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ISOCOUMARINS FROM THE BARK OF *LINDERA CAUDATA*

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Abstract – Three new isocoumarins, caudacoumarins A - C (**1 - 3**), together with two known isocoumarins (**4 - 5**) were isolated from the bark of *Lindera caudata*. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D- NMR techniques. Compounds **1 - 5** showed modest anti-tobacco mosaic virus (anti-TMV) activity with inhibition rate of 14.8 - 24.5%, respectively.

The plants of Lindera family (Lauraceae) are mainly distributed in tropical, subtropical to temperate regions of Asia and the Midwest of United States.[1] The plants from this family were traditionally used to treat stomach, urinary system diseases and rheumatic pain in Chinese folk.[2,3] *Lindera caudata* (Nees) Hook. f., an evergreen plant belongs to Lindera family, *Iteadaphne* subgenus, had been widely used as herbal medicine with the effects of hemostatic, analgesic and antipyretic.[4] However, the phytochemical studies on this plant had not been reported in literatures yet.

Isocoumarins are an important class of natural products widely occurring in plant kingdom and are known to exhibit a wide range pharmacological activities, such as antibacterial and antifungal,[5-7] cytotoxic,[8,9] antiviral,[10,11] antioxidative,[12] and anti-inflammatory[13] properties. With the aim of multipurpose utilization of medicine plants and identification of bioactive natural products, the phytochemical investigation on the bark of *L. caudata* was carried out. As a result, three new (**1 - 3**), and two known (**4 - 5**) isocoumarins were isolated from this plant. The structures of new compounds were elucidated on the basis of a comprehensive analysis of the ^{1}H, ^{13}C and 2D NMR spectra. In addition, the anti-tobacco mosaic virus (anti-TMV) activities of **1 - 5** were evaluated. In this paper, we report the isolation, structure elucidation of the new isocoumarins, caudacoumarin A - C (**1 - 3**), as well as their anti-TMV activity.

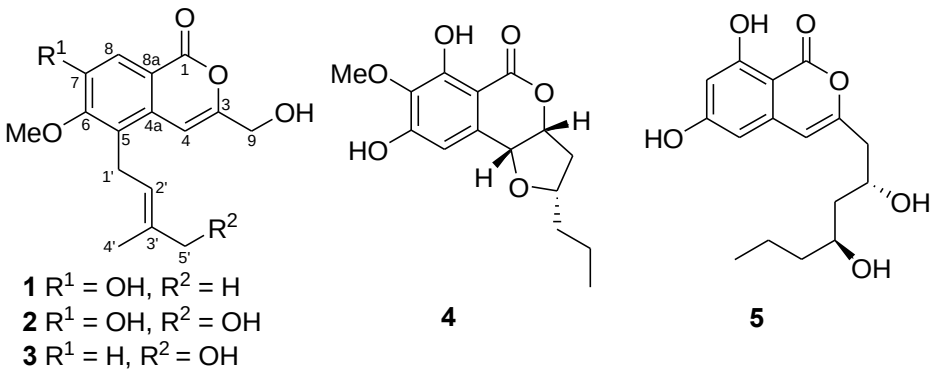

Figure 1. The structures of compounds **1** - **5**

A 70% aq. acetone extract prepared from the bark of *L. caudata* was subjected repeatedly to column chromatography on silica gel and preparative HPLC to afford compounds **1** - **5**, including three new isocoumarins, caudacoumarins A - C (**1** - **3**), together with two known isocoumarins, exserolide D (**4**)[6] and exserolide F (**5**).[6] The structures of the **1** - **5** were as shown in Figure 1, and the ^{1}H and ^{13}C NMR data of **1** - **3** were listed in Table 1.

Compound **1** was obtained as pale yellow gum. Its molecular formula $C_{16}H_{18}O_5$ was established on the basis of HRESIMS ($[M+Na]^+$, 313.1057, calcd 313.1052 for $C_{16}H_{18}NaO_5$), requiring 8 degrees of unsaturation. The IR spectrum showed absorption bands for hydroxyl group (3428 cm^{-1}), unsaturated lactone (1738 and 1665 cm^{-1}) and aromatic ring (1612, 1568, and 1476 cm^{-1}). The ^{1}H NMR signals revealed the presence of a 1,2,3,4,5-pentasubstituted benzene moiety (δ_H 7.40 s), a prenyl group [δ_H 3.40 d (7.2) 2H, 5.27 t (7.2) 1H, 1.54 s 3H, and 1.72 s 3H], an olefinic proton (δ_H 6.44 s), an oxygenated methylene proton (4.38 s 2H), a methoxy proton (δ_H 3.84 s), and a phenolic hydroxy proton (δ_H 9.10 s). Its ^{13}C NMR showed the presence of a 1,2,3,4,5-pentasubstituted benzene moiety (δ_C 129.8 s, 132.2 s, 157.9 s, 146.4 s, 115.1 d, and 121.9 s), a prenyl group (δ_C 26.9 t, 122.9 d, 133.3 s, 17.3 q, 25.4 q), a ester carbonyl (δ_C 161.4 s), a pair of olefinic carbon signals (δ_C 155.9 s, 106.1 d), an oxygenated methylene carbon (δ_C 62.0 t), and a methoxy carbon (δ_C 61.0 q). Its ^{1}H and ^{13}C NMR spectroscopic data were similar to those of 6-hydroxy-3-hydroxymethyl-8-methoxyisocoumarin at the positions C-1 ~ C-3, C-9,[14]

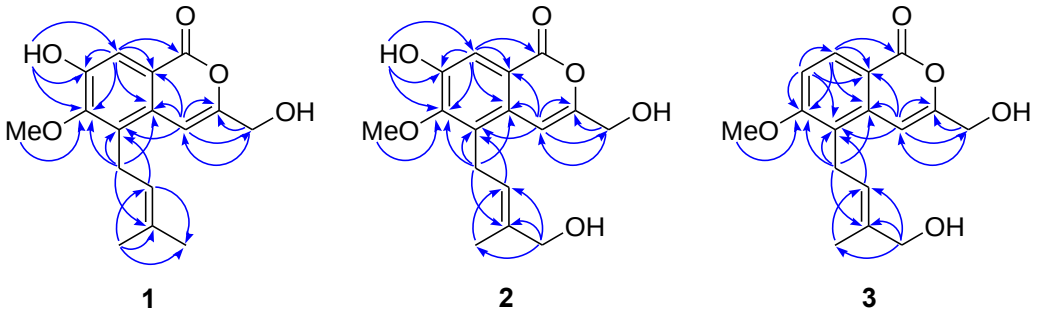

Figure. 2. Key HMBC ( ) correlations of (**1** - **3**)

indicated that **1** should be a 3-hydroxymethyl-isocoumarin. This deduction was also supported by the HMBC correlations (Figure 2) of H-9 (δ_H 4.38) with C-3 (δ_C 155.9) and C-4 (δ_C 106.1), and of H-4 (δ_H 6.44) with C-3 (δ_C 155.9), C-9 (δ_C 62.0), C-4a (δ_C 129.8), C-5 (δ_C 132.2), and C-8a (δ_C 121.9). Moreover, the HMBC correlation of methoxy proton (δ_H 3.84) with C-6 (δ_C 157.9) suggested the methoxy group located at C-6. The prenyl group located at C-5 was supported by the HMBC correlations of H-1′ (δ_H 3.40) with C-4a (δ_C 129.8), C-5 (δ_C 132.2), and C-6 (δ_C 157.9), and of H-2′ (δ_H 5.27) with C-5 (δ_C 132.2). The phenolic hydroxy group was assigned to C-7 on the basis of the HMBC correlations between the hydroxy proton (δ_H 9.10) and C-6 (δ_C 157.9), C-7 (δ_C 146.4) and C-8 (δ_C 115.1). Thus, compound **1** was assigned as shown in Figure 1, and named caudacoumarin A.

Table 1. ^{1}H and ^{13}C NMR data for compounds **1 - 3** (δ in ppm, 100 and 400 MHz)

No.	Compound **1** [a]		Compound **2** [a]		Compound **3** [b]	
	δ_C	δ_H (m, *J*, Hz)	δ_C	δ_H (m, *J*, Hz)	δ_C	δ_H (m, *J*, Hz)
1	161.4 s		162.1 s		161.0 s	
3	155.9 s		155.2 s		157.0 s	
4	106.1 d	6.44 s	106.3 d	6.41 s	106.2 d	6.47 s
4a	129.8 s		128.1 s		138.6 s	
5	132.2 s		132.6 s		132.2 s	
6	157.9 s		159.0 s		162.9 s	
7	146.4 s		145.0 s		114.3 d	6.79 (d) 8.2
8	115.1 d	7.40 s	115.0 d	7.42 s	128.0 d	6.72 (d) 8.2
8a	121.9 s		121.9 s		122.1 s	
9	62.0 t	4.38 s	62.6 t	4.48 s	62.6 t	4.33 s
1′	26.9 t	3.40 (d) 7.2	27.0 t	3.35 (d) 7.1	27.5 t	3.36 (d) 7.1
2′	122.9 d	5.27 (t) 7.2	124.9 d	5.42 (t) 7.1	125.8 d	5.39 (t) 7.1
3′	133.3 s	6.80 (d) 8.5	133.1 s		134.2 s	
4′	17.3 q	1.54 s	13.8 q	1.46 s	13.1 q	1.49 s
5′	25.4 q	1.72 s	67.2 t	3.97 s	67.8 t	3.93 s
6-OMe	61.0 q	3.84 s	61.0 q	3.83 s	56.0 q	3.81 s
Ar-OH		9.10 s		9.17 s		

[a] in $CDCl_3$; [b] in C_5D_5N

Caudacoumarin B (**2**) was assigned the molecular formula of $C_{16}H_{18}O_6$ by its HRESIMS at *m/z* 329.1008 $[M+Na]^+$. The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of compound **1**. The only difference was due to the disappearance of a methyl group (δ_C 25.4 q; δ_H 1.72 s) and appearance of an oxygenated methylene group (δ_C 67.2 t; 3.97 s) in **2**. These changes suggested that one methyl in prenyl group was replaced by an oxygenated methylene.[15] The HMBC correlations of H-5′ (δ_H 3.97) with C-2′ (δ_C 124.9),

C-3′ (δ_C 133.1), and C-4′ (δ_C 13.8), provided further evidence for the structural assignment. Accordingly, the structure of **2** was determined as shown. The ^{1}H and ^{13}C NMR spectra of **3** were also similar to those of compound **2**. The major difference resulted from the disappearance of a phenolic hydroxy group (δ_H 9.17 s). The remainder substituents, a 4-hydroxy-3-methylbutyl group at C-5 and a methoxy group at C-6 were deduced from the analysis of its HMBC collections (Figure 2). In addition, the HMBC correlations of H-8 with C-1, and the typical proton signals of C-7 [δ_H 6.79 (d) 8.2] and C-8 [δ_H 6.72 (d) 8.2] also supported the 5,6-substitution on the aromatic rings. Therefore, caudacoumarin C (**3**) was formulated as shown in Figure 1.

Since some isocoumarins are known to exhibit potential anti-virus activities,[10,11] compounds **1 - 5** were tested for their anti-TMV activities. The anti-TMV activities were tested using the half-leaf method,[16] using ningnanmycin (a commercial product for plant disease in China) as a positive control. Their antiviral inhibition rates at the concentration of 20 μM were listed in Table 2. The results revealed that compounds **1 - 5** showed modest anti-TMV activity with inhibition rate of 14.8 - 24.5%, respectively.

Table 2. TMV Infection Inhibition Activities of Compounds **1-5**

Compounds	Inhibition rate (%)	Compounds	inhibition rate (%)
1	22.4 ± 3.2	**4**	16.7 ± 2.8
2	18.6 ± 3.0	**5**	14.8 ± 2.2
3	24.5 ± 3.5	ningnamycin	28.6 ± 2.5

All results are expressed as mean ± SD; n = 3 for all groups.

EXPERIMENTAL

General. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. ECD spectra were measured on a JASCO J-810 spectropolarimeter. 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 500 instrument 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). Second separate was used an Agilent 1100 HPLC equipped with ZORBAX-C_{18} (21.2 mm × 250 mm, 7.0 μm) column and DAD detector.

Plant Material. The bark of *Lindera caudata* (Nees) Hook. f. was collected in Xishuangbanna Prefecture, Yunnan Province, People's Republic of China, in September 2012. The identification of the plant material was verified by Prof. Ren P. Y (Xishuangbanna Botanical Garden). A voucher specimen (YNNI-2012-97) has been deposited in our laboratory.

Extraction and Isolation. The bark of *L. caudata* (4.5 kg) was extracted four times with 70% acetone (4 ×6 L) at room temperature and filtered. The crude extract (158 g) was applied to silica gel (200 - 300

mesh) column chromatography, eluting with a $CHCl_3$-Me_2CO 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 B (9:1, 23.2 g) by silica gel column chromatography, eluted with petroleum ether-EtOAc (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures B1–B5. Fraction B2 (8:2, 5.84 g) was subjected to preparative HPLC (65% MeOH, flow rate 12 mL/min) to give **1** (11.4 mg), **3** (15.1 mg), and **5** (8.2 mg). Fraction B3 (7:3, 4.28 g) was subjected to preparative HPLC (60% MeOH, flow rate 12 mL/min) to give **2** (14.7 mg) and **4** (8.8 mg).

Anti-TMV Assays. The Anti TMV activities were tested using the half-leaf method,[17] and ningnanmycin, a commercial product for plant disease in China, was used as a positive control.

Caudacoumarin A (**1**): $C_{16}H_{18}O_5$, Obtained as a pale yellow gum; UV (MeOH) λ_{max} (log ε) 210 (4.08), 270 (3.75), 295 (3.52), 335 (3.64) nm; IR (KBr) ν_{max} 3428, 3062, 2935, 2864, 1735, 1668, 1615, 1562, 1498, 1382, 1210, 1132, 1080, 865, 752 cm^{-1}; ESIMS *m/z* (positive ion mode) 313 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 313.1057 $[M+Na]^+$ (calcd $C_{16}H_{18}NaO_5$ for 313.1052).

Caudacoumarin B (**1**): $C_{16}H_{18}O_6$, Obtained as a pale yellow gum; UV (MeOH) λ_{max} (log ε) 210 (4.02), 272 (3.78), 295 (3.57), 336 (3.68) nm; IR (KBr) ν_{max} 3432, 3068, 2939, 2862, 1738, 1665, 1612, 1568, 1476, 1389, 1248, 1135, 1068, 868, 759 cm^{-1}; ESIMS *m/z* (positive ion mode) 329 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 329.1008 $[M+Na]^+$ (calcd $C_{16}H_{18}NaO_6$ for 329.1001).

Caudacoumarin C (**3**): $C_{16}H_{18}O_5$, Obtained as a pale yellow gum; UV (MeOH) λ_{max} (log ε) 210 (4.12), 268 (3.65), 292 (3.52), 330 (3.64) nm; IR (KBr) ν_{max} 3438, 3065, 2937, 2867, 1740, 1667, 1610, 1560, 1479, 1385, 1246, 1138, 1067, 864, 756 cm^{-1}; ESIMS *m/z* (positive ion mode) 313 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 313.1047 $[M+Na]^+$ (calcd $C_{16}H_{18}NaO_5$ for 313.1052).

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