Three New Dicoumarins from *Daphne feddei*

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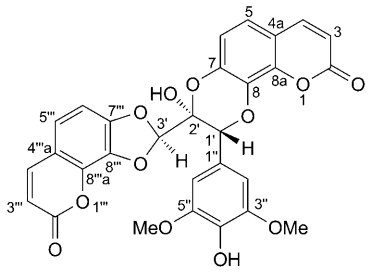
A novel dicoumarinolignoid, feddeiticin (**1**), the first example with a dicoumarinolignoid skeleton, along with the two new dicoumarin glucosides **2** and **3**, were isolated from the stem barks of *Daphne feddei*. The structures were elucidated on the basis of spectral analyses.

Introduction. – *Daphne feddei* LÉVL. is a common evergreen shrub cultivated in Yunnan, Sichuan, and Guizhou provinces in China. Its stem barks are used as a folk medicine for the treatment of injuries from falls and bruises [1]. In a previous chemical investigation of *D. feddei*, the occurrence of four diterpenes had been reported [2]. In the course of our studies on the constituents of thymelaeaceous plants [3–5], we investigated this plant and isolated a novel dicoumarinolignoid, feddeiticin[1]) (**1**), the first example with a dicoumarinolignoid skeleton, along with the two new dicoumarin glucosides **2** and **3** (coumarin = 2*H*-1-benzopyran-2-one). Herein, we report the isolation and structural elucidation of the three new compounds.



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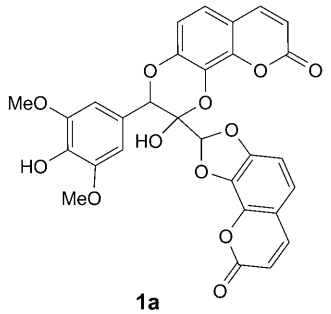
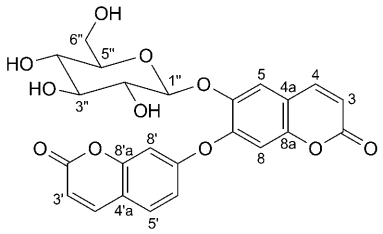


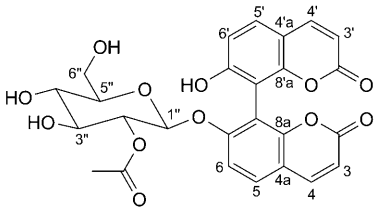
1a

Results and Discussion. – Feddeiticin (**1**) was obtained as a white powder (MeOH). The molecular formula $C_{29}H_{20}O_{12}$ was established by HR-ESI-MS (*m/z* 583.0848 ([*M* + Na]$^+$)). The assignments of the ^{1}H- and ^{13}C-NMR data (*Table 1*) were made by comparison with the data of daphneticin (= (2*R*,3*R*)-2,3-dihydro-3-(4-hydroxy-3,5-

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*

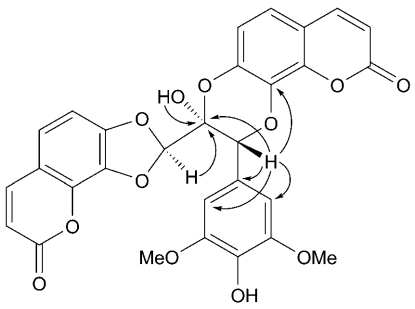


2



3

dimethylphenyl)-2-(hydroxymethyl)-9*H*-pyrano[2,3-*f*]-1,4-benzodioxin-9-one) [6] and confirmed by COSY, HMQC, HMBC (*Fig.*), and NOESY experiments. To the best of our knowledge, **1** is the first example with a dicoumarinolignoid skeleton isolated from a natural source.



1

Figure. *Selected HMBC of compound* **1**

The ^{13}C-NMR and DEPT spectrum of **1** revealed 29 resonances, including those of two Me and twelve CH groups, and of fifteen quaternary C-atoms. In the ^{1}H-NMR spectrum, two pairs of *d* with an *AB* coupling pattern (δ(H) 6.30 (J = 9.6 Hz, H–C(3)) and 7.80 (J = 9.6 Hz, H–C(4)); δ(H) 7.34 (J = 9.6 Hz, H–C(5)) and 7.14 (J = 9.6 Hz, H–C(6)), along with another two pairs of *d* with an *AB* coupling pattern H-atom resonance (δ(H) 6.31 (J = 9.6 Hz, H–C(3‴)) and 7.94 (J = 9.6 Hz, H–C(4‴)); δ(H) 7.27 (J = 9.6 Hz, H–C(5‴)) and 7.00 (J = 9.6 Hz, H–C(6‴))), indicated the existence of two 7,8-dioxygenated coumarin groups [6]. In the ^{1}H-NMR spectrum, a *s* at δ(H) 6.94 (H–C(2″) and H–C(6″)) integrating for two aromatic H-atoms, together with the presence of two identical MeO groups at δ(H) 3.76 (*s*, 6 H), indicated a typical 4-hydroxy-3,5-dimethoxy-substituted benzene ring. This was confirmed by the nearly identical NMR spectra in the corresponding region of daphneticin, isolated form *Daphne tangutica* [6]. A three-C-atom sequence, CH(O)–C(OH)–CH(O)$_2$ (C(1′), C(2′), and C(3′)), was deduced from the presence of a *s* at δ(H) 5.34 (H–C(1′)), a *d* at δ(H) 8.82 (J = 7.2 Hz, OH–C(2′)), and a *d* at δ(H) 4.81 (J = 7.2 Hz, H–C(3′)), as well as from the corresponding C-atom resonances at δ(C) 76.3 (C(1′)), 93.0 (C(2′)), and 90.9 (C(3′)). The HMBCs δ(C) 93.0 (C(2′))/δ(H) 5.34 (H–C(1′)) and 4.81 (H–C(3′)) (*Fig.*) further confirmed this three-C-atom sequence. The HMBC δ(H) 5.34 (*s*, H–C(1′))/δ(C) 106.3 (C(2″) and C(6″)) suggested that the three-C-atom sequence was attached to the 4-hydroxy-3,5-dimethoxyphenyl group. The fact that δ(H) 8.82 (*d*, J = 7.2 Hz, OH–C(2′)) had a correlation with δ(C) 93.0 (C(2′)) suggested that the OH group was attached to C(2′). On the basis of the above data, the other 7,8-

Table 1. *^{13}C- and ^{1}H-NMR Data* ((D_6)DMSO) *of Compound* **1**[1]). δ in ppm, J in Hz.

	1[1])			**1**[1])	
	δ(C)	δ(H)		δ(C)	δ(H)
C(2)	159.4		H−C(6″)	106.3	6.94 (*s*)
H−C(3)	113.4	6.30 (*d*, *J* = 9.6)	C(2‴)	159.3	
H−C(4)	144.5	7.80 (*d*, *J* = 9.6)	H−C(3‴)	113.4	6.31 (*d*, *J* = 9.6)
C(4a)	113.9		H−C(4‴)	144.4	7.94 (*d*, *J* = 9.6)
H−C(5)	121.5	7.34 (*d*, *J* = 9.6)	C(4‴a)	114.0	
H−C(6)	113.6	7.14 (*d*, *J* = 9.6)	C(5″)	147.6	
C(7)	145.9		H−C(5‴)	121.6	7.27 (*d*, *J* = 9.6)
C(8)	127.7		H−C(6‴)	113.6	7.00 (*d*, *J* = 9.6)
C(8a)	143.2		C(7‴)	144.2	
H−C(1′)	76.3	5.34 (*s*)	C(8‴)	127.1	
C(2′)	93.0		C(8‴a)	143.0	
H−C(3′)	90.9	4.81 (*d*, *J* = 7.2)	*Me*O−C(3″)	55.9	3.76 (*s*)
C(1″)	122.0		*Me*O−C(5″)	55.9	3.76 (*s*)
H−C(2″)	106.3	6.94 (*s*)	OH−C(2′)		8.82 (*d*, *J* = 7.2)
C(3″)	147.6		OH−C(4‴)		8.65 (*s*)
C(4″)	136.6				

dioxygenated coumarin group was located at C(3′) through a 1,3-dioxolane ring. The $^{1}H,^{1}H$-COSY and HMBC data (*Fig.*) confirmed the above deductions. This type of skeleton of **1** is similar to that of daphneticin and isodaphneticin [7]. Therefore, two structures, **1** and **1a**, are possible for feddeiticin. The final evidence in favor of **1** was the presence of the HMBC δ(H) 5.34 (*s*, H−C(1′))/δ(C) 127.7 (C(8)), and the absence of a HMBC δ(H) 5.34 (H−C(1′))/δ(C) 145.9 (C(7)). The relative configurations of H−C(1′), OH−C(2′), and H−C(3′) in **1** were determined to be β, α, and α, respectively, based on the NOE OH−C(2′)/H−C(3′) and the absence of the NOEs H−C(1′)/OH−C(2′) and H−C(1′))/H−C(3′). Furthermore, compound **1** was optically inactive and showed no ellipticity in the CD spectrum, which suggested that it occurs as a racemate.

Compound **2** was obtained as a white, optically active powder (MeOH). The molecular formula $C_{24}H_{20}O_{11}$ was established by HR-ESI-MS (*m*/*z* 507.0902 ($[M + Na]^+$)). The structure of **2** was established by comparing the NMR data (*Table 2*) with those of 6-hydroxy-7-[(2-oxo-2*H*-1-benzopyran-7-yl)oxy]-2*H*-1-benzopyran-2-one [8], and confirmed by COSY, HMQC, HMBC, and NOESY experiments.

The ^{13}C-NMR and DEPT spectra of **2** revealed 24 resonances, including those of one CH_2 and 13 CH groups, and of nine quaternary C-atoms. In the ^{1}H-NMR spectrum, two *d* with an *AB* coupling pattern (δ(H) 6.37 (*J* = 9.6 Hz, H−C(3)) and 7.94 (*J* = 9.6 Hz, H−C(4))), along with two *s* (δ(H) 7.56 (H−C(5)) and 7.32 (H−C(8))), indicated the existence of a 6,7-dioxygenated coumarin moiety. Another pair of *d* with an *AB* coupling pattern (δ(H) 6.32 (*J* = 9.6 Hz, H−C(3′)) and 7.99 (*J* = 9.6 Hz, H−C(4′))), along with an *ABX* coupling pattern (δ(H) 7.65 (*d*, *J* = 9.6 Hz, H−C(5′)), 6.94 (*dd*, *J* = 1.8, 9.6 Hz, H−C(6′)), and 6.86 (*d*, *J* = 1.8 Hz, H−C(8′))), suggested the presence of a monosubstituted coumarin moiety. The latter was assigned to be 7-oxygenated based on the NOE correlation H−C(4′)/H−C(5′). The ^{13}C-NMR spectrum suggested that **2** contained a glucose unit (δ(C) 100.3, 77.3, 76.7, 73.1, 69.7, and 60.8). The anomeric H−C(1″) of the glucose moiety was determined to be β-oriented on the basis of the coupling constant for H−C(1″) (δ(H) 5.07 (*d*, *J* = 8.4 Hz)). The HMBC H−C(1″)/C(6) (δ(C) 152.1) suggested that the sugar moiety was attached at C(6). The NMR spectra of **2** were very similar to those of 6-hydroxy-7-[(2-oxo-2*H*-1-benzopyran-7-yl)oxy]-2*H*-1-benzopyran-2-one [8], except for the additional

Table 2. *^{13}C- and ^{1}H-NMR Data* ((D_6)DMSO) *of Compounds* **2** *and* **3**. δ in ppm, J in Hz.

	2		**3**	
	δ(C)	δ(H)	δ(C)	δ(H)
C(2)	160.3		160.1	
H–C(3)	113.7	6.37 (*d*, *J* = 9.6)	113.2	6.32 (*d*, *J* = 8.4)
H–C(4)	143.9	7.94 (*d*, *J* = 9.6)	144.6	8.08 (*d*, *J* = 8.4)
C(4a)	113.4		113.7	
H–C(5)	121.0	7.56 (*s*)	129.2	7.78 (*d*, *J* = 8.4)
C(6) or H–C(6)	152.1		111.7	7.29 (*d*, *J* = 8.4)
C(7)	152.3		157.9	
H–C(8) or C(8)	104.7	7.32 (*s*)	109.5	
C(8a)	140.1		152.4	
C(2′)	160.4		160.5	
H–C(3′)	113.7	6.32 (*d*, *J* = 9.6)	111.0	6.18 (*d*, *J* = 8.4)
H–C(4′)	144.3	7.99 (*d*, *J* = 9.6)	145.1	8.00 (*d*, *J* = 8.4)
C(4′a)	114.2		111.3	
H–C(5′)	130.0	7.65 (*d*, *J* = 9.6)	129.3	7.60 (*d*, *J* = 8.4)
H–C(6′)	114.2	6.94 (*d*, *J* = 9.6)	112.8	6.94 (*d*, *J* = 8.4)
C(7′)	160.8		158.9	
H–C(8′) or C(8′)	104.1	6.86 (*d*, *J* = 2.4)	106.0	
C(8′a)	155.0		153.2	
H–C(1″)	100.3	5.07 (*d*, *J* = 8.4)	98.2	5.13 (*d*, *J* = 7.8)
H–C(2″)	73.1	3.03–3.07 (*m*)	72.5	4.47 (*dd*, *J* = 9.6, 7.8)
H–C(3″)	77.3	3.39–3.43 (*m*)	73.8	3.40–3.43 (*m*)
H–C(4″)	69.7	3.03–3.07 (*m*)	69.7	3.15–3.19 (*m*)
H–C(5″)	76.7	3.23–3.26 (*m*)	77.4	3.47–3.52 (*m*)
CH_2(6″)	60.8	3.64–3.67, 3.39–3.43 (2*m*)	60.4	3.72–3.76, 3.47–3.52 (2*m*)
Me*C*O			168.3	
*Me*CO			20.5	1.82 (*s*)

signals due to a β-glucosyl group. Thus, compound **2** was deduced as 6-(β-glucopyranosyloxy)-7-[(2-oxo-2*H*-1-benzopyran-7-yl)oxy]-2*H*-1-benzopyran-2-one.

Compound **3** was obtained as a white, optically active powder (MeOH). The molecular formula $C_{26}H_{22}O_{12}$ was established by HR-ESI-MS (*m*/*z* 549.1006 ($[M + Na]^+$)). The structure of **3** was identified by comparing the NMR data with those of giraldoid A (= 7-(β-D-glucopyranosyloxy)-7′-hydroxy-[8,8′-bi-2*H*-1-benzopyran]-2,2′-dione) [9] and confirmed by COSY, HMQC, HMBC, and NOESY experiments.

The ^{13}C-NMR and DEPT spectra of **3** revealed 26 resonances, including those of one Me, one CH_2, and 13 CH groups, and of eleven quaternary C-atoms. In the ^{1}H-NMR spectrum, two pairs of *d* with *AB* coupling patterns (δ(H) 6.32 (*J* = 8.4 Hz, H–C(3)) and 8.08 (*J* = 8.4 Hz, H–C(4)); δ(H) 7.78 (*J* = 8.4 Hz, H–C(5)) and 7.29 (*J* = 8.4 Hz, H–C(6))) indicated the existence of a 7,8-dioxygenated coumarin moiety. Another two pairs of *d* with *AB* coupling patterns (δ(H) 6.18 (*J* = 8.4 Hz, H–C(3′)) and 8.00 (*J* = 8.4 Hz, H–C(4′)); δ(H) 7.60 (*J* = 8.4 Hz, H–C(5′)) and 6.94 (*J* = 8.4 Hz, H–C(6′))) revealed another 7,8-dioxygenated coumarin moiety. The observation of six resonances at δ(C) 98.2, 77.4, 73.8, 72.5, 69.7, and 60.4 in the ^{13}C-NMR spectrum of **3** disclosed the presence of a glucose moiety. Its anomeric configuration was determined to be β on the basis of the coupling constant for H–C(1″) (δ(H) 5.13 (*d*, *J* = 7.8 Hz)). The HMBC H–C(1″)/C(7) (δ(C) 157.9) suggested that the sugar moiety was attached at

C(7). The ^{1}H- and ^{13}C-NMR (DEPT) spectra also showed signals of an Ac group (δ(H) 1.82 (*s*), 3 H); δ(C) 168.3 (Me*C*O) and 20.5 (*Me*CO). The HMBC δ(H) 4.47 (*dd*, $J = 7.8$, 9.6 Hz, H–C(2″))/δ(C) 168.3 (Me*C*O) suggested that the Ac group was attached to C(2″). The NMR data were very similar to those of giraldoid A, except for this additional Ac group. Thus, compound **3** was deduced to be 2″-*O*-acetylgiraldoid A.

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Experimental Part

General. Column chromatography (CC): silica gel *H* (SiO_2, 10–40 μm) from *Zhifu Huangwu Silica Gel D & R Plant*, Yantai, China; *Sephadex LH-20* and *ODS* from *Pharmacia* and *Merck*, resp. TLC: plates precoated with SiO_2 *H* F_{254} (5–7 μm) from *Zhifu Huangwu Silica Gel D & R Plant*, Yantai, China. Optical rotations: *Perkin-Elmer-343* polarimeter. CD Spectra: *Jasco-J810* spectrometer. UV Spectra: *Shimadzu-UV-2550* UV/VIS spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Bruker-Vector-22* spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker-DRX-600* spectrometer; at 600 (^{1}H) and 150 MHz (^{13}C, DEPT); (D_6)DMSO solns. with Me_4Si as internal standard; δ in ppm, J in Hz. HR-TOF-MS: ESI mode; *Q-Tof-Micro-Mass* spectrometer; in *m*/*z*.

Plant Material. The plant material was collected in July 2006 in Kunming City, Yunnan Province, China, and identified as *Daphne feddei* LÉVL. by Prof. *Li-Shan Xie* of the Kunming Institute of Botany. A voucher specimen was deposited with the Herbarium of the School of Pharmacy, Second Military Medical University, Shanghai (No. 200607-12).

Extraction and Isolation. The air-dried and powdered stem barks of *D. feddei* (6.5 kg) were percolated with MeOH (25 l) at r.t. for 3 × 4 h. The solvent was evaporated. Then, the extract was suspended in H_2O and partitioned with petroleum ether, AcOEt, and BuOH, successively. The AcOEt extract (400 g) was subjected to CC (SiO_2 (1 kg), 9 × 100 cm column, $CHCl_3$/MeOH 100 : 1, 50 : 1, 25 : 1, 10 : 1, 8 : 1, and 5 : 1): *Frs. 1–18*. *Fr. 13* (6.5 g) was subjected to CC (SiO_2 (150 g), 6 × 80 cm, $CHCl_3$/MeOH 20 : 1 and 15 : 1) to give impure **1**, which was further purified by CC (*Sephadex LH-20* (200 ml), MeOH): **1** (20 mg). *Fr. 15* (2.5 g) was subjected to CC (SiO_2 (75 g), 6 × 80 cm, $CHCl_3$/MeOH 10 : 1) to give impure **2** and **3**, which were further purified by CC (*ODS* (100 g), MeOH/H_2O 35 : 65): **2** (10 mg) and **3** (70 mg).

*(2*RS*,3*SR*)-2,3-Dihydro-3-hydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-3-[(2*RS*)-8-oxo-8*H*-1,3-dioxolo[4,5-*h*][1]benzopyran-2-yl]-9*H*-pyrano[2,3-*f*]1,4-benzodioxin-9-one* (**1**): White powder (MeOH). M.p. 188–189°. UV (MeOH): 248 (4.11), 312 (4.21). $[\alpha]_D^{18} = 0$ ($c = 0.09$, MeOH). CD ($c = 0.24$, MeOH): $\Delta\varepsilon_{400-190} = 0$. IR: 3431, 3396, 3086, 2938, 2839, 1744, 1704, 1457, 1263, 1118, 1036, 836. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-TOF-MS: 583.0848 ($[M + Na]^+$, $C_{29}H_{20}NaO_{12}^+$; calc. 583.0852).

*6-(β-Glucopyranosyloxy)-7-[(2-oxo-2*H*-1-benzopyran-7-yl)oxy]-2*H*-1-benzopyran-2-one* (**2**): White powder (MeOH). M.p. 147–148°. UV (MeOH): 291 (3.84), 325 (4.00). $[\alpha]_D^{17} = -23$ ($c = 0.10$, DMSO). IR: 3368, 3002, 2961, 2932, 2855, 1735, 1702, 1620, 1576, 1563, 1504, 1449, 1288, 1120, 845, 650. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-TOF-MS: 507.0902 ($[M + Na]^+$, $C_{24}H_{20}NaO_{11}^+$; calc. 507.0903).

*7-[(2-*O*-Acetyl-β-glucopyranosyl)oxy]-7′-hydroxy-[8,8′-bi-2*H*-1-benzopyran]-2,2′-dione* (**3**): White powder (MeOH). M.p. 179–180°. UV (MeOH): 320 (4.57). $[\alpha]_D^{19} = +71$ ($c = 0.17$, DMSO). IR: 3367, 3080, 2934, 2932, 2876, 1754, 1735, 1692, 1602, 1402, 1234, 1075, 838, 617. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-TOF-MS: 549.1006 ($[M + Na]^+$, $C_{26}H_{22}NaO_{12}^+$; calc. 549.1009).

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