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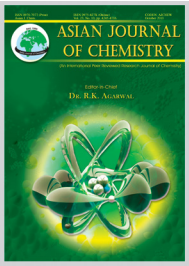

NOTE

A New Flavonoid from *Daphne feddei* and Its Anti-HIV-1 Activity

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A new flavonoid, feddeinoid A was isolated from the leaves and stems of *daphne feddei*. Its structure was determined by means of HRESIMS, extensive ^{1}D and ^{2}D NMR spectroscopic studies and chemical evidence. Feddeinoid A was also tested for their anti-HIV-1 activities. The results showed that feddeinoid A has modest anti-HIV-1 activities.

Key Words: *Daphne feddei*, Flavonoid, Feddeinoid A, anti-HIV-1 activity.

Daphne feddei levl. is a common evergreen shrub native to Yunnan, Sichuan and Guizhou Provinces in P.R. China. It has been used as a traditional Chinese medicine named Dian Rui Xiang for the treatment of the injuries from falls and bruises[1]. The previous phytochemical researches on *Daphne feddei* has revealed that diterpenes[2], phenylpropanoids[3], as well as flavonoids[4] are major components isolated from this plant.

Motivated by search for bioactive metabolites from this plant, the phytochemical investigation on *Daphne feddei* was carried out. As a result, a new flavonoid was isolated from this plant. In addition, its Anti-HIV-1 activitie was evaluated. This article deals with the isolation, structural elucidation and biological activities of the new compound.

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. ^{1}D and ^{2}D NMR spectra were recorded on DRX-500 spectrometers with TMS as internal standard. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. HRESIMS was performed on an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer, respectively. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a ZORBAX PrepHT GF (21.2 mm × 25 cm, 7 μm) column or a Venusil MP C_{18} (20 mm × 25 cm, 5 mm) column. Column chromatography was performed with Si gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China), Lichroprep RP-18 gel (40-63 μm, Merck, Darmstadt, Germany) and MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by TLC and spots were visualized by heating Si gel plates sprayed with 5 % H_2SO_4 in EtOH.

The leaves and stems of *Daphne feddei* were collected in Dali Prefecture, Yunnan Province, People's Republic of China, in September 2009. The identification of the plant material was verified by Prof. Chen Y. J. (Yunnan Nationalities University). A voucher specimen (YNNI 09-9-12) has been deposited in our laboratory.

Extraction and isolation: The air-dried and powdered leaves and stems of *Daphne feddei* (2.5 kg) were extracted four times with 70 % methanol (4 × 2.5 L) at room temperature and filtered. The crude extract (153 g) was applied to silica gel (200-300 mesh) column chromatography, eluting with a chloroform-acetone gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A-F. The further separation of fraction E (6:4, 38.4 g) by silica gel column chromatography, eluted with chloroform-methanol (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures E1-E5. Fraction E2 (8:2, 3.68 g) was subjected to preparative HPLC (40 % methanol, flow rate 12 mL/min) to give feddeinoid A (22.8 mg).

Anti-HIV-1 assay: The cytotoxicity assay against C8166 cells (CC_{50}) was assessed using the MTT method and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50})[11].

Feddeinoid A (1): Obtained as pale yellow gum; m.p. 159 ºC; $[\alpha]_D^{24.9}$ -25.8 (c 0.24, CH_3OH); UV (MeOH) λ_{max} (log ε) 362 (3.97), 286 (4.18), 268 (4.26), 210 (4.88) nm; IR (KBr, ν_{max}, cm^{-1}) 3428, 1685, 1622, 1571, 1460; ^{1}H and ^{13}C NMR data (C_5D_5N, 500 MHz), Table-1; positive ESIMS *m/z* 341

$[M+Na]^+$; HRESIMS *m/z* 341.1031 $[M-H]^+$ (calcd. 341.1025 for $C_{19}H_{17}O_6$).

A 70 % aq. methanol extract prepared from the leaves and stems of *Daphne feddei* was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and Preparative HPLC to afford feddeinoid A. The structure of the feddeinoid A was shown in Fig. 1 and its ^1H and ^{13}C NMR spectroscopic data were listed in Table-1.

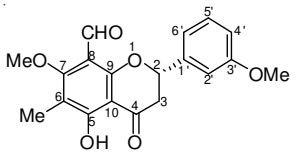

Fig. 1. Structure of feddeinoid A

TABLE-1
^1H NMR AND ^{13}C NMR DATA (C_5D_5N) OF FEDDEINOID A

Position	^{13}C	^1H
2	79.3 d	5.40, dd, *J* = 10.2, 4.6
3	45.0 t	3.02, dd, *J* = 17.0, 10.2
		2.89, dd, *J* = 4.5, 16.8
4	189.5 s	
5	164.2 s	
6	112.4 s	
7	165.2 s	
8	115.6 s	
9	163.0 s	
10	110.2 s	
1'	139.8 s	
2'	113.2 d	7.01, m
3'	162.2 s	
4'	113.8 d	6.88, d, *J* = 8.0
5'	130.6 d	7.22, t, *J* = 8.0
6'	118.5 d	6.92, m
7-OMe	63.6 q	3.77, s
3'-OMe	55.8 q	3.92, s
-Me	8.2 q	2.08, s
-CHO	188.8 s	10.12, s
5-OH		11.23, brs

The ESIMS spectrum of feddeinoid A presented a molecular ion peak at *m/z* 341.1031 [M-H] (calcd. 341.1025). The ^1H NMR spectrum showed the presence of five singlets at δ_H 11.23 (1H), 10.12 (1H), 3.77 (3H), 3.92 (3H) and 2.08 (3H) consistent with the presence of one chelated hydroxyl, one aldehyde, two methoxyl and one methyl groups, respectively. The presence of three signals at δ_H 5.40 (dd,1H, *J* = 10.2 and 4.6 Hz), 3.02 (dd, 1H, *J* = 17.0 and 10.2 Hz), 2.89 (dd, 1H, *J* = 4.5 and 16.0 Hz) and also signals for four coupled aromatic protons at δ_H 7.01 (m, 1H), 7.22 (t, 1H, *J* = 8.0 Hz), 6.88 (d, 1H, *J* = 8.0 Hz) and 6.92 (m, 2H), suggested a flavanone nucleus with a 3'-monosubstituted B ring[5], deduced by analysis of the multiplicity and coupling constants of the aromatic protons[6]. The above data and UV spectrum, with max at 268 nm, reinforced a flavanone nature for feddeinoid A[7]. Since no further aromatic protons were evident, ring A should be fully substituted. The presence of the signal to methane carbon at δ_C 188.8 in the ^{13}C NMR APT (attached proton test) spectrum was taken as a proof for the presence of an aldehyde group and the low-field hydroxyl hydrogen [δ_H 11,23 (s, 1H)] evidenced the chelated hydroxyl at C-5 with the -C=O of the a,b-unsaturated carbonyl group[7]. In the HMBC spectrum, the presence of cross peaks at δ_H 11.23 with δ_C 164.2, 165.2 and 112.4 evidenced the existence of hydroxyl at C-5, besides the cross peak at δ_H 2.08 with δ_C 164.2, 165.2 and 112.4, evidenced the existence of a methyl group at C-6. The HMBC of correlations of δ_H 3.77 with δ_C 165.2 and δ_H 3.92 with δ_C 162.2 also suggested the attachment position of the two methoxyl groups at C-7 and C-3'. The correlation of the peak at δ_H 10.12 (aldehyde hydrogen) with δ_C 165.2, δ_C 115.6, δ_C 163.0 suggested the placement of the formyl group at C-8 (Fig. 2). Thus, the structure of feddeinoid A was established as shown.

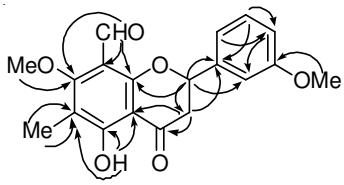

Fig. 2. Selected HMBC (→) correlations of feddeinoid A

Since some of flavonoid exhibited modest or strong anti-HIV activities[8-10], feddeinoid A was tested for their potencies in preventing the cytopathic effects of HIV-1 in C8166 and cytotoxicity measured in parallel with the determination of antiviral activity, using AZT as a positive control (EC_{50} = 0.0045 mg/mL and CC_{50} > 200 mg/mL)[11]. Feddeinoid A showed modest anti-HIV-1 activities with EC_{50} values of 5.58 mg/mL and minimal cytotoxicity against C8166 cells (CC_{50} > 200 mg/mL). The therapeutic index (TI) values (CC_{50}/EC_{50}) of feddeinoid A was more than 35.8.

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REFERENCES

1. L. Putian and Y. Jiang, Flora of China, Chinese Science Press: Beijing, (1977).
2. H. Kobayashi, H. Karasawa, T. Miyase and S. Fukushima, *Chem. Pharm. Bull*., **33**, 1452 (1985).
3. S. Liang, Y.H. Shen, J.M. Tian, Z.J. Wu, H.Z. Jin, W.D. Zhang, S.K. Yan, *J. Nat. Prod*., **71**, 1902 (2008).
4. S. Liang, J. Tang, Y.H. Shen, H.Z. Jin, J.M. Tian, Z.J. Wu, W.D. Zhang and S.K. Yan, *Chem. Pharm. Bull*., **56**, 1729 (2008).
5. T. Asai, N. Hara, S. Kobayashi and Y. Fujimoto, *Phytochemistry,* **69**, 1234 (2008).
6. D. Alavez-Solano, R. Reyes-Chilpa, M. Jiménez-Estrada, F. Gómez-Garibay, I. Chavez-Uribe and M. Sousa-Sánchez, *Phytochemistry,* **55**, 953 (2000).
7. C.-L. Ye, Y.-H. Lu and D.-Z. Wei, *Phytochemistry,* **65**, 445 (2004).
8. V. Veljkovic, J.F. Mouscadet, N. Veljkovic, S. Glisic and Z. Debyser, *Bioorg. Med. Chem. Lett.*, **17**, 1226 (2007).
9. V. Reutrakul, N. Ningnuek, M. Pohmakotr, C. Yoosook, C. Napaswad, J. Kasisit, T. Santisuk and P. Tuchinda, *Planta Med.*, **73**, 683 (2007).
10. G. Casano, A. Dumètre, C. Pannecouque, S. Hutter, N. Azas and M. Robin, *Bioorg. Med. Chem.*, **18**, 6012 (2010).
11. J.H. Wang, S.C. Tam, H. Huang, D.Y. Yang, Y.Y. Wang and Y.T. Zheng, *Biochem. Biophys. Res. Commun*., **317**, 965 (2004).