

Bioactive Phenylpropanoids from *Daphne feddei*

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A investigação de extrato acetônico de folhas e caules de *Daphne feddei* forneceu três novos fenilpropanóides, feddeicetonas A-C (**1-3**), juntamente com nove fenilpropanóides conhecidos (**4-11**). Suas estruturas foram elucidadas com base em métodos espectroscópicos. Os compostos **1-3** foram testados com relação as suas atividades anti-HIV-1 e citotoxicidades frente a células cancerígenas. Os resultados mostraram que as atividades citotóxicas e anti-HIV-1 dos compostos **1-3** são modestas.

Investigation of acetone extract of the leaves and stems of *Daphne feddei* afforded three new phenylpropanoids, feddeiketones A-C (**1-3**), together with nine known phenylpropanoids (**4-11**). Their chemical structures were elucidated on the basis of spectroscopic methods. Compounds **1-3** were tested for their anti-HIV-1 activities and cytotoxicities against cancer cells. The results showed that compounds **1-3** have modest cytotoxic and anti-HIV-1 activities.

Keywords: *Daphne feddei*, phenylpropanoids, feddeiketones A-C, anti-HIV-1 activity, cytotoxic activity

Introduction

The genus *Daphne* (*Thymelaeaceae*) comprises about 70 species, 35 of which grow in China. *Daphne feddei* Levl., which is known as “Dian Rui Xiang” (Chinese), is distributed in Yunnan, Sichuan and Guizhou Provinces of China. It is a kind of evergreen shrub. Its leaves and stems have been used as folk medicine to treat the injuries from falls and bruises.¹ Previous chemical studies on *D. feddei* Levl. led to the identification of diterpenes, monomeric coumarins, dicoumarins, flavonoids, biflavonoids, phenylpropanoids and lignans.²⁻⁸ Among them, phenylpropanoids are important plant secondary metabolites with various biological activities, including antioxidant, anti-inflammatory, healing, antiviral and antimicrobial.⁹⁻¹¹

Our previous studies on *Daphne feddei* collected from Dali Prefecture of Yunnan Province resulted in the isolation of several lignans and phenylpropanoids including two new secolignans and one new neolignan.¹² As a continuation of investigation on chemical constituents of the same *Daphne feddei* plant, three new phenylpropanoids (**1-3**), together

with nine known phenylpropanoids (**4-12**), were isolated. In addition, the anti-HIV-1 and cytotoxic activities of compounds **1-3** were evaluated. This article deals with the isolation, structural elucidation and biological activities of the new compounds.

Results and Discussion

The 70% aq. acetone extract of *D. feddei* was suspended in H₂O and partitioned with ethyl acetate. The ethyl acetate partition was repeatedly subjected to column chromatography over Si gel and preparative HPLC (high performance liquid chromatography) to afford compounds **1-12**, including three new phenylpropanoids, named feddeiketones A-C (**1-3**), together with nine known phenylpropanoids, *p*-coumaric acid (**4**),¹³ caffeic acid (**5**),¹⁴ ferulic acid (**6**),¹⁵ coniferoside (**7**),¹⁶ isoconiferin (**8**),¹⁷ turbinataphenol A (**9**),¹⁸ nervolan B (**10**),¹⁹ nervolan C (**11**)²⁰ and 1,2-*O*-dicaffeoylcyclopenta-3-ol (**12**).²¹ The structures of compounds **1-12** are shown in Figure 1. In addition to three new compounds, compounds **8, 9, 10, 11** and **12** were isolated from the plant of *Daphne* genus for the first time.

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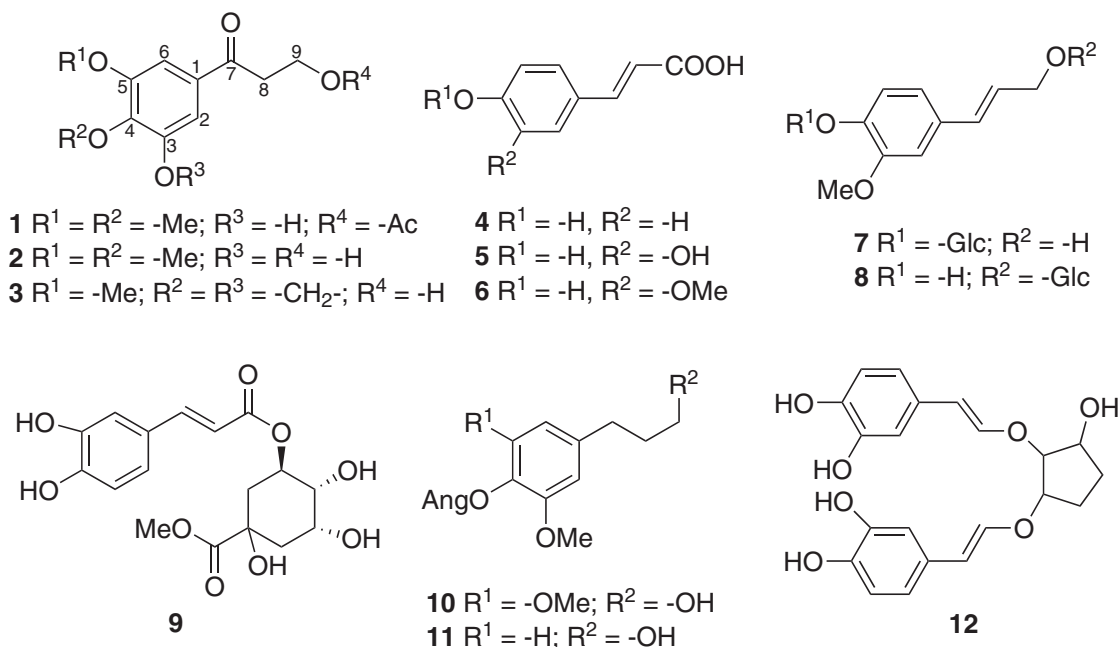


Figure 1. Chemical structures of compounds 1-12.

Compound **1** was isolated as pale yellow gum. Its molecular formula was established as C₁₃H₁₆O₆ by a *pseudo*-molecular-ion peak in the HRESIMS (high-resolution electron spray ionization mass spectrometry) (*m/z* 291.0852 [M + Na]⁺, calc. 291.0845). Its ¹H and ¹³C NMR (nuclear magnetic resonance) spectra (Table 1) showed signals to 16 hydrogens and 13 carbons, respectively, corresponding to one aromatic ring (δ_c 105.5, 111.6, 130.5, 138.6, 148.8, 152.8) with two

aromatic protons (δ_H 6.82 *d*, *J* 1.8 Hz; 7.06 *d*, *J* 1.8 Hz), one methylene group (δ_c 42.0), one methylenedioxy group (δ_c 60.0), one carbonyl group (δ_c 198.0), two methoxyl groups (δ_c 55.7, 60.9), an acetoxy group (δ_c 169.7, 21.3) and a phenolic hydroxyl group (δ_H 10.98). Strong absorption bands accounting for hydroxyl (3382 cm⁻¹), carbonyl (1715, 1705 cm⁻¹) and aromatic group (1636, 1508, 1452 cm⁻¹) could also be observed in its IR (infrared) spectrum. The UV spectrum of **1** showed absorption

Table 1. ¹H and ¹³C NMR data (in C₃D₃N) of compounds 1-3

Position	1		2		3	
	δ _c (m)	δ _H (m, <i>J</i> / Hz)	δ _c (m)	δ _H (m, <i>J</i> / Hz)	δ _c (m)	δ _H (m, <i>J</i> / Hz)
1	130.5 (s)		130.4 (s)		131.4 (s)	
2	111.6 (d)	7.06 (d, <i>J</i> 1.8)	111.4 (d)	7.05 (d, <i>J</i> 1.9)	111.1 (d)	7.06 (d, <i>J</i> 1.8)
3	148.8 (s)		149.0 (s)		143.7 (s)	
4	138.6 (s)		138.4 (s)		137.1 (s)	
5	152.8 (s)		152.7 (s)		148.5 (s)	
6	105.5 (d)	6.82 (d, <i>J</i> 1.8)	106.0 (d)	6.82 (d, <i>J</i> 1.9)	103.9 (d)	7.32 (d, <i>J</i> 1.8)
7	198.0 (s)		198.1 (s)		198.8 (s)	
8	42.0 (t)	3.43 (t, <i>J</i> 6.2)	43.8 (t)	3.44 (t, <i>J</i> 6.2)	43.7 (t)	3.32 (t, <i>J</i> 6.2)
9	60.0 (t)	4.66 (t, <i>J</i> 6.2)	58.6 (t)	4.43 (t, <i>J</i> 6.2)	58.9 (t)	4.44 (t, <i>J</i> 6.2)
OMe-4	60.9 (q)	3.70 (s)	60.7 (q)	3.70 (s)		
OMe-5	55.7 (q)	3.78 (s)	55.7 (q)	3.74 (s)	56.0 (q)	3.70 (s)
-OCH ₂ O-					101.3 (t)	5.83, 5.89 (s)
Ar-OH		10.98 (brs)		10.85 (brs)		
1'	169.7 (s)					
2'	21.3 (q)	1.99 (s)				

maxima at 289 nm confirming the existence of the aromatic function. The ^1H - ^1H COSY (correlation spectroscopy) of H-8/H-9, together with HMBC (heteronuclear multiple bond correlation) correlations (Figure 2) of aromatic protons, H-2 (δ_{H} 7.06), H-6 (δ_{H} 6.82) with C-7 (δ_{C} 198.0), of H-8 (δ_{H} 3.43) with C-1 (δ_{C} 130.5) and of H-9 (δ_{H} 4.66) with C-1' (δ_{C} 169.7) suggested that **1** is a 3-acetoxy-1-phenyl-1-propanone (Ar-CO-CH₂-CH₂OAc) and possess two methoxyl groups and a phenolic hydroxyl group on the aromatic ring. The HMBC correlations of aromatic hydroxyl proton signal (δ_{H} 10.98) with C-2 (δ_{C} 111.6), C-3 (δ_{C} 148.8), C-4 (δ_{C} 138.6) indicated that the hydroxyl group should be located at C-3. The HMBC correlations of two methoxyl proton signals (δ_{H} 3.70, 3.78) with C-4 (δ_{C} 138.6) and C-5 (δ_{C} 152.8) indicated that two methoxyl groups should be located at C-4 and C-5, respectively. Therefore, the structure of **1** was elucidated as that shown in Figure 1, and it has been trivially named as feddeiketone A.

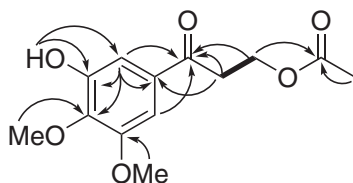


Figure 2. Selected HMBC (—) and ^1H - ^1H COSY (—) correlations of compound **1**.

Compound **2**, that was also obtained as pale yellow gum, has a molecular formula C₁₁H₁₄O, based on the HRESIMS showing a sodiated molecular ion at m/z 249.0731 [M + Na]⁺ (calc. m/z 249.0739). The ^1H and ^{13}C NMR spectra of **2** were very similar to those of **1**. The IR and UV spectra were also similar to those of compound **1**. The differences from **1** were the absence of the acetoxy group signal and the appearance of the hydroxyl proton signal (δ_{H} 4.99 brs) in **2**. Therefore, the structure of **2** was established and was named feddeiketone B.

Compound **3** was also obtained as pale yellow gum. The ^1H and ^{13}C NMR spectra of **3** were very similar to those of **2**. The obvious chemical shift differences resulted from the substituent group variations in the aromatic ring. For compound **3**, the HMBC spectrum showed that a methoxyl group was located at C-3, and a methylenedioxy group at C-4 and C-5. Accordingly, the structure of **3** was determined as shown and named feddeiketone C.

In the anti-HIV-1 tests, the cytotoxicity assay against C8166 cell CC₅₀ (cause 50% cells' death) was assessed using the MTT (1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan) method, and the anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC₅₀).¹³ Compound **1** shows anti-HIV-1 activity with EC₅₀

3.96 $\mu\text{g mL}^{-1}$, CC₅₀ 64.5 $\mu\text{g mL}^{-1}$ and TI (therapeutic index) 16.3. Compound **2** shows anti-HIV-1 activity with EC₅₀ 3.16 $\mu\text{g mL}^{-1}$, CC₅₀ 96.3 $\mu\text{g mL}^{-1}$ and TI 30.5. Compound **3** shows anti-HIV-1 activity with EC₅₀ 2.47 $\mu\text{g mL}^{-1}$, CC₅₀ 43.4 $\mu\text{g mL}^{-1}$ and TI 17.6. The results showed that compounds **1-3** have modest anti-HIV-1 bioactivities.

The cytotoxicity tests for the isolates were performed using a previously reported procedure.²² All treatments were performed in triplicate. In the MTT assay, the IC₅₀ was defined as the concentration of the test compound resulting in a 50% reduction of absorbance compared with untreated cells. The cytotoxic abilities of compound **1-3** against HL-60, Hep-G2, KB and MDA-MB-231 tumor cell lines were performed using the MTT-assay (with doxorubicin as the positive control). The results were shown in Table 2 and showed that compounds **1-3** have significant potential cytotoxic activities.

Table 2. Cytotoxicities of compounds **1-3**

Compounds	Cell lines			
	HL-60 ^b	HepG2 ^c	KB ^d	MDA-MB-231 ^e
1	3.27	1.55	1.35	2.47
2	2.08	1.18	2.84	10.5
3	1.96	4.62	1.28	3.67
Doxorubicin ^a	0.1	0.2	0.1	0.1

^aData of IC₅₀ values in $\mu\text{mol}\cdot\text{L}^{-1}$. For a compound to be deemed effective, an IC₅₀ value < 100 $\mu\text{mol}\cdot\text{L}^{-1}$ is required. Doxorubicin was used as a positive control; ^bHL-60, human acute promyelocytic leukemia; ^cHep-G2, human hepatocellular carcinoma; ^dKB, human oropharyngeal epidermoid carcinoma; ^eMDA-MB-231, human breast cancer cells.

Conclusions

The spectroscopic analyses of compounds isolated from leaves and stems of *Daphne feddei*. revealed three new phenylpropanoids (named feddeiketones A-C (**1-3**)), together with nine known phenylpropanoids (**4-11**). These new compounds had their cytotoxic activities and anti-HIV-1 bioactivity studied, with modest activities.

Experimental

General procedures

IR spectra were measured on a Bruker Tensor 27 spectrometer with KBr pellets. UV spectra were measured on a Shimadzu UV-2401A spectrophotometer. Optical rotations were measured on a Horiba SEPA-300 polarimeter. ^1H and ^{13}C NMR and 2D NMR spectra were recorded on Bruker DRX-500, with chemical shifts (δ) in ppm relative to tetramethylsilane (TMS) as internal standard and coupling constants in Hz. HRESIMS data were measured

using an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph system equipped with a ZORBAX PrepHT GF (21.2 mm × 25 cm, 7 μm) column or a Venusil MP C₁₈ (20 mm × 25 cm, 5 μm) column. Silica gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China) was used for column chromatography. Fractions were monitored by TLC (Qing-dao Marine Chemical, Inc., Qingdao, China), and spots were visualized by heating Si gel plates sprayed with 5% H₂SO₄ in EtOH and under UV light.

Plant material

The leaves and stems of *D. feddei* were collected from Dali Prefecture, Yunnan Province, People's Republic of China, in September 2009, and authenticated by Professor Chen Y. J. (Yunnan University of Nationalities). A voucher specimen (YNNI 09-9-12) is deposited at the Herbarium of Key Laboratory of Ethnic Medicine Resource Chemistry, Yunnan University of Nationalities.

Extraction and isolation

Air-dried and powdered leaves and stems of *D. feddei* (2.5 kg) were extracted with 70% aqueous acetone (4 × 2.5 L) at room temperature. After evaporating the solvents in vacuum, a residue was obtained (153 g). This residue was dissolved in H₂O and extracted with EtOAc (3 × 1 L). The EtOAc (82.5 g) extract was chromatographed on a silica gel (200-300 mesh) column and eluted with gradient mixtures of chloroform-acetone (20:1, 9:1, 8:2, 7:3, 6:4, 5:5) to afford five fractions A-F. Fraction E (6:4, 38.4 g) was subjected to silica gel column chromatography, eluted with chloroform-methanol (9:1, 8:2, 7:3, 6:4, 1:1), yielded subfractions E1-E5. Subfraction E1 (9:1, 2.47 g) was further purified by preparative HPLC eluting with H₂O-MeOH (50:50, flow rate 12 ml min⁻¹) to give **1** (21.2 mg), **3** (11.4 mg), **9** (22.6 mg) and **10** (8.62 mg). Subfraction E2 (8:2, 3.68 g) was subjected to preparative HPLC eluting with H₂O-MeOH (58:42, flow rate 12 mL min⁻¹) to afford **2** (18.5 mg), **11** (35.4 mg) and **12** (4.58 mg). Subfraction E3 (3:2, 9.2 g) was also further purified by preparative HPLC eluting with H₂O-MeOH (80:20, flow rate 12 mL min⁻¹) to afford **4** (50.5 mg), **5** (36.5 mg), **6** (24.6 mg), **7** (63.5 mg) and **8** (41.2 mg).

Anti-HIV-1 assay

Cytopathic effect inhibitory assay using C8166 cells infected with HIV-1 was used to evaluate anti-HIV-1

activity (EC₅₀). The cytotoxicities of compounds **1-3** against C8166 cells (CC₅₀) were also evaluated using the MTT method and allowed the determination of therapeutic index (TI).²¹

Cytotoxicity assay

Cytotoxic activities of compounds **1-3** against cancer cell lines HL-60, Hep-G2, KB and MDA-MB-231 were measured by the MTT method using doxorubicin as the positive control.²²

Feddeiketone A (**1**)

Pale yellow gum; UV (MeOH) λ_{max}/nm (log ε) 324 (δ_H 2.47), 289 (δ_H 4.18), 250 (δ_H 3.26), 210 (δ_H 4.82); IR (KBr) ν_{max}/cm⁻¹ 3382, 2923, 2852, 1715, 1705, 1636, 1508, 1452, 1432, 1360, 1280, 1172, 1138, 1089, 1043, 976, 825; ¹H NMR (C₅D₅N, 500 MHz) and ¹³C NMR data (C₅D₅N, 125 MHz) see Table 1; positive-mode ESIMS *m/z* 291 [M + Na]⁺; HRESIMS *m/z* 291.0852 [M + Na]⁺ (calc. for C₁₃H₁₆NaO₆, 291.0845).

Feddeiketone B (**2**)

Pale yellow gum; UV (MeOH) λ_{max}/nm (log ε) 325 (δ_H 2.44), 288 (δ_H 4.16), 250 (δ_H 3.18), 210 (δ_H 4.89); IR (KBr) ν_{max}/cm⁻¹ 3408, 2924, 2857, 1718, 1632, 1516, 1458, 1362, 1276, 1160, 1085, 974, 820; ¹H NMR (C₅D₅N, 500 MHz) and ¹³C NMR data (C₅D₅N, 125 MHz) see Table 1; positive-mode ESIMS *m/z* 249 [M + Na]⁺; HRESIMS *m/z* 249.0731 [M + Na]⁺ (calc. for C₁₁H₁₄NaO₅, 249.0739).

Feddeiketone C (**3**)

Pale yellow gum; UV (MeOH) λ_{max}/nm (log ε), 325 (δ_H 2.57), 287 (δ_H 4.22), 250 (δ_H 3.26), 210 (δ_H 4.82); IR (KBr) ν_{max}/cm⁻¹ 3412, 2925, 2862, 1718, 1628, 1518, 1462, 1453, 1273, 1156, 1082, 951, 862; ¹H NMR (C₅D₅N, 500 MHz) and ¹³C NMR data (C₅D₅N, 125 MHz) see Table 1; positive-mode ESIMS *m/z* 247 [M + Na]⁺; HRESIMS *m/z* 247.0587 [M + Na]⁺ (calc. for C₁₁H₁₂NaO₅, 247.0582).

Supplementary Information

¹³C NMR, DEPT and ¹H NMR spectra of feddeiketones A-C, HSQC, HMBC, HRESIMS spectra of feddeiketone A, are available free of charge at <http://jbcbs.sbg.org.br> as PDF file.

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