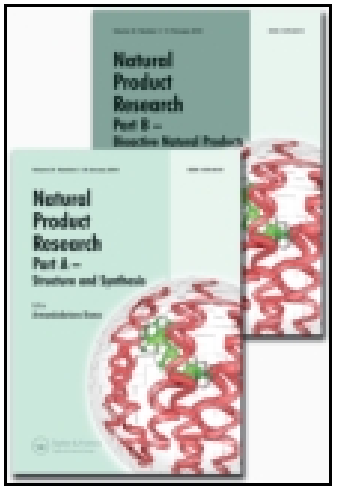

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A new cytotoxic iridoid from *Callicarpa nudiflora*

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A new iridoid, named nudifloside (**1**), together with three known compounds, was isolated from the EtOH extract of the aerial parts of *Callicarpa nudiflora* Hook. The structures were elucidated by a study of their physical and spectral data. Nudifloside (**1**) and the isolated known compound linearoside (**2**) displayed inhibitory effects towards chronic myelogenous leukaemia K562 cell line, with IC_{50} values of 20.7 and 36.0 $\mu g\,mL^{-1}$, respectively.

Keywords: *Callicarpa nudiflora*; iridoid; nudifloside; cytotoxicity

1. Introduction

The genus *Callicarpa* (Verbenaceae) consists of more than 190 species, of which 46 are distributed in China. The plants of *Callicarpa* are widely used as traditional Chinese herbal medicines for the treatments of inflammation and bleeding (The Chinese Academy of Science Flora of China Editional Board, 1982; Jiangsu New Medical College, 1986). Previous chemical studies established the occurrence of diterpenoids, triterpenoids, flavonoids and volatile constituents from this genus, some of them being biologically active (Ahmad & Zaman, 1973; Chatterjee, Desmukh, & Chandrasekharan, 1972; Hu, Shen, Gan, & Hao, 2002; Kobaisy, Tellez, Dayan, & Duke, 2002; Liu et al., 2006; Re et al., 2003; Talapatra, Polley, & Talapatra, 1994). *Callicarpa nudiflora* Hook. is a small shrub widely distributed from southern China to Malaysia. It is commonly used as a traditional Chinese folk medicine for the treatment of respiratory tract infections, hepatitis and bleeding (Guangdong Institute of Botany, 1977). In our screening for cytotoxic agents from tropical medicinal plants, the ethanol extract from aerial parts of *C. nudiflora* showed inhibitory effect towards chronic myelogenous leukaemia K562 cell line. A previous phytochemical investigation of *C. nudiflora* yielded triterpenoids and flavonoids (Wang, Han, Cui, & Dai, 2007). Further investigation on the ethanol extract of this plant resulted in the isolation of a new iridoid, nudifloside (**1**), along with three known compounds, linearoside (**2**), verbascoside (**3**), and 5,7-dihydroxy-3,3′,4′,-trimethoxy flavone (**4**). Compounds **1** and **2** showed cytotoxicity against a K562 cell line, with IC_{50}

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values of 20.7 and 36.0 μg mL^{-1}, respectively. In this article, we describe the isolation, structural elucidation and cytotoxicity of **1**–**4**.

2. Results and discussion

Nudifloside (**1**) was obtained as yellow gel, $[\alpha]_D^{26} = -160.3$ (*c* 0.5, MeOH). Its molecular formula was determined to be $C_{24}H_{28}O_{13}$ on the basis of the HRESIMS, which showed a pseudomolecular ion peak at *m/z* 547.1421 $[M+Na]^+$ (Calcd for $C_{24}H_{28}O_{13}Na$, 547.1427), which was supported by ^{13}C NMR and DEPT spectra. Compound **1** showed UV maximum peaks at 329 (4.20), 295 (3.92), 246 (3.80) and 216 (3.96) and IR absorption bands at 3419 (br.), 2927, 1700, 1657, 1630 and 1500 cm^{-1}, which are suggestive of the skeleton of an iridoid and a substituent of a caffeoyl group (Wang et al., 2007). The ^{13}C NMR spectrum (Table 1) showed 24 resonances, including six for a sugar unit, nine for a caffeoyl moiety and the remaining nine for an iridoid aglycone moiety. The gross structure of compound **1** was determined from 1D and 2D NMR (COSY, HMQC, HMBC, ROESY) experiments. The ^{13}C NMR spectrum of **1** was very similar to that of the known compound verminoside (Sticher & Afifi-Yazar, 1979), while the ROESY spectrum showed the stereochemistry difference between compound **1** and

Table 1. ^1H and ^{13}C NMR (CD_3OD) data of **1** (δ in ppm, *J* in Hz).

	δ_C	δ_H	^1H–^1H COSY selected	HMBC selected
1 (CH)	95.1	5.19 (d, 9.2)	H-9	H-3,5,9,1″
3 (CH)	142.4	6.39 (dd, 1.2, 5.8)	H-4	H-4
4 (CH)	103.0	5.00 (dd, 4.1, 5.9)	H-3,5	H-3,6
5 (CH)	36.8	2.60 (m)	H-4,6,9	H-3,4,6,7,9
6 (CH)	81.3	5.04 (br d, 7.0)	H-5,7	H-5,7,9
7 (CH)	60.3	3.72 (br s)	H-6	H-5,6,9,10
8	66.9			H-1,5,9,10
9 (CH)	43.2	2.65 (t, 7.7, 8.9)	H-1,5	H-1,4,5,7,10
10 (CH_2)	61.3	3.85 (d, 13.0, H-α), 4.19 (d, 13.2, H-β)		H-7, 9
1′	127.6			H-5′,7′,8′
2′ (CH)	115.2	7.09 (d, 1.9)	H-6′	H-6′,7′
3′	146.9			H-5′
4′	149.8			H-2’, 5’, 6’
5′ (CH)	116.6	6.81 (d, 8.2)	H-6′	H-6′
6′ (CH)	123.2	6.99 (dd, 1.9, 8.2)	H-2′,5′	H-2′,5′,7′
7′ (CH)	147.7	7.62 (d, 15.8)	H-8′	H-6′
8′ (CH)	114.5	6.34 (d, 15.9)	H-7′	H-7′
9′	169.0			H-6,7′,8′
1″ (CH)	99.7	4.82 (d, 7.9)	H-2″	H-1,3″,5″
2″ (CH)	74.9	3.30 (dd, 8.2, 9.2)	H-1″,3″	H-1″,3″
3″ (CH)	77.7	3.44 (t, 9.0)	H-2″,4″	H-1″,2″
4″ (CH)	71.8	3.29 (t, 9.6)	H-3″,5″	H-2″,3″
5″ (CH)	78.6	3.37 (m)	H-4″,6″	H-4″,6″
6″ (CH_2)	63.0	3.67 (dd, 6.6, 12.0, H-α), 3.95 (dd, 1.8, 12.0, H-β)	H-5″	H-4″,5″

verminoside at C-1 and C-9 (Figure 1). *Trans*-orientations at the C-9/C-5 and C-9/C-1 bond were determined by the ROESY experiment of **1**, exhibiting that H-1 (δ 5.19) correlated with H-5 (δ 2.60), and H-10 (δ 3.85,4.19) correlated with H-6 (δ 5.04), H-7 (δ 3.72) and H-9 (δ 2.65), while it did not correlate with H-1 (δ 5.19) or H-5 (δ 2.60) (Figure 1). This result indicated that H-10, H-6, H-7 and H-9 were on the same side. When they were in α-orientations, H-1 and H-5 should be in β-orientations. Based on the above evidence, the structure of **1** was established, as shown in Figure 2, and named nudifloside.

Compounds **1**–**4** were evaluated for their cytotoxic activity against the chronic myelogenous leukaemia K562 cell line using the MTT method (Mosmann, 1983). Compounds **1** and **2** showed cytotoxic activity against the K562 cell line with IC_{50} values of 20.7 and 36.0 $\mu g\,mL^{-1}$, respectively, while compounds **3** and **4** were inactive (>100 $\mu g\,mL^{-1}$).

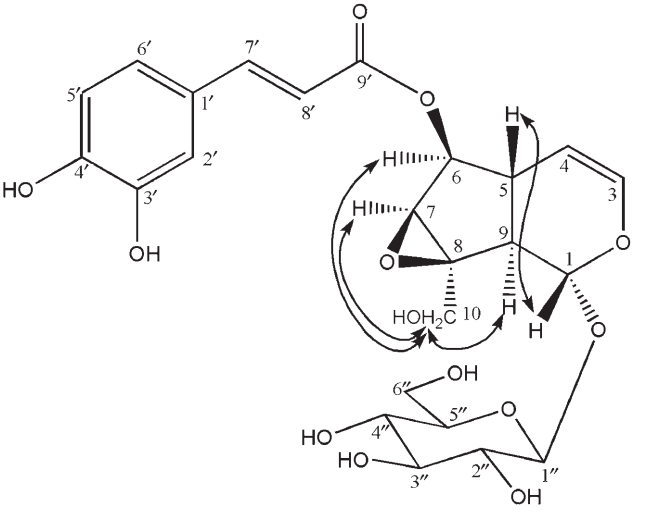

Figure 1. Key ROESY correlations and relative configurations assigned for **1**.

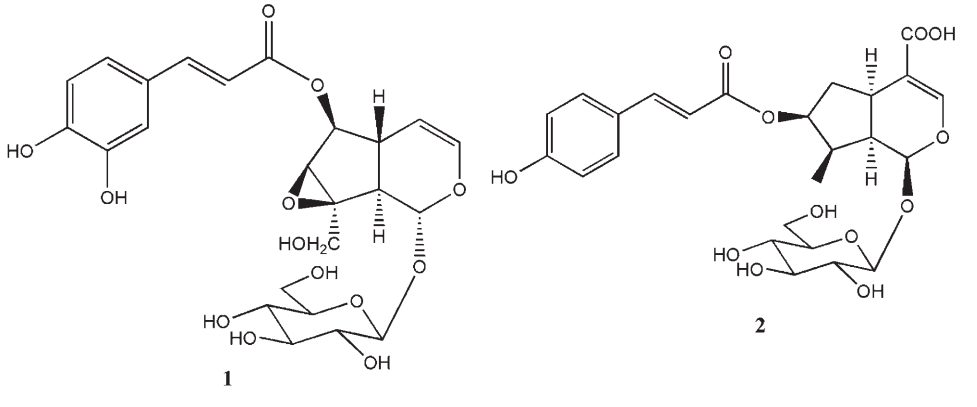

Figure 2. The structures of compounds **1** and **2**.

3. Experimental

3.1. *General experimental procedures*

TLC: precoated TLC plates (Si gel G) from Qingdao Marine Chemical Factory, Qingdao, P.R. China. Column chromatography (CC): silica gel (200–300 and 80–100 mesh) from Qingdao Marine Chemical Factory, Qingdao, P.R. China. Sephadex LH-20 from Amersham Bioscience. Optical rotations: Jasco DIP-370 digital polarimeter. IR Spectra: Bio-Rad FTS-135 IR spectrometer; KBr pellets; in cm^{-1}. NMR spectra: Bruker AM-400 or DRX-500 instruments; $SiMe_4$ as internal standard; δ in ppm, J in Hz. MS: VG Auto-Spec-3000 and APIQSTAR-Pulsar-i spectrometer; m/z (%).

3.2. *Plant material*

The aerial parts of *C. nudiflora* Hook. were collected in Wuzhishan county (August 2005), Hainan Province of P.R. China. The plant was identified by Associate Prof. Zheng-Fu Dai of the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, where a voucher specimen (No. 2050801) is deposited.

3.3. *Extraction and isolation*

The air-dried and powdered aerial parts of *C. nudiflora* (1.7 kg) were extracted with EtOH at room temperature (4×, three days for each time). The EtOH extract was concentrated *in vacuo* and partitioned between petrol-ether and H_2O and then between EtOAc and H_2O. Removal of the solvent of the EtOAc extract gave 124.0 g of residue, which was subjected to column chromatography (CC) over silica gel and eluted with $CHCl_3$ with increasing gradient of MeOH, to give 10 fractions (Fr.1–10) (monitored by TLC). Fr.7 (11.9 g) was chromatographed over silica gel using $CHCl_3$–MeOH (6 : 1) as eluant, and the sub-fraction was further subjected to CC on Sephadex LH-20 gel, eluted with EtOH (95%) to afford compound **4** (21.0 mg). Fr.8 (34.7 g) was subjected to CC on silica gel using $CHCl_3$–MeOH (4 : 1 to 2 : 1) as eluant to give 15 sub-fractions (Fr.8-1–15). Fr.8-9 (192 mg) was further purified by CC over Rp-18 and eluted with MeOH/H_2O (6 : 4) to yielded compound **2** (43.0 mg). Fr.8-10 (223 mg) was further chromatographed over silica gel using $CHCl_3$–MeOH (4 : 1) as eluant; after purification by successive CC on Rp-18 using MeOH–H_2O (6 : 4), compound **1** (86.0 mg) was obtained. Fr.8-12 (126 mg) was subjected to CC over Rp-18 using MeOH–H_2O (1 : 1) as eluant to afford compound **3** (30.0 mg).

Nudifloside (1): yellow gel; $[\alpha]_D^{26} = -160.3$ (c 0.5, MeOH), IR (KBr): $v = 3419$ (br), 2927, 1700, 1657, 1630, 1500, 1384, 1074. UV: 329 (4.20), 295 (3.92), 246 (3.80) and 216 (3.96). ^1H and ^{13}C NMR: Table 1. HR-ESI-MS: $m/z = 547.1421$ ($[M + Na]^+$).

Linearoside (2): pale-yellow gum; UV, FAB-MS, ^{13}C and ^1H NMR data were identical to those reported in the literature (Bergeron, Marston, Gauthier, & Hostettmann, 1997).

Verbascoside (3): yellow needles; m.p. 275–277°C, FAB-MS, ^{13}C and ^1H NMR data were identical to those reported in the literature (Andary, Wylde, Laffite, Privat, & Winternitz, 1982).

5,7-Dihydroxy-3,3′,4′-trimethoxy flavone (4): yellow needles; m.p. 248–250°C; EI-MS, ^{13}C and ^{1}H NMR data were identical to those reported in the literature (Dai, Mei, Wu, Li, Wang, 2006).

3.4. *Cytotoxic activity*

Compounds **1–4** were examined for their cytotoxic activity against the chronic myelogenous leukaemia K562 cell line. Cancer cells were incubated for 3 days at 37°C in the presence of various concentrations of compounds from DMSO-diluted stock solutions. The growth inhibitory property was determined using 3-(dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as described by Mosmann (1983).

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