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Phenols with Anti-HIV Activity from *Daphne acutiloba*

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Abstract

The present paper describes the study of the phytochemical properties and anti-HIV activity of the phenolic isolates of the plateau medicinal plant *Daphne acutiloba* Rehd. (Thymelaeceae). Two new lignans named daphnenin (**1**) and daphnetone (**2**), along with 11 known ones, were isolated, and their structures were elucidated by 1D and 2D NMR spectroscopy as well as HR-ESI-MS. In the anti-HIV activity study, daphnenin (**1**) and caffeic acid *n*-octadecyl ester (**13**) showed definite anti-HIV activity with EC_{50} values of 0.39 and 0.16 µg/mL, respectively.

Key words
Daphne acutiloba · Thymelaeceae · anti-HIV activity · phenols

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Daphne acutiloba Rehd. (Thymelaeceae) is a shrub widely distributed in the Southwest of China and used traditionally in folk medicine [1]. The Thymelaeceae plants are claimed to be a good medicine against tumor [2], inflammation [1], and as antihyperglycemics [3] and neurotrophics [4]. Previous studies have focused on common species of this genus, such as *D. genkwa*, and reported a series of lignans [5–7] and biflavonoids [8]. In order to study the bioactivity of this plant, the phytochemical investigation of the phenols extract has been carried out, and two new lignans, named daphnenin (**1**) and daphnetone (**2**), together with 11 known ones, (+)-7-ethoxymatairesinol (**3**) [9], 4,4′-dihydroxy-3,3′-dimethoxy-9-ethoxy-9,9′-epoxylignan (**4**) [10], (−)-epinortrachelogenin (**5**) [11], (2*R*,3*S*)-*cis*-matairesinol (**6**) [12], haplomyrfolin (**13**) [13], dihydrocubebin (**8**) [14], (+)3-hydroxy-1,5-diphenyl-1-pentanone (**9**) [15, 16], daphnenone 1 (**10**) [17], daphneolone (**11**) [7], daphnenone 2 (**12**) [17], and caffeic acid *n*-octadecyl ester (**13**) [18], were isolated. Their structures were elucidated by 1D and 2D NMR spectroscopy as well as HR-ESI-MS (**Fig. 1**). This paper describes the isolation and structural elucidation of the two new compounds and the anti-HIV-1 activities of the compounds **1**, **4**, **11**, **12**, and **13**.

Daphnenin was obtained as colorless amorphous powder, and its molecular formula was assigned as $C_{37}H_{40}O_8$ by HR-ESI-MS (*m/z* 611.2632 $[M - H]^-$ calcd. for $C_{37}H_{39}O_8$ 611.2644) and NMR data (**Table 1**), indicating eighteen degrees of unsaturation. The IR spectrum displayed the presence of hydroxyl (3407 cm^{-1}), carbonyl (1661 cm^{-1}), and phenyl groups (1603, 1584, 1514, 1452 cm^{-1}) absorptions. Analysis of the ^{13}C NMR and DEPT spectra (**Table 1**) showed the presence of 33 carbon resonances, including 4 overlapping signals. These carbons were assigned to two methoxy groups, six methylenes (one oxygenated), nineteen methines (two oxygenated including one acetal), and ten quaternary carbons (one carbonyl). The ^1H and ^{13}C NMR data (**Table 1**) of **1** indicated that it was a conjugated of 4,4′-dihydroxy-3,3′-dimethoxy-9-ethoxy-9,9′-epoxylignan (**4**) [10] and daphneolone (**11**) [7] via the connection of C-3-O-C-9″ after losing the ethoxy group (δ_c 62.8 and 15.2) in **4**. The formation of C-3-O-C-9″ was verified by the oxygenated methane shifts down-field from δ 67.4 d in **4** to δ 74.5 d in **1** and the key HMBC (**Fig. 2**) correla-

Table 1 ^1H NMR and ^{13}C NMR data of **1**.

No.	δ_C	δ_H (mult, *J* in Hz)	No.	δ_C	δ_H (mult, *J* in Hz)
1	197.8 s	/	3″	145.6 s	/
2	44.3 t	3.38 (dd, 6.8, 15.2) 2.91 (dd, 6.0, 15.2)	4″	143.9 s	/
3	74.5 d	4.17 (ddt, 6.0, 6.2, 6.8)	5″	114.1 d	6.77 (d, 8.0)
4	36.1 t	1.79 (m)	6″	121.5 d	6.58 (dd, 8.0, 1.6)
5	31.3 t	2.48 (m)	7″	38.9 t	2.48 (m)
6	141.7 s	/	8″	52.7 d	2.12 (m)
7	128.2 d	7.04 (d, 7.2)	9″	108.2 d	4.93 (d, 1.2)
8	128.3 d	7.24 (dd, 7.2, 7.6)	1‴	131.6 s	/
9	125.7 d	7.15 (t, 7.6)	2‴	111.0 d	6.42 (d, 1.6)
10	128.3 d	7.24 (dd, 7.2, 7.6)	3‴	146.4 s	/
11	128.2 d	7.04 (d, 7.2)	4‴	143.7 s	/
1′	130.1 s	/	5‴	114.0 d	6.77 (d, 8.0)
2′	115.3 d	6.83 (d, 8.7)	6‴	121.2 d	6.49 (d, 8.0, 1.6)
3′	131.0 d	7.86 (d, 8.7)	7‴	38.6 t	2.48 (m)
4′	160.7 s	/	8‴	45.8 d	2.05 (m)
5′	131.0 d	7.86 (d, 8.7)	9‴	72.0 t	3.48 (dd, 8.0, 8.4) 3.86 (dd, 7.6, 8.4)
6′	115.3 d	6.83 (d, 8.7)	3″ -OMe	55.7 q	3.78 (s)
1″	132.4 s	/	3‴-OMe	55.7 q	3.76 (s)
2″	111.2 d	6.50 (d, 1.6)			

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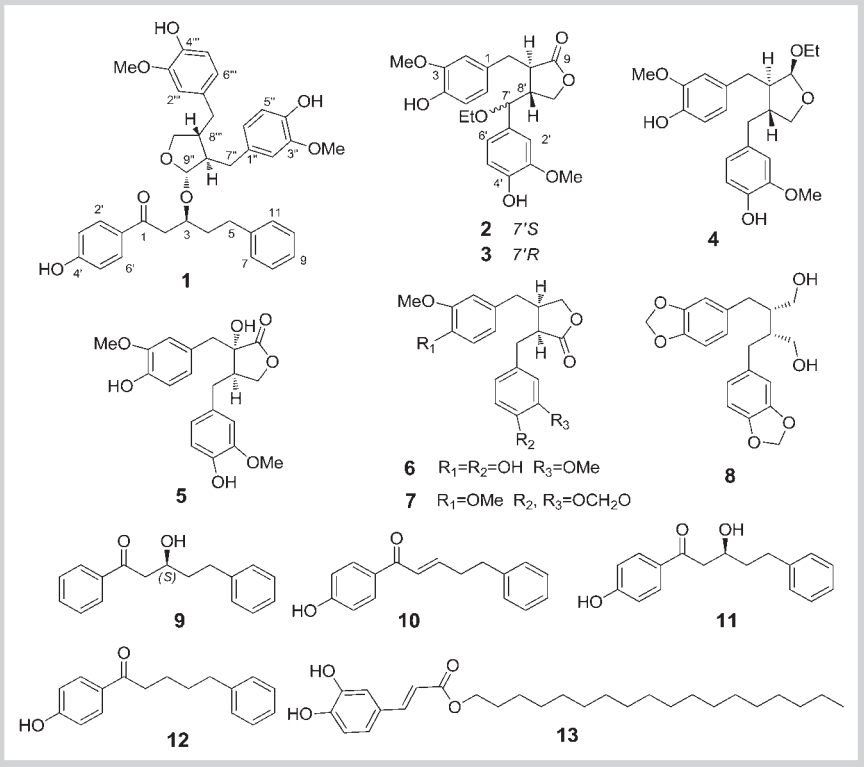

Fig. 1 Chemical structures of compounds **1**–**13**.

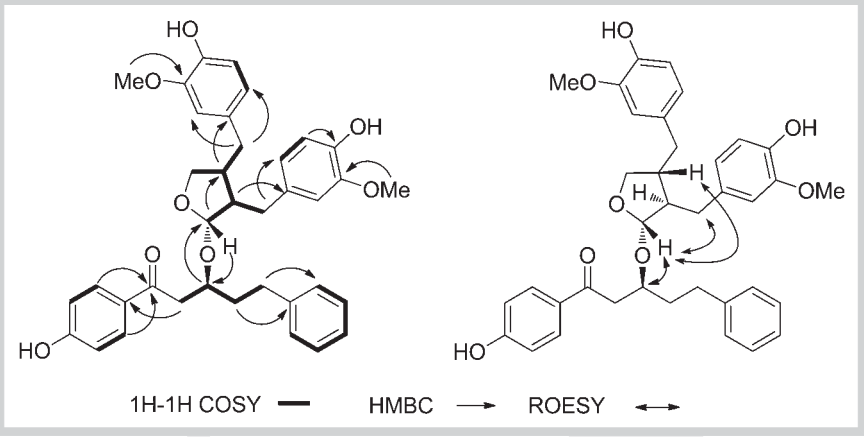

Fig. 2 The ^{1}H-^{1}H COSY, HMBC, and ROESY correlations of compound **1**.

tions of **1** from H-3 [δ_H 4.17 (1H, ddt, J = 6.0, 6.8, 6.2 Hz)] to C-9″ (δ_C 108.2 d) as well as the NOE of H-3/H-9″. The other correlations in the HMBC and ^{1}H-^{1}H COSY spectrum further confirmed the atom connectivity in **1**. The relative configurations of the stereogenic centers (C-8″ and C-8‴) of **1** were elucidated by ROESY (**Fig. 3**) and determined to be the same as those of compound **4**, β-orientation with H-8‴ and α-orientations of H-8‴. The β-orientation of H-9″ was established by key NOE of H-9″/H-7″ and H-9″/H-8‴. Moreover, the configuration of C-3 in compound **11** was determined to be *S* by comparing its positive rotation ($[\alpha]_D^{25}$ + 4.7 (*c* 1.23, MeOH)) with those of compound **9** ($[\alpha]_D^{18}$ + 4.0 [*c* 0.275, MeOH]) [11] which had the *S* configuration of C-3. Biogenetically, **1** might be derived from **11** and accordingly possessed the same *S* configuration of C-3. Thus, the structure of compound **1** was assigned as showed in **Fig. 2**, and this compound was named daphnenin.

Daphnetone was obtained as colorless amorphous powder, and its molecular formula was determined as $C_{22}H_{26}O_7$ by HR-EI-MS (*m/z* 425.1569 $[M + Na]^+$ calcd. for $C_{22}H_{27}O_7$ 425.1576) and NMR data (**Table 2**). The IR spectrum displayed the presence of hydroxyl (3434 cm^{-1}), carbonyl (17641 cm^{-1}), and phenyl groups (1616, 1516, 1464, 1432 cm^{-1}) absorptions. The ^{13}C and DEPT NMR spectra of compound **2** revealed 22 carbon resonances due to two methoxy groups, one methyl, three methylenes (two oxygenated), nineteen methines (one oxygenated), and seven quaternary carbons (one carbonyl). Its similar ^{1}H and ^{13}C NMR data (**Table 2**) with (+)-7-ethoxymatairesinol (**3**) [5] were reminiscent of the epimer of **3**. And the NMR spectra of C-7′ shifted

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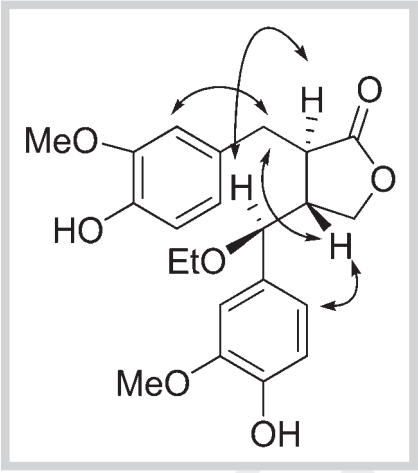

Fig. 3 The selected ROESY correlations of compound **2**.

Table 2 ^{1}H NMR and ^{13}C NMR data of **2**.

No.	δ_C	δ_H (mult, *J* in Hz)	No.	δ_C	δ_H (mult, *J* in Hz)
1	129.5 s	/	2′	108.4 d	6.63 (d, 1.9)
2	112.1 d	6.66 (d, 1.8)	3′	146.4 s	/
3	146.8 s	/	4′	144.3 s	/
4	145.5 s	/	5′	114.2 d	6.86 (dd, 1.9, 8.4)
5	113.9 d	6.62 (d, 8.0)	6′	119.7 d	6.67 (d, 8.4)
6	122.7 d	6.78 (dd, 1.8, 8.0)	7′	82.7 d	4.12 (d, 4.1)
7	34.8 t	2.06 (m) 2.90 (m)	8′	44.2 d	2.91 (m)
8	44.6 d	2.55 (m)	9′	68.4 t	3.79 (m) 3.76 (m)
9	179.2 s	/	3′-OMe	55.7 q	3.82 (s)
3-OMe	55.8 q	3.86 (s)	7′-O-CH_2-	64.3 t	3.43 (dq, 6.9, 14.6) 3.29 (dq, 6.9, 14.6)
1′	131.2 s	/	-CH_3	15.2 q	1.20 (dd, 6.9, 6.9)

down-field from δ_C 81.7 d (δ_H 3.80) in **3** to δ_C 82.7 (δ_H 4.12) in **2**, suggesting that **2** was the 7′-epimer of **3**. Since the configuration of C-7′ in **3** was *R*, the configuration of C-7′ in **2** should be *S*. The relative configuration of C-8′ and C-8 in **2** were the same as **3** with *β*-orientation of H-8′ and *α*-orientations of H-8 by the key ROESY correlations of H-7/H-8′ and H-8/H-7′ (**Fig. 3**). Based on the above analysis, the structure of compound **2** was assigned as showed in **Fig. 3**, and this compound was named daphnetone.

In the anti-HIV experiment the compounds **1**, **4**, **11**, **12**, **13** have been tested. As a result, daphnenin (**1**) and caffeic acid *n*-octadecyl ester (**13**) showed definite activity with EC_{50} values of 0.39 and 0.16 g/mL, respectively. The other ones showed weak bioactivity (**Table 3**).

In conclusion, two new compounds were isolated from *D. acutiloba* for the first time. Moreover, among the compounds isolated from *D. acutiloba*, daphnenin and caffeic acid *n*-octadecyl ester showed anti-HIV-1 bioactivity. This novel discovery helps us to better understand the biological properties of the phenols in the *Daphne* genus, among which the anti-HIV-1 bioactivity especially deserves further investigation.

Table 3 Summary of anti-HIV-1 activity of compounds **1**, **4**, **11**, **12**, and **13**.

No.	Cytotoxicity IC_{50} (μg/mL)	Anti-HIV-1 activity EC_{50} (μg/mL)	Selectivity index SI (IC_{50}/EC_{50})
1	9.21	0.39	23.62
4	19.23	2.09	9.20
11	93.02	10.86	8.57
12	15.52	2.14	7.25
13	86.14	0.16	538.28
3′-Azido-3′-deoxythymidine	869.95	4.25×10^{-3}	204690

Materials and Methods

The stems of *Daphne acutiloba* were collected in Hexi country, Yunnan Province, People's Republic of China. A voucher specimen (HUANG0004) identified by Prof. H. Sun and Dr. L.L. Yue (Kunming Institute of Botany, Chinese Academy of Sciences) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, People's Republic of China. The dried and powdered stems of *Daphne acutiloba* (7 kg) were extracted with 95% EtOH under reflux for three times (3 × 10 L). The extract was concentrated and suspended in water followed by successive partition with petroleum ether, EtOAc, and *n*-BuOH, respectively. The EtOAc extract (300 g) was separated by silica gel column using a gradient solvent $CHCl_3$/MeOH (9 : 1–3 : 1, 10 L) to afford fractions A–C. Fraction A (120 g) was separated by silica gel column using a gradient solvent petroleum ether/EtOAc (20 : 1–1 : 2, 8 L) to afford fractions A1–A10. Fractions A1–A10 (5–10 g) were gel-filtrated on Sephadex LH-20 ($CHCl_3$/MeOH 1 : 1) to give two subfractions, A1a–A10a and A1b–A10b. Fractions A1b–A8b (1–5 g) were subjected to repeated RP-18, silica gel column, and semi-preparative HPLC (MeOH/H_2O, 55 : 45) to yield **12** (40 mg) and **13** (76 mg) from A1b; **11** (134 mg) from A2b; **10** (478 mg) and **9** (16 mg) from A3b; **8** (32 mg) from A4b; **7** (11 mg) from A5b; **6** (21 mg) and **5** (46 mg) from A6b; **4** (12 mg) and **3** (12 mg) from A7b; as well as **2** (22 mg) and **1** (9 mg) from A8b, respectively.

Daphnenine (**1**): Colorless amorphous powder; $[\alpha]_D^{24}$ + 71.6 (*c* 3.4, MeOH); UV (MeOH) λ_{max} (log ε) 281 (4.2), 204 (4.7); IR (KBr) ν_{max} 3407, 3025, 2935, 2850, 1661, 1603, 1584, 1514, 1452, 1432, 1364, 1272, 1236, 1212, 1169, 1154, 1123, 1091,1034, 986, 936, 839, 819, 796, 745, 700, 563; ^{1}H and ^{13}C NMR data, see **Table 1**; ESIMS negative *m/z* $[M - H]^-$ 611 (100), 227 (25); HR-ESI-MS *m/z* $[M - H]^-$ 611.2632 (calcd. for $C_{37}H_{39}O_8$, 611.2644).

Daphnetone (**2**): Colorless amorphous powder; $[\alpha]_D^{25}$ + 14.5 (*c* 1.53, MeOH); UV (MeOH) λ_{max} (log ε) 282 (3.75), 229 (4.07), 207 (4.39); IR (KBr) ν_{max} 3434, 2966, 2934, 1764, 1616, 1516, 1464, 1432, 1274, 1239, 1208, 1155, 1123, 1031; ^{1}H and ^{13}C NMR data, see **Table 2**; ESIMS positive *m/z* $[M + Na]^+$ 425 (100); HR-ESI-MS *m/z* $[M + Na]^+$ 425.1569 (calcd. for $C_{22}H_{27}O_7$, 425.1576).

The anti-HIV activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}) and cytotoxicity assay against C8166 cell line (IC_{50}) using MTT methods as described in the literature [19]. AZT (3′-azido-3′-deoxythymidine; Sigma-Aldrich 99%) was used as positive control. The concentration of the antiviral sample reducing HIV-1 replication by 50% (EC_{50})

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was determined from the dose-response curve and calculated by the Reed and Muench method [20]. The selectivity index (SI) was calculated from the IC_{50}/EC_{50} ratio.

Supporting information

Details about the isolation methods of the compounds, the NMR spectra data of the new compounds, and the detailed protocols for the anti-HIV-1 bioactivity assay are available as Supporting Information.

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Conflict of Interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

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