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NOTE

Isolation and Characterization of Sesquiterpenoids from *Daphne acutiloba* Rehd.

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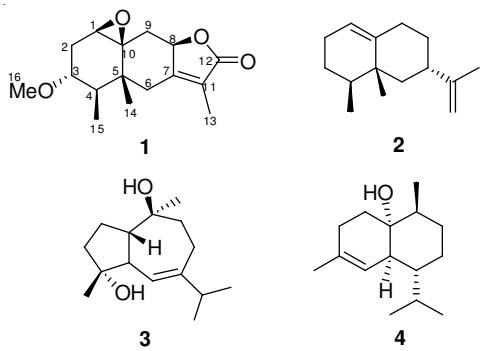
A new sesquiterpenoid (daphnelnoid A), together with three known, were isolated from the leaf and steam of *Daphne acutiloba* Rehd. Their structures were determined by means of HRESIMS, extensive 1D and 2D NMR spectroscopic studies and chemical evidence. The anti-HIV-1 activity of daphnelnoid A was also evaluated and it shows a moderate anti-HIV-1 activity with a therapeutic index above 45.2.

Key Words: *Daphne acutiloba* Rehd, Sesquiterpenoid, Daphnelnoid A, Anti-HIV-1 activity.

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 the major principles isolated from this plant are daphnane diterpenes, coumarines and lignans[4-8].

In our continuing efforts to identify bioactive natural products from the thymelaeaceae medicinal plants, a chemical investigation on the leaf and steam of *Daphne acutiloba* Rehd., indigenous to the Dali Prefecture of Yunnan Province of China was carried out and a new sesquiterpenoid (daphnelnoid A)[1], together with three known sesquiterpenoids *e.g.*, eremophilane[2], guaianediol[3], (+)-ent-epicubenol[4] (Fig. 1) were separated from this plant. In addition, the anti-HIV-1 activities of daphnelnoid A were evaluated. In this paper, the structure elucidation and biological activities of these sesquiterpenoids are described.



1 2 3 4

Fig. 1. Structure of sesquiterpenoids in *Daphne acutiloba* Rehd.

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 2D 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 mm, Qingdao Marine Chemical Inc., China). On second separate used Agilent 1100 HPLC equipped with ZORBAX-C18 (9.4×250 nm, 5.0 mm) column and DAD detector.

The leaf and steam of *Daphne acutiloba* Rehd. was collected in Heqing 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 (4.0 L × 3) and EtOAc (4.0 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 9:1 fraction (3.18 g) was further purified by HPLC with mobile phase (MeOH-H_2O 75:25) to yield **1** (13.6 mg), **2** (7.62 mg), **3** (8.52 mg) and **4** (10.6 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})[12].

Compound **1** was obtained as a white powder. The IR spectrum displayed a band at 1742 cm^{-1} attributed to a conjugated γ-lactone. The ^{13}C NMR spectrum exhibited 16 signals which were assigned, by DEPT 135 and HSQC experiments to a methoxy group, three methyls, three methylenes, four methines, three sp^2 carbons and one of this being a carbonyl group. Its HR-ESI-MS spectrum contains an ion peak of $[M+H]^+$ at m/z 275.1591, consistent with the molecular formula $C_{16}H_{22}O_4$ which was in accordance with the NMR data. The ^1H NMR spectrum of **1** showed three signals δ_H 1.89, 0.86 and 1.10 attributed to CH_3-13, CH_3-14 and CH_3-15, respectively. ^1H NMR spectrum exhibited the presence of a deshielded signal assignable to an oxymethine hydrogen at δ_H 3.15 (1H, m, H-3); this signal correlated in COSY spectrum with the δ_H 1.82 (1H, m, H-2β); 2.38 (1H, m, H-2α); 1.76 (1H, m, H-4). H-4 showed coupling with the methyl group at δ_H 1.01 (3H, J = 6.8 Hz, CH_3-15). The presence of an epoxy group was confirmed by the chemical shifts of H-1 (δ 2.97), C-1 (δ 58.5) and C-10 (δ 62.7). The COSY spectrum (Fig. 2) exhibited correlation peaks among the oxymethine hydrogen at δ_H 4.87 (1H, m, H-8) with hydrogens at δ_H 2.09 (1H, m, H-9α); 1.87 (1H, m, H-9β); 1.89 (3H, s CH_3-13). HSQC analysis indicated the presence of one tetra substituted double bond which was associated with the carbons at δ_C 158.2; 123.4 (C-7 and C-11, respectively). On the other hand another double bond was observed in the ^{13}C NMR spectrum, with characteristic chemical shifts of a carbonyl group at δ_C 175.6 attributed to C-12. The HSQC spectrum exhibited correlations among the diasterotopic hydrogens at δ_H 2.18 (1H, d, J = 13.4 Hz, H-6α) and δ_H 2.72 (1H, d, J = 13.4 Hz, H-6β) with the carbon at δ_C 35.6. The HMBC spectrum showed coupling of H-6 with C-5, C-7, C-8, C-10, C-11 and CH_3-14. The ^1H, ^{13}C, COSY and HMBC NMR analysis showed that the compound **1** are similar to those of cupressolide A[13]. The obvious differences is the appearance of singal of a methoxy group in **1**. The HMBC correlation of δ_H 3.08 (3H, s, CH_3-16) with δ_C 85.8 (C-3) indicated the methoxy group was located at C-3. This was also supported by the chemical shift of C-3 with δ_C 67.4 in cupressolide A downfield shift to δ_C 85.8 in **1**. Thus, the planar structure of **1** was established (Fig. 2).

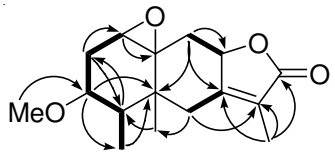


Fig. 2. Selected HMBC (!u) and ^1H-^1H cosy (—) correlations of **1**

The relative stereochemistry of **1** was elucidated using nOe and COSY spectroscopy. The β orientation of H-3 was inferred from the nOe correlation with CH_3-14 and CH_3-15. H-8 (δ 4.87) should be a oriented as indicated by the homoallylic coupling with the methyl (CH_3-13, δ 1.89) bounded to C-11 observed in the COSY experiment. This homoallylic coupling requires a 90º dihedral angle of the methyl group at C-11 with H-6α and H-8, witch was in agreement with the nOe experiment. Furthermore, the nOe spectrum showed correlations of H-6α with H-4 (δ 1.76) and of H-6β with CH_3-13, CH_3-14 and CH_3-15. Therefore, the structure of **1** was determined unambiguously as shown in Fig. 1 and given the name as daphnelnoid A.

Since certain of sesquiterpenoid exhibit potential anti-HIV activities, the new compounds **1** was tested for their potencies in preventing the cytopathic effects of HIV-1 in C8166 cells. Cytotoxicity was measured in parallel with the determination of antiviral activity, using AZT as a positive control (0.0043 μg/mL and CC_{50} >200 μg/mL)[12]. The results indicated that compound **1** showed moderate anti-HIV-1 activities with EC_{50} of 2.3 μg/mL and therapeutic index (TI) values of 45.2.

Daphnelnoid A (1αR,3R,4R,4αR,8αR,9αS)-3-methoxy-4,4α,6-trimethyl-1α,2,3,4,4αR,8α,9-octahydro-7*H*-oxirene-[8,8α] naphtho[2,3β]furan-7-one: $C_{16}H_{22}O_4$, white powder; $[\alpha]_D^{23.8}$ -8.15 (c 0.028, $CHCl_3$); UV ($CHCl_3$), λ_{max} (log ε) 205 (4.96), 230 (3.15), 280 (2.12) nm; IR (KBr, ν_{max}, cm^{-1}): 3472, 3046, 2947, 2876, 1742, 1655, 1438, 1375, 1082, 1011; ^{13}C NMR and ^1H NMR data ($CDCl_3$, 500 MHz) (Table-1); positive ESIMS m/z 275 $[M+H]^+$; HRESIMS m/z 275.1591 $[M+H]^+$ (calcd. for $C_{16}H_{22}O_4$, 279.1596).

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

No.	δ_C (mult.)	δ_H (mult, *J*, Hz)	No.	δ_C (mult.)	δ_H (mult, *J*, Hz)
1	58.1 d	2.97, d, *J* = 4.6	9	38.6 t	2.09, m
2	30.2 t	2.38, m			1.87, m
		1.82, m	10	62.7 s	–
3	85.8 d	3.15, m	11	123.4 s	–
4	38.4 d	1.76, m	12	175.6 s	–
5	38.4 s	–	13	8.5 q	1.89, s
6	35.6 t	2.18, d, *J* = 13.4	14	16.2 q	0.86, s
		2.72, d, *J* = 13.4	15	11.4 q	1.01, *J* = 6.8
7	158.2 s	–	16	56.8 q	3.08 s
8	77.5 d	4.87, m			

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