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A New Daphne Diterpenoids from *Daphne acutiloba* Rehd.

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A new daphne diterpenoid (daphnediterp A) was isolated from the leaf and steam of *Daphne acutiloba* Rehd. Its structure was determined by means of HRESIMS, extensive ^{1}D and ^{2}D NMR spectroscopic studies and chemical evidence. The anti HIV-1 activity was also evaluated and it shows an anti HIV-1 activity with a therapeutic index above 31.4.

Key Words: *Daphne acutiloba* Rehd; daphne diterpenoid; 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 daphne diterpenoid (daphnediterp A). The structure was established by means of HRESIMS and extensive NMR spectra. It activity of anti HIV-1 activity was also evaluated.

EXPERIMENTAL

Optical rotation was measured in Horiba SEPA-300 High Sensitive Polarimeter. IR spectra was 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 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

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used Agilent 1100 HPLC equipped with ZORBAX-C_{18} (9.4 nm × 250 nm, 5 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 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 kg) were extracted with 70 % aqueous Me_2CO (5 L × 3, 24 h each) at room temperature and the extract was partitioned successively with petroleum ether (8 L × 3) and EtOAc (8 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 to give fractions 1-5. Fr.3 (9:1) was further purified by HPLC with mobile phase (MeOH-H_2O 70:30) to yield daphnediterp A (22.6 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})[7].

RESULTS AND DISCUSSION

Daphnediterp A (Fig. 1) was obtained as colourless resin. The molecular formula of this compound was determined as $C_{25}H_{36}O_9$ from its HRESIMS at m/z 505.2328 $[M + Na]^+$ (calcd. 505.2324). In the ^1H NMR spectrum, proton signals at δ_H 6.02 (1H, m), 1.50 (3H, s) and 1.83 (3H, d, J = 7.2) suggested the existence of a angeloyl group, two olefinic protons at δ_H 5.22 (br s, 2H) were ascribed to a terminal double bond and methyl groups at δ_H 1.87 (s, 3H), 1.02 (d, J = 7.2 Hz, 3H) and δ_H 1.24 (d, J = 7.2 Hz, 3H), were consistent with the presence of a tertiary methyl group and two secondary methyl groups, respectively. After subtraction of the angeloyl group mentioned above, there were 20 carbon signals remaining in the ^{13}C NMR spectrum, which were sorted by ^{13}C DEPT NMR experiment into one keto group, eight oxygenated carbons (including one primary, three secondary and four tertiary), a terminal double bond, two methylenes, four methines and three methyls. These NMR data were very similar to those of known compound (1,2α-dihydro-5β-hydroxy-6α,7α-epoxy-resiniferonol-14-benzonate)[8]. The obvious differences are the benzoyl group was replaced by angeloyl group in daphnediterp A. In the ^1H NMR spectrum, instead of the signals of an olefinic proton and a vinylic methyl belonging to the α, β unsaturated carboxy group, protons of a methine group (δ_H 2.26, m, 2H) and a secondary methyl group (δ_H 1.24, d, J = 7.2, 3H) were observed. Furthermore, a downfield shift around 10 ppm of the ketone carbon signal (δ_C 219.2, C-3) on ring A suggested the absence of the conjugate effect. All these analysis implied that the endocyclic 1(2)-double bond of daphne diterpene is saturated. In the HMBC spectrum, key correlations of H3-19/C-3, H-5/C-4, H-20/C-6, H3-18/C-11, H3-17/C-3, H-16/C-17 confirmed the presumed daphne diterpene skeleton of daphnediterp A and the cross peaks of oxymethine proton signal at δ_H 5.52 (1H, br s, H-14) with C-13 and C-8, suggested the esterification of the angeloyl group on C-14.

The stereochemistry of daphnediterp A was identical with those known daphne diterpene derivatives[9-12] based on the analysis of the NOESY spectrum. The β orientation of C-19 (the methyl group at C-2) was determined by the NOE correlations of H3-19/H-1β and H-2/H-10, the a position of the benzoyl group was deduced from the key NOE correlations between H-14 and H-8 (Fig. 2). Thus, the chemical structure of daphnediterp A was determined as 1,2α-dihydro-5β-hydroxy-6α,7α-epoxy-resiniferonol-14-angelate and given the name as daphnediterp A.

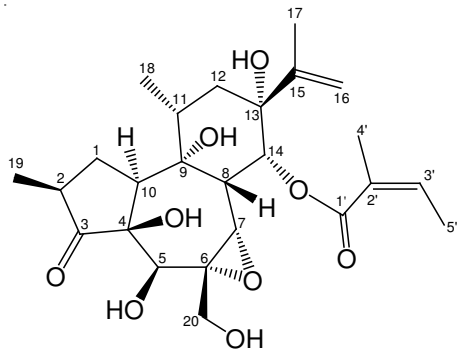

Fig. 1. Structure of daphnediterp A

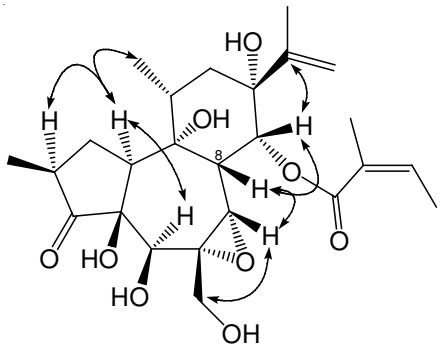

Fig. 2. Selected NOE correlations of daphnediterp A

TABLE-1
^{1}H NMR AND ^{13}C NMR DATA OF DAPHNEDITERP A IN $CDCl_3$

No.	δ_C (mult.)	δ_H (mult, *J*, Hz)	No.	δ_C (mult.)	δ_H (mult, *J*, Hz)
1	31.8 t	2.19 m	13	73.9 s	
		1.72 m	14	74.3 d	5.52 br s
2	43.1 d	2.31 m	15	143.2 s	
3	219.2 s		16	115.5 t	5.22 br s
4	76.7 s		17	18.6 q	1.87 s
5	74.6 d	4.87 br s	18	15.2 q	1.02 d (*J* = 7.2)
6	62.4 s		19	17.3 q	1.24 d (*J* = 7.2)
7	66.8 d	3.24 s	20	66.9 t	4.11 d (*J* = 11.6)
8	40.1 d	3.18 d (*J* = 3.1)			3.58 d (*J* = 11.6)
9	74.2 s		1'	166.8 s	
10	54.2 d	2.08 m	2'	128.2 s	
11	35.0 d	1.48 m	3'	138.8 d	6.02 m
12	33.8 t	2.13 m	4'	20.5 q	1.50 s
		1.65 m	5'	15.9 q	1.83 d (*J* = 7.3)

The potencies of daphnediterp A in preventing the cytopathic efects of HIV-1 in MT_4 cells, as well as compound-induced cytotoxicity in MT_4 cells in parallel with the antiviral activity were evaluated[7]. The results from the cell-based assays demonstrated potent anti HIV-1 activity with EC_{50} (median effect concentration) value of 6.54 μg/mL and a therapeutic index of greater than 31.4. Daphnediterp A shows weak anti HIV activity.

Daphnediterp A: $C_{25}H_{36}O_9$, colourless resin; $[\alpha]_D^{24.2}$ + 38.5 (c 0.08, $CHCl_3$); UV (MeOH), λ_{max} (log ε) 228 (4.82), 205 (5.96) nm; IR (KBr, ν_{max}, cm^{-1}):3550, 2948, 2915, 1722 (br), 1648, 1614, 1447, 1372, 1121, 1027; ^{13}C and ^{1}H NMR data ($CDCl_3$, 500 MHz), Table-1; HRESIMS (positive ion mode) m/z 505.2328 $[M + Na]^+$ (calcd. 505.2324 for $C_{25}H_{36}O_9$).

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