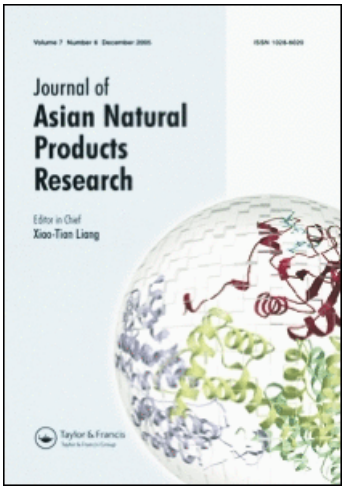

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Wang, Lei , Zhang, Chao-Feng , Wang, Zheng-Tao , Zhang, Mian and Xu, Luo-Shan(2009) 'Five new compounds from *Dendrobium crystallinum*', Journal of Asian Natural Products Research, 11: 11, 903 — 911
To link to this Article: DOI: 10.1080/10286020903128942
URL: http://dx.doi.org/10.1080/10286020903128942

Journal of Asian Natural Products Research
Vol. 11, No. 11, November 2009, 903–911

Five new compounds from *Dendrobium crystallinum*

Lei Wang[a], Chao-Feng Zhang[a]*, Zheng-Tao Wang[bc]*, Mian Zhang[a] and Luo-Shan Xu[a]

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(*Received 9 March 2009; final version received 19 June 2009*)

Five new compounds, dencryol A (**1**), dencryol B (**2**), crystalltone (**3**), crystallinin (**4**), and 3-hydroxy-2-methoxy-5,6-dimethylbenzoic acid (**5**), together with six known compounds, dendronobilin B (**6**), syringic acid (**7**), apigenin (**8**), isoviolanthin (**9**), 6‴-glucosyl-vitexin (**10**), and palmarumycin JC2 (**11**), have been isolated from the stems of *Dendrobium crystallinum*, of which compounds **9**–**11** were isolated from the genus *Dendrobium* for the first time, and all the other compounds were first obtained from this plant. Their structures were established on the basis of spectroscopic analysis and literature data.

Keywords: *Dendrobium crystallinum*; dencryol A; dencryol B; crystalltone; crystallinin; 3-hydroxy-2-methoxy-5,6-dimethylbenzoic acid

1. Introduction

Dendrobium (Orchidaceae) is a large and polymorphic genus, which has a variety of about 1100 species in the world, and about 80 species are distributed in China [1]. Previous phytochemistry studies of the genus led to the isolation of a series of diverse compounds, including bibenzyls, phenanthrenes, alkaloids, fluorenones, sesquiterpenes, and so on; some of the compounds were found to possess antimutagenic and antitumor activities [2–4]. *Dendrobium crystallinum* Rchb.f., locally known as 'Shihu' or 'Huangcao', is widely distributed in south-western China, and the phytochemical study of this plant has been reported previously [5]. In order to know more about the chemical constituents of this plant, a systematic investigation has been undertaken. We herein report the isolation and structural elucidation of 11 compounds, including: two bibenzyl derivatives dencryol A (**1**) and dencryol B (**2**); one new phenanthrene lactone crystalltone (**3**); two sesquiterpenes, crystallinin (**4**) and dendronobilin B (**6**) [6]; two organic acids, 3-hydroxy-2-methoxy-5,6-dimethylbenzoic acid (**5**) and syringic acid (**7**) [7]; together with three flavones, apigenin (**8**) [8], isoviolanthin (**9**) [9], and 6‴-glucosyl-vitexin (**10**) [10]; and palmarumycin JC2 (**11**) [11]. This paper reports the isolation and structural elucidation of five new compounds in detail.

2. Results and discussion

Compound **1** was obtained as an orange amorphous powder (MeOH), mp 89–90°C, $[\alpha]_D^{20}$ +7.1 ($c = 0.05$, MeOH). Its molecular formula $C_{30}H_{28}O_7$ was deduced by the

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020903128942
http://www.informaworld.com

positive HR-ESI-MS giving a quasi-molecular ion peak at *m/z* 523.1709 $[M+Na]^+$. The UV absorption maxima at 281, 216, and 214 nm were characteristic of a bibenzyl skeleton [12,13]. The presence of phenol groups was indicated by the color reaction with $FeCl_3$ (violet) on silica gel thin layer chromatography and its IR absorption bands at 3452 cm^{-1} (OH). The IR spectrum also exhibited absorptions at 1632 and 1512 cm^{-1} (aromatic residues). The ^1H NMR spectrum of **1** (Table 1) was analyzed by the $^1H-^1H$ COSY and HMQC experiments, suggesting 11 aromatic protons, seven aliphatic protons, and two methoxyl proton signals at δ_H 3.78 (3H, s) and 3.68 (3H, s). Among the 11 aromatic protons, there were two pairs of a typical AB system of *ortho*-coupled doublet, assigned to H-2′, 6′ at δ 6.97 (2H, dd, $J = 8.5$, 2.1 Hz), H-3′, 5′ at δ 6.67 (2H, dd, $J = 8.5$, 2.1 Hz), H-2″, 6″ at δ 6.47 (2H, dd, $J = 8.7$, 2.0 Hz), and H-3″, 5″ at δ 6.61 (2H, dd, $J = 8.7$, 2.0 Hz) and three aromatic proton singlets at δ_H 6.45 (1H, s, H-6), 6.30 (1H, s, H-2), and 6.10 (1H, s, H-6″).

In the HSQC spectrum, the signals at δ_H 2.66–2.70 (m, 2H, H-8′) and 2.71–2.78 (m, 2H, H-7′) among the seven aliphatic protons corresponded to the carbon signals

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectral data of **1** and **2** (**1** in MeOD, **2** in acetone-d_6, δ in ppm, J in Hz).

	1		**2**	
Position	δ_H (multiplicity, J)	δ_C	δ_H (multiplicity, J)	δ_C
1		142.6		147.8
1a		116.7		120.0
2	6.45 (s)	112.8	6.66 (s)	109.2
3		142.7		130.3
4	6.30 (s)	102.5		134.8
4a		155.7		142.6
5		138.8		131.2
5a		141.8		140.8
6		137.9		137.8
7		157.9		142.3
8	6.10 (s)	110.7	6.13 (s)	110.4
8a		118.3		118.7
9	3.96 (s)	40.0	4.08 (s)	40.5
1′		134.5		135.4
2′	6.97 (dd, 8.5, 2.1)	130.8	7.13 (dd, 8.7, 2.1)	130.9
3′	6.67 (dd, 8.5, 2.1)	116.8	6.84 (dd, 8.7, 2.1)	115.2
4′		157.1		159.2
5′	6.67 (dd, 8.7, 2.1)	116.8	6.84 (dd, 8.7, 2.1)	115.2
6′	6.97 (dd, 8.7, 2.1)	130.8	7.13 (dd, 8.7, 2.1)	130.9
7′	2.71–2.78 (m)	38.3	2.85 (m)	38.3
8′	2.66–2.70 (m)	35.8	2.75–2.80 (m)	35.2
1″		132.4		131.1
2″	6.47 (dd, 8.7, 2.0)	132.2	6.58 (dd, 8.7, 2.3)	116.1
3″	6.61 (dd, 8.7, 2.0)	114.6	6.52 (dd, 8.7, 2.3)	132.0
4″		160.1		157.3
5″	6.61 (dd, 8.7, 2.0)	114.6	6.52 (dd, 8.7, 2.3)	132.0
6″	6.47 (dd, 8.7, 2.0)	132.2	6.58 (dd, 8.7, 2.3)	116.1
7″	2.60–2.66 (2H, m)	46.3	2.67–2.76 (m)	46.2
1-OCH_3			3.82 (s)	57.4
6-OCH_3	3.78 (s)	62.1	3.92 (s)	61.9
4′-OCH_3			3.74 (s)	56.1
4″-OCH_3	3.68 (s)	56.1		

at δ_C 35.8 (C-8′) and 38.3 (C-7′), which suggested the presence of two methylene groups of a bibenzyl unit. The other three aliphatic protons consisted of a methylene group at δ_H 2.60–2.66 (m, 2H, H-7″) and a methine proton at δ_H 3.96 (H-9), which correlated to the carbon signals at δ_H 46.3 (C-7″) and 40.0 (C-9), respectively. By an extensive analysis of the $^1H-^1H$ COSY spectrum, the correlation between the methine proton signal at δ_H 3.96 (H-9) and a methylene proton signal at δ_H 2.60–2.66 (H-7″) could be observed, which is reminiscent of a bibenzyl unit [14].

The ^{13}C NMR and HSQC spectra revealed that **1** contained 12 quaternary, 12 methine, three methylene, and two methoxyl carbons. By the analysis of the DEPT, HSQC and HMBC spectra, a dimeric structure of two bibenzyl moieties linked by a C—C (sp^2—sp^3) bond [15] was assumed. In the HMBC spectrum of **1**, the proton signal at δ_H 3.96 (1H, m, H-9) showed the significant long-range correlations with aromatic carbon signals at δ_C 155.7 (C-4a), 142.6 (C-1), 141.8 (C-5a), 118.3 (C-8a), 116.7 (C-1a), and 110.7 (C-8), and the proton signal at δ_H 6.10 (H-8) showed correlations with carbon signals at δ_C 141.8 (C-5a), 137.9 (C-6), and 40.0 (C-9). So, the C—C (sp^2—sp^3) bond between C-9 and C-1a was confirmed (Figure 1). The other important long-range correlations in the HMBC spectrum were also obtained as follows: proton signal at δ_H 2.60–2.66 (2H, m, H-7″) with carbon signals at δ_C 118.3 (C-8a), 40.0 (C-9), and 132.2 (C-2″); proton signal at δ_H 6.97 (2H, d, $J = 8.5$, 2.1 Hz, H-2′, 6′) with carbon signals at δ_C 157.1 (C-4′) and 38.3 (C-7′); proton signal at δ_H 6.30 (1H, d, $J = 2.4$ Hz, H-4) with carbon signals at δ_C 116.7 (C-1a) and 112.8 (C-2), and thus the structure of *p*-hydroxyl phenethyl (CH_2—CH_2—Ar′) moiety was obtained.

By an extensive analysis of the HMBC and $^1H-^1H$ COSY spectra, correlations can be obtained between proton signals at δ_H 6.47 (2H, dd, $J = 8.7$, 2.0 Hz, H-2″, 6″) and carbon signals at δ_C 160.1 (C-4″) and 46.3 (C-7″), between proton signal at δ_H 6.61 (2H, dd, $J = 8.7$, 2.1 Hz, H-3″, 5″) and carbon signal at δ_C 132.4 (C-1″), between proton signal at δ_H 3.96 (1H, m, H-9) and carbon signals at δ_C 46.3 (C-7″) and 132.4 (C-1″) in the HMBC spectrum (Figure 1), and thus the moiety of *p*-methoxybenzyl was linked finally at C-9. In the HSQC spectrum, the two methoxyl proton signals at δ_H 3.68 (3H, s) and 3.78 (3H, s) showed correlations with carbon signals at δ_C 160.1 (C-4″) and 137.9 (C-6), respectively. In addition, correlations in the NOESY spectrum were observed between the methoxyl proton at δ_H 3.68 and H-3″, H-5″ (δ_H 6.61), which indicated that the substitution at C-4″ was a methoxyl group.

Therefore, based on the above extensive analyses, the structure of **1** was deduced as 3-(4′-hydroxyphenethyl)-9-(4″-metho-

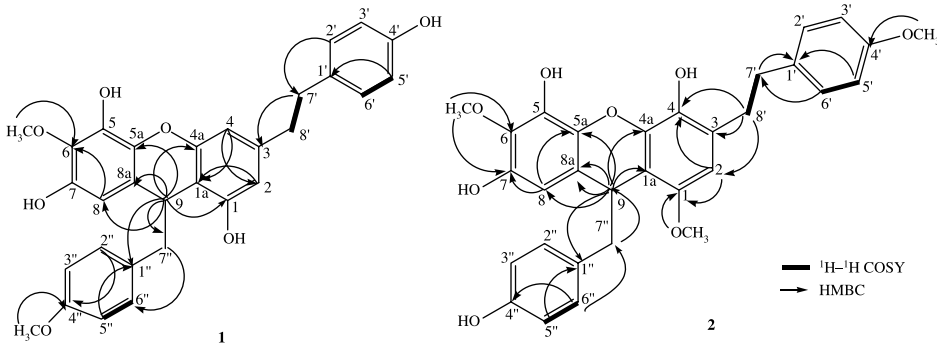

Figure 1. Main correlations in the $^1H-^1H$ COSY and HMBC spectra of compounds **1** and **2**.

xybenzyl)-6-methoxy-9H-xanthene-1,5,7-triol and named as dencryol A.

Compound **2** was obtained as a pale-orange amorphous powder (acetone-d_6), mp 89–90°C, $[\alpha]_D^{20}$ −12.8 ($c = 0.05$, MeOH). The UV absorption maxima at 214, 279, and 284 nm were characteristic of a bibenzyl skeleton. The presence of the phenolic groups was also indicated by its IR absorption bands 3455 cm^{-1} (OH), while its molecular formula $C_{31}H_{30}O_8$ was deduced by the positive HR-ESI-MS at *m/z* 553.1833 $[M+Na]^+$. In the ^1H NMR spectrum of **2**, two pairs of *ortho*-coupled proton signals at δ_H 7.13 (2H, dd, $J = 8.7$, 2.1 Hz) and 6.84 (2H, dd, $J = 8.7$, 2.1 Hz), and two aromatic proton signals at δ_H 6.66 (1H, s) and 6.13 (1H, s) can be obtained. The ^1H NMR spectral signals due to the seven aliphatic protons were superimposed between **2** and **1**, which suggested that compound **2** may possess the same skeleton as **1**, the main differences between the structures of **1** and **2** being the number and position of methoxyl and hydroxyl.

In the HMBC spectrum, aromatic proton signals at δ 6.66 (1H, s, H-2) showed correlations with carbon signals at δ_C 120.0 (C-1a), 35.2 (C-8′), 147.8 (C-1), and 134.8 (C-4), aliphatic proton signal at δ_H 2.75–2.80 (2H, m, H-α) correlated with δ_C 109.2 (C-2) and 134.8 (C-4), and one methoxyl proton signal at δ_H 3.82 (3H, s) showed correlation with the carbon signal at δ_C 147.8 (C-1). In the NOESY spectrum, the methoxy proton at δ_H 3.82 showed correlations with the protons at δ_H 6.66 (H-2) and 4.08 (1H, m, H-9) and with C-1 (δ_C 147.8) in HSQC, which indicated that the substitution at C-1 was a methoxyl group. From the correlations of the methoxyl proton at δ_H 3.74 and H-3′, H-5′ (δ_H 7.13) in the NOESY spectrum, the methoxyl group at δ_H 3.74 was assigned to C-4′. Thus, the structure of **2** was determined as 3-(4′-methoxyphenethyl)-9-(4″-hydroxybenzyl)-1,6-dimethoxy-9H-xanthene-4,5,7-triol, named as dencryol B (Figure 1).

Compound **3** was obtained as a white amorphous powder and the molecular formula assigned as $C_{18}H_{14}O_5$ from positive HR-ESI-MS at *m/z* 333.0721 $[M+Na]^+$, indicating 12 degrees of unsaturation. The UV spectrum of **3** in MeOH showed absorption maxima at 236, 276 (sh), 285, 318, 333 (sh), and 373 nm, suggesting that **3** has a phenanthrene skeleton [16]. The ^1H and ^{13}C NMR spectral data of compound **3** were similar to those of fimbiatone [16], except for the appearance of new signals of the ethoxyl group. A detailed analysis of the ^1H–^1H COSY and HMQC spectra indicated that compound **3** might be a phenanthrene lactone derivative with an oxygenated ethyl group. The HMBC spectrum (Figure 2) unambiguously confirmed that the OCH_2CH_3 group was connected at C-2 due to the long-range correlations of C-2 with H-3 and OCH_2 at δ_H 4.28, and the methoxyl was connected at C-7 due to the

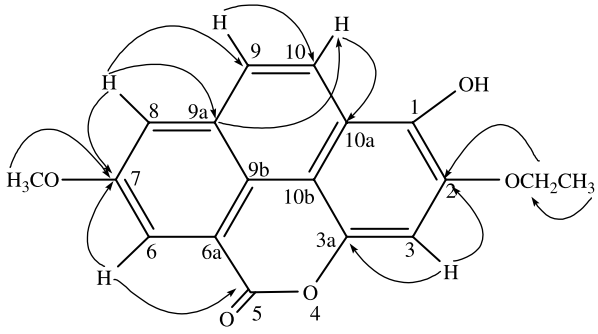

Figure 2. Structure and key HMBC correlations of **3**.

correlations of C-7 with H-6, H-8, and HCH_2O. Therefore, the structure of **3** was elucidated as 2-ethoxy-1-hydroxy-7-methoxy-5H-naphtho[8,1,2-cde]chromen-5-one, named crystalltone.

Compound **4** was obtained as white needles, with $[\alpha]_D^{25}$ +100.9 ($c = 0.1$, MeOH). The pseudomolecular ion peak at *m/z* 305.1355 $[M+Na]^+$ in its HR-TOF-MS corresponded with the molecular formula $C_{15}H_{22}O_5$. In the IR spectrum, absorption bands at 3387 and 1746 cm^{-1} revealed the existence of hydroxyl and γ-lactone groups, respectively. The ^{13}C NMR spectrum of **4** showed 15 carbon signals including three methyl, one methylene, eight methine, and three quarterary carbons. The ^1H NMR spectrum demonstrated the presence of three methyl groups at δ_H 1.51, 1.02, and 1.00, two oxygenated methylene protons at δ_H 4.20 and 4.22, and three oxygenated methine protons at δ_H 3.81, 4.45, and 4.60. The ^{13}C NMR spectral data and the presence of five degrees of unsaturation in compound **4** suggested that it was a sesquiterpene with a double bond posessing a picrotoxane-type skeleton. When compared with the known compound flakinin B [17], their NMR spectral data were very similar (Table 2). The only difference is that there is a hydroxyl group but no oxymethyl group in compound **4**. Analysis of the NOESY and HSQC spectra showed the correlations between H-2 and H-3, H-13, H-14; H-3 and H-13, H-14; H-6 and H-10, which indicated the methyl group at C-1, H-2, H-6 and the isopropyl group at C-4 were all in the same orientation. The correlation between H-5 and H-7 showed that the hydroxy at C-7 was α-oriented. Accordingly, **4** was confirmed to be a new picrotoxinin-type sesquiterpene, as shown in Figure 3, and assigned the trivial name crystallinin.

Compound **5** was obtained as white needles, and its molecular formula was established as $C_{10}H_{12}O_4$ by HR-TOF-MS giving a pseudomolecular ion at *m/z* 197.0816 $[M+H]^+$, indicating five degrees of unsaturation. The ^1H NMR spectrum of **5** showed two methyl groups at δ_H 2.02 (3H, s,) and 2.42 (3H, s,), one methoxyl group at δ_H 3.91 (3H, s), one aromatic proton at δ_H 6.33 (1H, s, H-4), and two hydroxyl protons at δ_H 8.85 and 11.97. In the ^{13}C NMR and HMBC spectra, the hydroxy proton of carboxyl at δ_H 11.97 was correlated with C-1 (δ_C 163.8), simultaneously the correlation of 2-$HOCH_2$ at δ_H 3.90 and the methyl proton at δ_H 2.02 with C-2 (δ_C 161.1) and carboxyl (δ_C 173.4), respectively, was observed, so we can infer that the methoxy and a methyl group (δ_H 2.02) were at the *ortho* position of the carboxyl. The significant relations of H-4 with C-2 (δ_C 161.1), C-6 (δ_C 109.5), and C-5 (δ_C 104.9) in the HMBC spectrum (Figure 4) indicated that the aromatic proton at δ_H 6.33 was at the *meta* position of C-2 and C-6. At the same time, the methyl protons at δ_H 2.42 correlated with C-5 (δ_C 104.9) and C-3 (δ_C 140.5) and the aromatic proton at δ_H 6.33 in the NOSEY experiment showed that another methyl group was connected at C-5. Thus, compound **5** was determined as 3-hydroxy-2-methoxy-5,6-dimethylbenzoic acid.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a JASCO P-1020 digital automatic polarimeter. The UV spectra were recorded on a Shimadzu UV-2501 spectrometer (Kyoto, Japan). The IR spectra were taken on a Nicolet Impact 410 infrared spectrophotometer (Madison, WI, USA). HR-ESI-MS were obtained on an Agilent G3250AA LC/MSD TOF mass spectrometer (Santa Clara, CA, USA). The NMR experiments were performed on a Bruker AV-500 spectrometer (Fllanden, Switzerland) with TMS as an internal standard. Silica gel (200–300 mesh for column chromatography and GF254 for TLC) was obtained from Qingdao Marine Chemical Com-

Table 2. ^{1}H (500 MHz) and ^{13}C (75 MHz) NMR spectral data of **3** and **4** (**3** and **4** in acetone-d_6, δ in ppm, J in Hz).

	3		Fimbriatone[a]			**4**		Flakinin B[b]	
Position	δ_H (m.[c], J)	δ_C	δ_H (m., J)	δ_C	Position	δ_H (m., J)	δ_C	δ_H (m., J)	δ_C
1		158.3	7.23 (d, 1.9)	107.1	1		54.1		53.0
2		164.5		157.6	2	3.81 (s)	75.5	3.65 (brs)	73.8
3	7.03 (s)	99.7	7.07 (d, 1.9)	102.5	3	4.45 (d, 5.5)	85.3	4.55 (d, 5.5)	85.5
3a		163.9		150.5	4	2.08 (m)	52.9	2.09 (m)	51.5
5		184.6		160.8	5	2.17 (d, 3.7)	52.3	2.48 (d, 4.0)	49.9
6	6.97 (d, 8.7)	101.9	8.03 (s)	117.8	6	2.48 (m)	45.9	2.22 (brd, 6.0, 3.5)	47.2
6a		154.6		113.2	7	4.59 (d, 2.6)	78.5	4.15 (d, 2.0)	86.1
7		162.3		147.9	8	5.59 (d, 1.8)	130.7	5.74 (d, 2.0)	129.7
8	7.26 (d, 8.7)	105.2		147.9	9		155.5		154.2
9	8.07 (d, 9.0)	123.9	7.99 (d, 9.3)	120.8	10	1.51 (s)	31.4	1.41 (s)	29.6
9a		110.8		122.6	11a	4.20 (s)	61.3	4.01 (d, 12.6)	58.9
9b		123.2		122.6	11b	4.22 (s)		4.20 (d, 12.6)	
10	7.65 (d, 9.0)	133.2	7.84 (d, 9.3)	126.3	12	1.76–1.78 (m)	26.7	1.70 (m)	25.3
10a		113.5		130.8	13	1.01 (d, 6.7)	21.7	0.97 (d, 6.5)	20.8
10b		116.2		107.2	14	1.01 (d, 6.7)	20.5	0.97 (d, 6.5)	19.5
2-OCH_2	4.28 (dd, 1.9, 13.8)	66.5			15		178.3		179.8
CH_3	1.50 (t, 6.9)	15.7			OMe			3.28 (s)	56.7
7-OCH_3	3.99 (s)	56.9							
8-OCH_3			4.08 (s)	60.9					
2-OH			10.30 (s)						
7-OH			10.41 (s)						

[a] ^{1}H and ^{13}C NMR spectral data of fimbriatone in DMSO.
[b] ^{1}H and ^{13}C NMR spectral data of flakinin B in $CDCl_3$–CD_3OD (9:1) at 300 MHz.
[c] m., multiplicity.

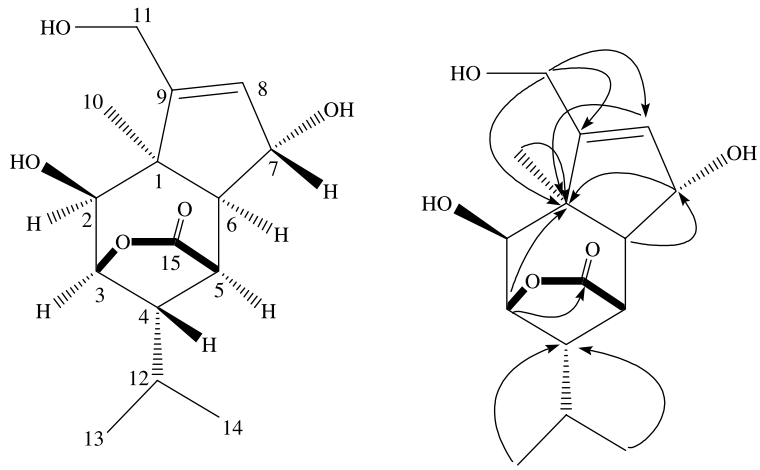

Figure 3. Structure and key HMBC correlations of **4**.

pany, Qingdao, China. Sephadex LH-20 and ODS were purchased from Pharmacia Company, Kalamazoo, MI, USA. MCI GEL was obtained from Mitsubishi Chemical Corporation, Tokyo, Japan.

3.2 Plant material

The stems of *D. crystallinum* Rchb.f. were collected from Menglian, Yunnan Province, China, in 2004 and authenticated by Prof. Luo-Shan Xu. A voucher specimen (JM-2004-05) has been deposited in the Research Department of Pharmacognosy, China Pharmaceutical University.

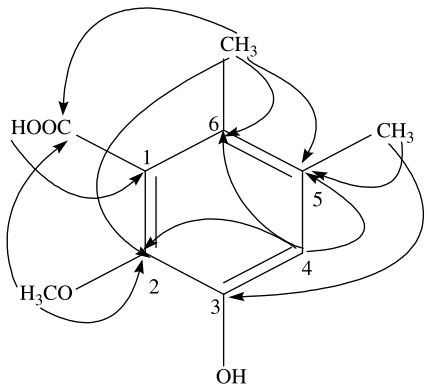

Figure 4. Structure and key HMBC correlations of **5**.

3.3 Extraction and isolation

The air-dried stems of *D. crystallinum* (10 kg) were extracted with 90% ethanol under reflux and evaporated *in vacuo* to yield a syrupy residue (850 g). The residue was suspended in water and partitioned with EtOAc (3000 ml × 5) and *n*-BuOH (3000 ml × 4) successively and evaporated *in vacuo*, respectively, to yield the corresponding fractions (400 and 185 g). The EtOAc extract was subjected to column chromatography [silica gel (200–300 mesh, 2000 g), petroleum ether–Me_2CO (99:1–0:100)] to afford 15 fractions. Fraction 2 (12 g) was chromatographed on silica gel with a Pt–EtOAc gradient system (20:1–2:1) to yield **5** (3 mg). Fraction 5 (14 g) was subjected to column chromatography [silica gel, $CHCl_3$–MeOH (10:1, v/v)] and purified by a Sephadex LH-20 column ($CHCl_3$–MeOH, 1:1, v/v) to afford compounds **7** (4 mg), **8** (60 mg), and **11** (6 mg). Fraction 9 (12 g) was treated as fraction 5 to provide compounds **1** (5 mg) and **2** (11 mg). Fraction 10 (20 g) was further separated by column chromatography [silica gel, petroleum ether–acetone (25:1, v/v)] and purified by a Sephadex LH-20 column ($CHCl_3$–MeOH, 1:1, v/v) to afford com-

pounds **3** (5 mg), **4** (9 mg), and **6** (5 mg). The *n*-BuOH extract (185 g) was subjected to column chromatography [silica gel, $CHCl_3$–MeOH (50:1–1:1, v/v)] to give six fractions (A–F). Fraction D (30 g) was separated by column chromatography (MCI gel, MeOH–H_2O, 1:5–1:0), and then purified on ODS eluting with the MeOH–H_2O gradient system (1:4–1:0) to yield compounds **9** (35 mg) and **10** (44 mg).

*3.3.1 3-(4′-Hydroxyphenethyl)-9-(4″-methoxybenzyl)-6-methoxy-9H-xanthene-1,5,7-triol (**1**)*

An orange amorphous powder (MeOH), mp 89–90°C, $[\alpha]_D^{20}$ +7.1 ($c = 0.05$, MeOH), UV (MeOH) λ_{max} (nm): 281, 216, 214; IR (KBr) ν_{max} (cm^{-1}): 3452, 1632, 1512; ^1H and ^{13}C NMR spectral data are listed in Table 1; ESI-MS *m/z*: 499 $[M-H]^-$, HR-ESI-MS *m/z*: 523.1709 $[M+Na]^+$ (calcd for $C_{30}H_{28}O_7Na$, 523.1727).

*3.3.2 3-(4′-Methoxyphenethyl)-9-(4″-hydroxybenzyl)-1,6-dimethoxy-9H-xanthene-4,5,7-triol (**2**)*

A pale-orange amorphous powder (acetone-d_6), mp 89–90°C, $[\alpha]_D^{20}$ −12.8 ($c = 0.05$, MeOH), UV (MeOH) λ_{max} (nm): 284, 279, 214; IR (KBr) ν_{max} (cm^{-1}): 3454, 1637, 536; ^1H and ^{13}C NMR spectral data are listed in Table 1; ESI-MS *m/z*: 529 $[M-H]^-$, HR-ESI-MS *m/z*: 553.1833 $[M+Na]^+$ (calcd for $C_{31}H_{30}O_8Na$, 553.1833).

*3.3.3 2-Ethoxy-1-hydroxy-7-methoxy-5H-naphtho[8,1,2-cde]chromen-5-one (**3**)*

A white amorphous powder, mp 260–262°C; UV (MeOH) λ_{max} (nm): 236, 276 (sh), 285, 318, 333 (sh), 373; IR (KBr) ν_{max} (cm^{-1}): 3453, 1635, 1467, 1401, 1160, 1053, 839; ^1H and ^{13}C NMR spectral data are listed in Table 2. ESI-MS *m/z*: 309 $[M-H]^-$, HR-ESI-MS *m/z*: 333.0721 $[M+Na]^+$ (calcd for $C_{18}H_{14}O_5Na$, 333.0733).

*3.3.4 Crystallinin (**4**)*

White needles; $[\alpha]_D^{25}$ +100.9 ($c = 0.1$, MeOH); ^1H and ^{13}C NMR spectral data are listed in Table 2. ESI-MS *m/z*: 587 $[2M+Na]^+$, HR-TOF-MS *m/z*: 305.1355 $[M+Na]^+$ (calcd for $C_{15}H_{22}O_5Na$, 305.1355).

*3.3.5 3-Hydroxy-2-methoxy-5,6-dimethylbenzoic acid (**5**)*

White needles; ^1H NMR (acetone-d_6, 300 MHz) δ: 11.97 (1H, s, COOH), 8.85 (1H, s, 3-OH), 6.33 (1H, s, H-4), 3.91 (3H, s, 2-OCH_3), 2.42 (3H, s, 5-CH_3), 2.02 (3H, s, 6-CH_3); ^{13}C NMR (acetone-d_6, 75 MHz) δ: 163.8 (C-1), 161.1 (C-2), 140.5 (C-3), 112.5 (C-4), 104.9 (C-5), 109.5 (C-6), 173.4 (C—COOH), 52.1 (C—OCH_3), 24.8 (5-CH_3), 8.7 (6-CH_3). ESI-MS *m/z*: 195 $[M-H]^-$, HR-TOF-MS *m/z*: 197.0816 $[M+H]^+$ (calcd for $C_{10}H_{13}O_4$, 197.0816).

Acknowledgements

We are grateful to Dr Dong-Jun Chen and Associate Professor De-Ran Xu of China Pharmaceutical University for NMR and ESI-MS spectra. This work was supported by the Natural Science Foundation of China (NSFC) for Luo-Shan Xu (No. 3017114), the 211 program for Chao-Feng Zhang (No. 2004), and the Foundation of Young Teachers of China Pharmaceutical University (No. B0606).

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