaternary carbons at δ_C 157.3, 147.7 and 106.8 in ^{13}C NMR spectrum, which implied the trisubstituted ring A. In the ^{1}H NMR spectrum of compound **1**, a pair of two-proton doublet signals at δ_H 7.21 (2H, d, $J = 8.0$ Hz, H-2′, 6′) and 6.71 (2H, d, $J = 8.0$ Hz, H-3′, 5′) revealed 4′-OH substitution of the ring B. A *p*-hydroxybenzoxy group was assigned by the characteristic signals at δ_C 163.7 (C-1″), 119.2 (C-2″), 132.0 (C-3″, 7″), 115.5 (C-4″, 6″), and 162.6 (C-5″) in the ^{13}C NMR spectrum, as well as their corresponding proton resonances at δ_H 7.90 (2H, d, $J = 8.0$ Hz, H-3″, 7″) and 6.85 (2H, d, $J = 9.0$ Hz, H-4″, 6″) in ^{1}H NMR spectrum, and the *p*-hydroxybenzoxy group was attached to C-5 by the HMBC correlations

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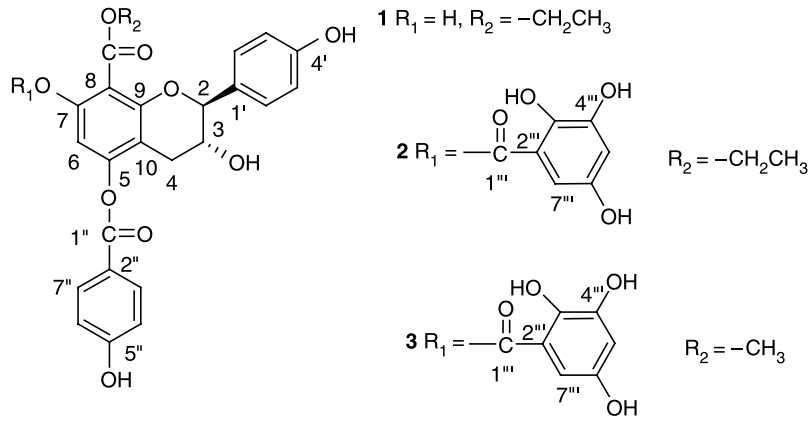

Figure 1. Structures of compounds **1**–**3**.

of H-6 with C-1″ and C-10. Also, an ethoxycarbonyl group substituted at C-8 was determined by the carbon signals at δ_C 164.0 (C═O), 60.2 (OCH_2), and 13.6 (Me) and the corresponding proton signals at δ_H 3.94 (2H, m, OCH_2) and 0.90 (3H, t, Me). A hydroxyl was assigned to C-7 by the HMBC correlation of H-6 with C-7. On the basis of the above evidences, compound **1** was determined as 5-*p*-hydroxybenzoxy-7-hydroxyl-8-ethoxycarbonyl-(−)-afzelechin.

Compound **2**, a yellow powder, possesses the molecular formula $C_{32}H_{26}O_{13}$ due to its HRESIMS at *m/z* 641.1269 $[M + Na]^+$. The ^1H NMR spectrum showed characteristic resonances for flavanol-3-ol at δ_H 4.92 (1H, d, $J = 6.0$ Hz), 4.02 (1H, m) and 2.57 (2H, m), and the ^{13}C NMR spectrum exhibited the corresponding carbon resonances at δ_C 81.0, 65.1, and 27.2. As deduced in compound **1**, a *p*-hydroxybenzoxy and an ethoxycarbonyl were determined by the interpretation of the NMR spectral data of compound **2** and were attached to C-5 and C-8, respectively. Moreover, two aromatic proton resonances at δ_H 6.14 (1H, d, $J = 2.0$ Hz) and 5.71 (1H, d, $J = 2.0$ Hz), as well as seven carbon resonances at δ_C 100.4, 101.7, 102.8, 151.6, 161.6, 161.8, and 162.0 in the ^{13}C NMR spectrum, revealed the presence of a 2, 3, 5-trihydroxybenzoxy. The H-6 was observed as an HMBC correlation to C-1‴, hinting the linkage of 2, 3, 5-trihydroxybenzoxy at C-7. Therefore, the structure of compound **2** was elucidated as 5-*p*-hydroxybenzoxy-7-(2,3,5-trihydroxybenzoxy)-8-ethoxycarbonyl-(−)-afzelechin.

Compound **3** was isolated as yellow powder. The HRESIMS of compound **3** showed a quasimolecular ion at *m/z* 627.1114 $[M + Na]^+$, resulting in a molecular formula $C_{32}H_{26}O_{13}$. Its molecular weight was 14, less than that of compound **2**. The ^1H and ^{13}C NMR spectra of compound **3** were quite similar to those of compound **2** except for one methoxy group in **3** instead of the ethoxy group in **2**. The proton resonance at δ_H 3.57 (MeO) showed the HMBC correlations with C═O (δ_C 167.69) and C-8, suggesting that methoxycarbonyl being attached to C-8. Thus, compound **3** was determined as 5-*p*-hydroxybenzoxy-7-(2,3,5-trihydroxybenzoxy)-8-methoxycarbonyl-(−)-afzelechin (Figure 1).

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Perkin–Elmer 341 polarimeter; UV spectra were performed on a Shimadzu UV210A, λ_{max} (log ε) in nm; IR spectra were obtained on a Perkin–Elmer 577 spectrometer, in cm^{-1}; ^1H and ^{13}C NMR, HSQC, HMBC and NOESY spectra were recorded on a Bruker

Table 1. ^{1}H NMR and ^{13}C NMR spectral data for compounds **1**–**3** in DMSO-d_6 (ppm).

	1		**2**		**3**	
Position	δ_c	δ_H (mult., *J*)	δ_c	δ_H (mult., *J*)	δ_c	δ_H (multi., *J*)
2	80.9	4.80 (d, 7.0)	81.0	4.92 (d, 6.0)	80.9	4.91 (d, 7.0)
3	65.2	3.99 (m)	65.1	4.02 (m)	65.0	4.05 (m)
4	27.2	2.47 (m)	27.2	2.57 (m)	27.0	2.57 (m) 2.76 (1H, m)
5	157.3		158.8		158.5	
6	101.5	6.28 (s)	102.2	6.33 (s)	101.9	6.33 (s)
7	147.7		149.5		148.9	
8	106.8		104.5		104.7	
9	153.1		154.9		154.4	
10	106.2		106.5		106.4	
1′	129.2		129.1		129.0	
2′	127.8	7.21 (d, 8.0)	127.7	7.18 (d, 9.0)	127.6	7.19 (d, 9.0)
3′	114.8	6.71 (d, 8.0)	114.8	6.71 (d, 9.0)	114.8	6.71 (d, 9.0)
4′	156.9		156.9		156.8	
5′	114.8	6.71 (d, 8.0)	114.8	6.71 (d, 9.0)	114.8	6.71 (d, 9.0)
6′	127.8	7.21 (d, 8.0)	127.7	7.18 (d, 9.0)	127.6	7.19 (d, 9.0)
1″	163.7		164.1		163.9	
2″	119.2		119.3		119.1	
3″	132.0	7.90 (d, 8.0)	132.2	7.90 (d, 8.0)	132.1	7.91 (d, 9.0)
4″	115.5	6.85 (d, 9.0)	115.5	6.85 (d, 9.0)	115.4	6.86 (d, 9.0)
5″	162.6		162.5		162.5	
6″	115.5	6.85 (d, 9.0)	115.5	6.85 (d, 9.0)	115.4	6.86 (d, 9.0)
7″	132.0	7.90 (d, 8.0)	132.2	7.91 (d, 8.0)	132.1	7.91 (d, 9.0)
1‴			161.6		161.5	
2‴			101.7		101.4	
3‴			161.8		161.9	
4‴			151.6		151.6	
5‴			102.8	5.71 (d, 2.0)	102.7	5.71 (d, 2.0)
6‴			162.0		162.1	
7‴			100.4	6.14 (d, 2.0)	100.4	6.14 (d, 2.0)
C = O	164.0		167.4		167.7	
OCH_2	60.2	3.94 (m)	60.9	4.04 (m)		
CH_3	13.6	0.90 (t, 7.0)	13.6	1.05 (t, 7.0)		
OCH_3					51.9	3.57 (s)

AVANCE^{II} 600 NMR; HRESI-MS were performed on a JMS-HX 110 instrument.

3.2 Plant material

The stem barks of *Daphne giraldii* Nitsche (Thymelaeaceae) were collected in May 2005 from Shanxi Province, China and were authenticated by Professor CeMing Tan. A voucher specimen (No. 2005051509) has been deposited in the School of Pharmacy, Second Military Medical University.

3.3 Extraction and isolation

The stem barks of *Daphne giraldii* (11 kg) were extracted three times with 95% EtOH at room temperature. After the removal of EtOH, the water suspension was partitioned with petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH. The EtOAc extract (200 g) was submitted to column chromatography over silica gel (100–200 mesh) eluting with the gradient $CHCl_3$–MeOH (15:1 to 5:1) to yield fractions 1–5. The fraction 2 (30 g) was separated by column chromatography over silica gel (100–200 mesh), eluting with the

gradient $CHCl_3$–MeOH (12:1 to 10:1), and were repeatedly purified through ODS column chromatography, eluting with MeOH–H_2O (5:5 to 6:4), to provide compounds **1** (98 mg), **2** (30 mg), and **3** (46 mg).

3.3.1 Compound *1*

Yellow powder; $[\alpha]_D^{20}$ −51.4 (*c* 0.319, MeOH); UV $[\lambda]_{max}^{MeOH}$ nm (log ε): 216 (3.33) and 260 (2.05); IR (KBr) cm^{-1}: 3313, 1724, 1698, 1606, 1519, 1441, 1293, 1172, 1057, 987 and 762; ^1H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) spectral data (see Table 1); HRESIMS: *m/z* 489.1159 $[M + Na]^+$ (calcd for $C_{25}H_{22}O_9Na$, 489.1162).

3.3.2 Compound *2*

Yellow powder; mp 177–178°C; $[\alpha]_D^{20}$ −93 (*c* 0.3075, MeOH); UV $[\lambda]_{max}^{MeOH}$ nm (log ε): 214 (0.567) and 260 (0.386); IR (KBr) cm^{-1}: 3365, 1701, 1662, 1517, 1519, 1445, 1288, 1166, 1054, 849, and 762; ^1H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) spectral data (see Table 1); HRESIMS: *m/z* 641.1269 $[M + Na]^+$ (calcd for $C_{32}H_{26}O_{13}Na$, 641.1271).

3.3.3 Compound *3*

Yellow powder; $[\alpha]_D^{20}$ −102 (*c* 0.1095, MeOH); UV $[\lambda]_{max}^{MeOH}$ nm (log ε): 214 (1.10) and 260 (0.724); IR (KBr) cm^{-1}: 3420, 1734, 1666, 1517, 1519, 1438, 1262, 1166, 1082, 849, and 763; ^1H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) spectral data (see Table 1); HRESIMS: *m/z* 627.1114 $[M + Na]^+$ (calcd for $C_{31}H_{24}O_{13}Na$, 627.1115).

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