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Arylnaphthalene Lignans from *Daphne acutiloba Rehd.*

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A new arylnaphthalene lignan (daphnelignan B), together with three known one, were isolated from the leaf and steam of *Daphne acutiloba Rehd.* Their structures were determined by means of HRESIMS, extensive ^{1}D and ^{2}D NMR spectroscopic studies and chemical evidence. The anti-HIV-1 activity of daphnelignan B was also evaluated and it shows an anti-HIV-1 activity with a TI (Therapeutic Index) above 35.62.

Key Words: *Daphne acutiloba* Rehd., Arylnaphthalene lignans, Daphnelignan B, Anti-HIV-1 activity.

INTRODUCTION

Daphne acutiloba Rehd. (thymelaeaceae), an evergreen shrub mainly distributed in west China, has been used as a traditional Chinese medicine named "Dian Rui Xiang" for the treatment of rheumatoid arthritis, apoplexia and stomach ache[1-3]. Previous phytochemical research on *Daphne acutiloba Rehd.* has revealed that daphnane diterpenes, coumarines as well as lignans are major principles isolated from this plant[4-6].

In order to investigates the components of the aerial parts and search for potential leads for drug development, phytochemical investigation on *Daphne acutiloba Rehd.* was carried out. This study led to the isolation of a new arylnaphthalene lignan, daphnelignan B (**1**), together with three known one, furfuracin A (**2**)[7], justicidin B (**3**)[8] and diphyllin (**4**)[9]. Their structures were established by means of HRESIMS and extensive NMR spectra. The anti-HIV-1 activity of daphnelignan B was also evaluated.

EXPERIMENTAL

Optical rotation was measured in Horiba SEPA-300 High Sensitive Polarimeter. IR spectra were obtained in KBr disc on a Bio-Rad Wininfmred spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. ^{1}H, ^{13}C and ^{2}D

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NMR spectra were recorded on Bruker DRX-500 instruments with TMS as internal standard. Column chromatography was performed on silica gel (200-300 mesh) or on silica gel H (10-40 μm, Qingdao Marine Chemical Inc., China). On second separate used Agilent 1100 HPLC equipped with ZORBAX-C_{18} (9.4 × 250 nm, 5.0 μm) column and DAD detector.

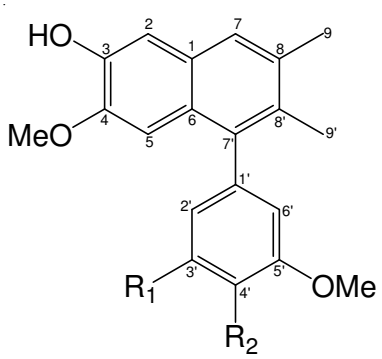
The leaf and steam of *Daphne acutiloba Rehd.* was collected in Lijian County, Yunnan Province, P. R. China, in June 2007 and was identified by Prof. N Yuan. A voucher specimen (No. YNNi 07-8-07) was deposited in our laboratory.

Extraction and isolation: The air-dried and powdered leaf and stem of *Daphne acutiloba Rehd.* (2.0 kg) were extracted with 70 % aqueous Me_2CO (5.0 L × 3, 24 h each) at room temperature and the extract was partitioned successively with petroleum ether (8.0 L × 3) and EtOAc (8.0 L × 3), respectively. The EtOAc extract (63.7 g) was subjected to column chromatography over silica gel eluting with a $CHCl_3$-Me_2CO (1:0-0:1, 30 L) gradient system. The 8:2 fraction (4.76 g) was further purified by HPLC with mobile phase (MeOH-H_2O 65:35) to yield daphnelignan B (11.8 mg), furfuracin A (36.5 mg), justicidin B (47.5 mg) and diphyllin (28.1 mg).

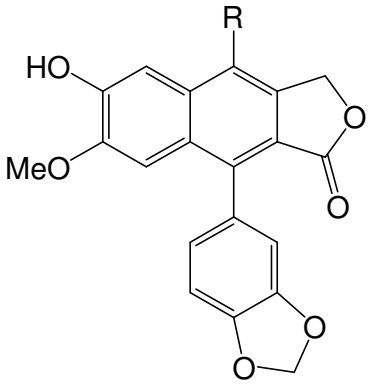
Anti-HIV-1 assay: The cytotoxicity assay against C8166 cells (CC50) was assessed using the MTT method and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC50)[10].

RESULTS AND DISCUSSION

Daphnelignan B (Fig. 1) was obtained as colourless amorphous crystals. Its UV spectrum showed strong absorption maximum at 238 and 287 nm, indicative of a naphthalene chromophore[9]. The IR spectrum showed absorption bands of hydroxyl groups at 3451 and 3415 cm^{-1}. The compound exhibited a quasimolecular ion at m/z 391.1524 $[M+Na]^+$ in its HRESI mass spectrum (calcd. 391.1521), establishing its molecular formula as $C_{22}H_{24}O_5$ with eleven degrees of unsaturation. The ^{13}C NMR spectrum (Table-1) exhibited 22 carbon signals which could be classified by DEPT experiments into those of 11 quaternary carbons, 5 methines, 2 methyl carbons and 4 methoxyl groups. The ^1H NMR (Table-1) spectra showed singlet signals of 2 methyl groups (δ_H 1.98 s and 2.40 s), 4 methoxyl groups (δ_H 3.72 s, 3.78 s, 3.83 s and 3.88 s), 1 hydroxyl protons (δ_H 11.07 s) and 5 aromatic protons (δ_H 6.35 s, 6.35 s, 6.82 s, 6.91 s, 7.38 s). These spectroscopic data and the calculated degrees of unsaturation support the arylnaphthalene basic structure of this compound. The HMBC correlation (Fig. 2) from the signal of H-2' (δ_H 6.35) to C-7' (δ_C 138.9 s) established the connectivity between the C-1' position on the benzene ring with the C-7' position on the naphthalene unit. The methyl resonance H-9 (δ_H 2.40 s) displayed HMBC correlations with C-7 (δ_C 126.3 d), C-8 (δ_C 133.8 s) and C-8' (δ_C 131.4 s), while another methyl signal H-9' (δ_C 1.98 s) showed HMBC cross peaks with C-7' (δ_C 138.9 s) and C-8' (δ_C 131.4 s), confirming their positions at C-9 and C-9' of the naphthalene moiety, respectively. Therefore, the basic skeleton of compound **1** could be deduced as an arylnaphthalene lignan. Four methoxy group located at C-4, C-3',



(1) R_1 = OMe, R_2 = OMe
(2) R_1 = H, R_2 = OH



(3) R=H (4) R=OH

Fig. 1. Structure of arylnaphthalene lignans in *Daphne acutiloba Rehd*.

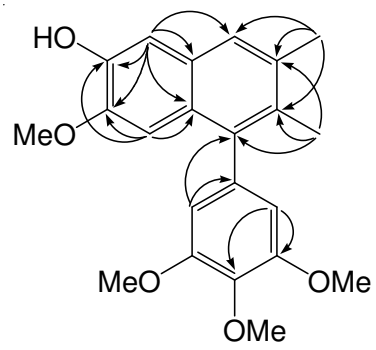


Fig. 2. Selected HMBC correlations daphnelignan B

TABLE-1
^{1}H NMR AND ^{13}C NMR DATA OF DAPHNELIGNAN B IN PYRIDINE-d_5

No.	δ_C (mult.)	δ_H (mult, *J*, Hz)	No.	δ_C (mult.)	δ_H (mult, *J*, Hz)
1	128.8 s		4’	140.1 s	
2	109.7 d	6.91 s	5’	151.2 s	
3	146.4 s		6’	105.3 d	6.35 s
4	148.4 s		7’	138.9 s	
5	106.3 d	6.82 s	8’	131.4 s	
6	129.5 s		9’	17.7 q	1.98 s
7	126.3 d	7.38 s	OMe-4	56.0 q	3.78 s
8	133.9 s		OMe-3’	55.7 s	3.72 s
9	21.2 q	2.40 s	OMe-4’	60.8 s	3.88 s
1’	133.0 s		OMe-5’	55.7 s	3.72 s
2’	105.3 d	6.35 s	OH-3		11.07 brs
3’	151.2 s				

C-4', C-5' in (1) was deduced by HMBC correlation of the proton signals (δ_H 3.78 s) with C-4 (δ_C 148.4 s), (δ_H 3.72 s) with C-3' (δ_C 151.2 s), C-5' (δ_C 151.2 s) and (δ_H 3.88 s) with C-4' (δ_C 140.1 s). The hydroxyl group located at C-4 was deduced by HMBC correlation of the proton signals (δ_H 11.1 s) with C-4 (δ_H 148.4 s). Thus, the chemical structure of **1** was established and given the name as daphnelignan B.

The potencies of daphnelignan B in preventing the cytopathic efects of HIV-1 in MT4 cells, as well as compound-induced cytotoxicity in MT4 cells in parallel with the antiviral activity were evaluated[10]. The results from the cell-based assays demonstrated potent anti-HIV-1 activity with EC50 (median effect concentration) value of 7.65 μg/mL and a TI (therapeutic index) of greater than 35.62, daphnelignan B shows weak anti-HIV activity.

Daphnelignan B: $C_{22}H_{24}O_5$, colourless amorphous crystals; UV (MeOH), λ_{max} (log ε) 205 (6.12), 238 (4.87), 287 (3.16) nm; IR (KBr, ν_{max}, cm^{-1}): 3451, 3415, 2936, 2908, 1655, 1634, 1618, 1526, 1462, 1422, 1375, 1045, 962, 874; ^{13}C NMR and ^{1}H NMR data (pyridine-d_5, 500 MHz), Table-1; HRESIMS (positive ion mode) m/z 391.1524 $[M + Na]^+$ (calcd. 391.1521 for $C_{25}H_{36}O_9$).

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