

## Antitumor Constituents from *Alternanthera philoxeroides*

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### Abstract:

Two new compounds, alternanthin B (**1**) and *N-trans*-feruloyl-3,5-dimethoxytyramine (**2**), along with four known compounds (**3-6**) were isolated from the aerial parts of *Alternanthera philoxeroides*. Their structures were elucidated on the basis of spectroscopic methods. The antitumor activity of the isolated compounds was also evaluated.

### Article:

#### INTRODUCTION

*Alternanthera philoxeroides* (Mart.) Griseb., is a folk medicinal herb widely distributed in Hubei, Hunan, Jiangsu and Zhejiang provinces of southern China, and has been used for the treatment of acute brain fever, measles and herpes zoster [1]. Pharmacological studies indicated that the extract of *A. philoxeroides* has antiviral, antibacterial, and molluscicidal activities [2-3].

In our previous study, the EtOAc extract of *A. philoxeroides* revealed significant cytotoxic effect on HeLa and L929 cell lines (97.24% and 84.75% at 200  $\mu\text{g/ml}$ , respectively). Here we describe the structural elucidation of two new and four known compounds (**1-6**) (figure 1) from the EtOAc extract of *A. philoxeroides*, as well as their antitumor activities.

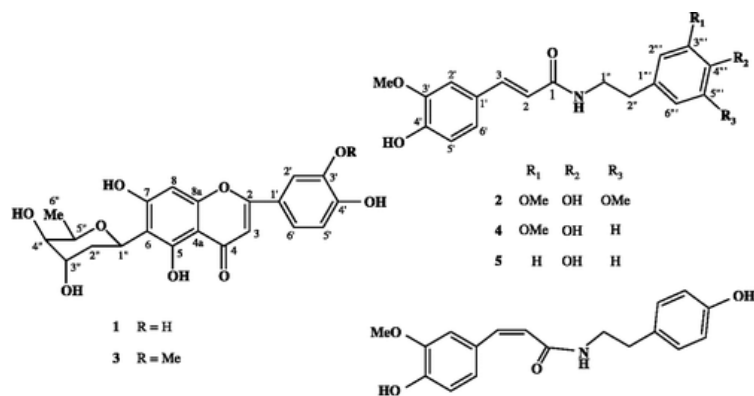


Figure 1: The structures of compounds 1-6.

## RESULTS AND DISCUSSION

Alternanthin B (**1**) was obtained as a yellow amorphous solid, exhibiting a quasi-molecular ion peak at  $m/z$  417.1180  $[M + H]^+$  by HRFTMS, indicating a molecular formula of  $C_{21}H_{20}O_9$  for **1**. The UV spectrum of **1** showed a typical absorption of flavonoid at 271 and 335 nm. Its IR spectrum showed the presence of hydroxyl groups ( $3394\text{ cm}^{-1}$ ) and a carbonyl carbon ( $1621\text{ cm}^{-1}$ ). The  $^1\text{H}$  NMR spectrum of **1** showed an ABX spin system due to a 3',4'-substituted benzyl group [ $\delta$  7.37 (br s), 6.94 (d,  $J = 8.5$  Hz), 7.37 (br d,  $J = 8.5$  Hz)], two olefinic protons [ $\delta$  6.53 (s), 6.46 (s)], one acetal proton ( $\delta$  5.48, dd,  $J = 12.0, 2.3$  Hz), and three oxygenated methine protons [ $\delta$  4.14 (q,  $J = 6.5$  Hz), 4.00 (d,  $J = 2.8$  Hz), 3.40 (d,  $J = 3.1$  Hz)]. In addition to one methylene ( $\delta$  2.24, br t,  $J = 11.9$  Hz, and 1.73, br d,  $J = 14.2$  Hz) and one secondary methyl ( $\delta$  1.28, d,  $J = 6.5$  Hz). Its  $^{13}\text{C}$  NMR spectrum showed 21 carbons, including one carbonyl, nine olefinic quaternary carbons, five olefinic methines, one methyl, one methylene and four oxygenated methine carbon signals. From above observations, compound **1** was deduced to be a flavone glycoside, its  $^{13}\text{C}$  NMR spectral data was similar to those of alternanthin (**3**) [4], except for the absence of a methoxyl group (table 1).

**Table 1:**  $^{13}\text{C}$  NMR spectral data of **1** and **3**.

Position	<b>1</b>	<b>3</b>
2	166.5	165.1
3	104.0	104.5
4	184.1	183.4
4a	105.1	105.1
5	158.8	158.9
6	111.7	111.9
7	164.6	164.4
8	96.1	96.0
8a	158.4	157.7
1'	123.9	123.0
2'	114.4	110.7
3'	147.1	152.8
4'	151.1	149.4
5'	116.9	117.4
6'	120.5	121.8
1''	70.2	69.9
2''	33.0	33.7
3''	68.9	68.5
4''	70.8	70.6
5''	72.8	72.4
6''	17.4	18.2
OCH <sub>3</sub>	–	56.5

From  $^1\text{H}$ - $^1\text{H}$  COSY and HSQC spectra, a coupling system (H-1'' to H-6'') of the glycoside moiety was proposed. And its  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data were also similar to the glycoside moiety of alternanthin (**3**) [4]. Thus, the glycoside moiety of **1** was elucidated as 2,6-dideoxy-xylo-hexose. In the HMBC spectrum of **1**, the proton signal at  $\delta$  5.48 (H-1'') correlated with the signals at  $\delta$  158.8 (C-5), 111.7 (C-6), and 164.6 (C-7), indicated the anomeric carbon of glycoside moiety was linked to C-6 through a C-linkage. And the chemical shift value of C-1'' ( $\delta$  70.2) also supported this conclusion. Therefore, compound **1** was determined as luteolin 6-C- $\beta$ -L-boivinopyranoside.

*N-trans*-feruloyl-3,5-dimethoxytyramine (**2**) revealed an ion peak at  $m/z$  374.1598  $[M + H]^+$ , corresponding to a molecular formula of  $C_{20}H_{23}NO_6$ . IR spectrum exhibited absorption bands for amide group ( $1657$  and  $1610\text{ cm}^{-1}$ ). The  $^1\text{H}$  NMR spectrum of **2** revealed two methylene protons [ $\delta$  2.82(2H, t,  $J = 6.8$  Hz), 3.62 (2H, q,  $J = 6.8$  Hz)], three methoxyl protons [ $\delta$  3.87 (6H,

s), 3.90 (3H, s)], two *trans*-olefinic protons [ $\delta$  6.19, 7.54 (each 1H, d,  $J = 15.5$  Hz)], and an ABX pattern spin system due to a 3',4'-substituted benzene [ $\delta$  6.97 (d,  $J = 1.8$  Hz), 6.90 (d,  $J = 8.2$  Hz) and 7.03 (dd,  $J = 8.2$  and 1.8 Hz)], in addition to two olefinic protons at  $\delta$  6.44 (2H, s). Its  $^{13}\text{C}$  NMR spectrum showed 20 carbons, including one carbonyl carbon ( $\delta$  166.2), seven olefinic quaternary carbons, seven olefinic methines, two methylenes, and three methoxyl signals. Compound **2** was assumed to be a phenolic amide, its  $^{13}\text{C}$  NMR data were similar to those of **4** [5], except for an additional methoxyl group.

In the HMBC spectrum, the proton signal at  $\delta$  3.62 (H-1'') correlated with the signals at  $\delta$  166.2 (C-1), 35.7 (C-2'') and 129.9 (C-1'''), the signal at  $\delta$  2.82 (H-2'') correlated with the signals at  $\delta$  40.9 (C-1''), and 105.4 (C-2''' and C-6'''), and the signal at  $\delta$  6.19 (H-2) correlated with the signals at  $\delta$  166.2 (C-1), 141.1 (C-3), and 127.2 (C-1'). Furthermore, the methoxyl signals at  $\delta$  3.87 (6H, s) showed the NOE correlation with the signals at  $\delta$  6.44 (H-2''' and 6'''), another methoxyl signal at  $\delta$  3.90 (3H, s) correlated with the signal at  $\delta$  6.97 (H-2'). Therefore, the structure of **3** was determined as *N-trans*-feruloyl-3,5-dimethoxytyramine.

Four known compounds were identified by their spectroscopic data in comparison with literature values as follows: alternanthin (**3**) [4], *N-trans*-feruloyl-3-methyltyramine (**4**) [5] *N-trans*-feruloyl tyramine (**5**) [5], and *N-cis*-feruloyl tyramine (**6**) [6].

In a search for antitumor active substances, we examined the inhibitory effect of compounds **1-5** on Hela and L929 cell lines, respectively. Compounds **2-5** showed significant inhibitory effects on Hela cell growth (table 3).

**Table 2:**  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectral data of **2**.

Position	$^1\text{H}$	$^{13}\text{C}$
Feruloyl		
1	–	166.2
2	6.19 d $J = 15.5$ Hz	118.1
3	7.54 d $J = 15.5$ Hz	141.1
1'	–	127.2
2'	6.97 d $J = 1.8$ Hz	109.5
3'	–	146.7
4'	–	147.4
5'	6.90 d $J = 8.2$ Hz	114.7
6'	7.03 d $J = 1.8, 8.2$ Hz	122.1
OCH <sub>3</sub>	3.90 s (3H)	55.9
Amine		
1''	3.62 q (2H) $J = 6.8$ Hz	40.9
2''	2.82 d (2H) $J = 6.8$ Hz	35.7
1'''	–	129.9
2'''	6.44 s	105.4
3'''	–	147.1
4'''	–	133.3
5'''	–	147.1
6'''	6.44 s	105.4
OCH <sub>3</sub> × 2	3.87 s (6H)	56.3

**Table 3:** Inhibitory activity of **1-5** against *Hela* and *L929*<sup>a</sup>.

Compounds	Inhibition (%)			
	Hela		L929	
	30 µg/ml	10 µg/ml	30 µg/ml	10 µg/ml
<b>1</b>	26.5	8.9	50.3	22.6
<b>2</b>	72.1	40.7	32.0	-0.7
<b>3</b>	55.9	49.0	74.9	-9.4
<b>4</b>	58.2	28.9	13.2	4.0
<b>5</b>	72.2	52.9	22.0	2.0

<sup>a</sup> ( $P < 0.05$ ,  $n = 6$ )

## EXPERIMENTAL

### General experimental procedures

NMR spectra were run on a Bruker AVANCE 300 instrument with TMS as internal standard. HRFTMS and ESIMS were obtained on IonSpec 4.7 Tesla FTMS and Alliance 2695 Quattro Micro TM ESI (Waters) instrument, respectively. Column chromatography was performed on silica-gel (Qingdao Haiyang Chemical Co., Ltd), Sephadex LH-20 (Amersham Pharmacia Biotech) and Toyopearl HW-40 (TOSOH). Flash chromatography was carried on a column (C18 HS 40M 1621-1, Biotage, Inc. USA). HPLC was a JASCO Gulliver Series with PU-1580 (pump), RI-1530 and UV-1575 (detector). Semi-Preparative HPLC column was used as below: ODS (YMC-Pack ODS-A, SH-343-5). IR spectra were recorded on a NICOLET 380 FT-IR spectrophotometer (Thermo Electron Corporation). UV spectra were taken on a Shimadzu UV-240 spectrophotometer. Optical rotation was measured with a MC 241 digital polarimeter (PERKIN-ELMER).

### Plant material

The aerial parts of *Alternanthera philoxeroides* (Mart.) Griseb were collected in Wuhan, Hubei province of China in November 2003 and identified by Prof. Ding-Rong Wan (School of Life Sciences, South-Central University for Nationalities). A voucher specimen (D20030802) has been deposited at School of Pharmacy, Tianjin Medical University, China.

### Extraction and isolation

The dried aerial parts (9.0 kg) of *A. philoxeroides* were crushed and extracted three times with EtOH (95%, 60 L each) under reflux for 6 hours. The EtOH extract was concentrated *in vacuo* to give a residue (960 g), which was suspended in H<sub>2</sub>O, and then partitioned with petroleum ether, EtOAc and n-BuOH, successively. The EtOAc extract (53 g) was chromatographed on silica gel column eluting with solvents of increasing polarity [petroleum ether—EtOAc (3:1, 2:1, 1:1, 1:2, 1:3, 100%EtOAc, EtOAc-5% MeOH, EtOAc-10%MeOH, EtOAc-20%MeOH, 100%MeOH)] to yield 22 fractions. Fraction 12 (4.0 g) was rechromatographed on silica gel column (CHCl<sub>3</sub>-MeOH, 95:5, then 9:1) to give 11 fractions (fr.12.1-12.11). Fraction 12.8 (0.7 g) was chromatographed on Toyopearl HW-40 (CHCl<sub>3</sub>/MeOH, 2:1) and then flash chromatography (C<sub>18</sub>, MeOH: H<sub>2</sub>O: HAc 50: 49: 1) to give **5** (89 mg) and **6** (25 mg). Fraction 12.9 (150 mg) was chromatographed on Toyopearl HW-40 (CHCl<sub>3</sub>/MeOH, 2:1) to give **3** (64 mg). Fraction 15 (4.3 g) was rechromatographed on silica gel column (CHCl<sub>3</sub>-MeOH, 95:5, 9:1, 8:2) to give ten fractions (fr.15.1-15.10). Fraction 15.3 (550 mg) was chromatographed on Toyopearl HW-40 and then preparative TLC to give **2** (18 mg) and **4** (15 mg). Fraction 15.5 (98 mg) was separated by HPLC (ODS-A, MeOH/H<sub>2</sub>O, 7:3) to give **1** (24 mg).

**Alternanthin B (1).** Yellow amorphous solid.  $[\alpha]_{\text{D}}^{25} +86.9$  (*c* 0.29, MeOH). UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 271 (4.02), 335 (4.14). IR (KBr)  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3394, 2924, 2855, 1621, 1574, 1487, 1454, 1367, 1296, 1271, 1207, 1170, 1118, 1057.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  6.53 (1H, s, H-3), 6.46 (1H, s, H-8), 7.37 (1H, br s, H-2'), 6.94 (1H, d,  $J = 8.5$  Hz, H-5'), 7.37 (1H, br d,  $J = 8.5$  Hz, H-6'), 5.48 (1H, dd,  $J = 12.0$  Hz, 2.3 Hz, H-1''), 1.73 (1H, br d,  $J = 14.2$  Hz, H-2a), 2.24 (1H, br t,  $J = 11.9$  Hz, H-2e), 4.00 (1H, d,  $J = 2.8$  Hz, H-3''), 3.40 (1H, d,  $J = 3.1$  Hz, H-4''), 4.14 (1H, q,  $J = 6.5$  Hz, H-5''), 1.28 (3H, d,  $J = 6.5$  Hz, H<sub>3</sub>-6'').  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ ) data were listed in Table 1. ESI-MS:  $m/z$  415  $[\text{M} - \text{H}]^-$ . HRFTMS (MALDI-DHB)  $m/z$  417.1180  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{21}\text{H}_{21}\text{O}_9$ , 417.1179).

***N-trans-feruloyl-3,5-dimethoxytyramine* (2).** Yellow solid.  $[\alpha]_{\text{D}}^{25} - 18.0$  (*c* 0.68, MeOH). UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 232 (4.13), 295 (3.98), 319 (4.09). IR (KBr)  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3387, 2934, 2847, 1657, 1610, 1517, 1460, 1428, 1365, 1330, 1275, 1216, 1160, 1118, 1033, 980, 820. ESI-MS:  $m/z$  372  $[\text{M} - \text{H}]^-$ . HRFTMS (MALDI-DHB)  $m/z$  374.1598  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{20}\text{H}_{24}\text{NO}_6$ , 374.1600).  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ) data were listed in table 2.

#### ACKNOWLEDGEMENTS

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