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New lignans with neuroprotective activity from *Adelostemma gracillimum*

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ABSTRACT

Adelostemma gracillimum is an herb used as nourishing and roborant drugs and in the treatment of convulsions in children. To date, a few molecular constituents have been isolated from the root of this herb and chemically characterized, but their biological activities have never been reported. Here, we demonstrate that the crude extract of *A. gracillimum* (AGE) can protect primary cortical neurons against *N*-methyl-D-aspartate (NMDA)-induced cytotoxicity. Further fractionations of AGE led to the isolation of four novel lignans (**1–4**), two known lignans (**5**,**11**), and five known acetophenones (**6–10**); their structures were elucidated by comparison with related literature, extensive analyses of NMR spectroscopy and high-resolution mass spectrometry. Of the eleven isolates, lignans **2**, **3** and **5** exhibit significant neuroprotection against NMDA-induced cell death. This is the first report of isolating lignans with neuroprotective activity from *A. gracillimum*.

1. Introduction

Adelostemma gracillimum (Wall. ex Wight) Hook. f. & Tsiang is a liana plant widely distributed in southwest China and Myanmar. Its root is used as a nourishing roborant and in the treatment of convulsions in children (Mu et al., 1992). The crude glycoside-containing extract of *A. gracillimum* (AGE) has been demonstrated to afford protection against seizures in rat models (Mu et al., 1992; Raza and Iqbal Choudhary, 2000). To date, only 4 aglycones from the hydrolyzed crude glycosides (Mu et al., 1992) and 6 glycosides (Gao et al., 2009) isolated from the root of this herb have been chemically characterized, but the biological activities of these chemical constituents have not been investigated. To identify new natural agents for treating neurological diseases that are characterized by neuronal cell death, we have conducted a phytochemical study of *A. gracillimum*. Herein, we report the unidentified neuroprotective property of AGE against *N*-methyl-D-aspartate (NMDA)-induced cytotoxicity and the isolation of four novel (**1–4**) and two known (**5**, **11**) lignans, and five known acetophenones (**6–10**) from AGE. The structural elucidation of the four novel lignans is described. Lignans **2, 3** and **5** offer significant neuroprotection against NMDA-induced cell death. This is the first report of isolating lignans from *A. gracillimum* which have neuroprotective property.

2. Results and discussion

2.1. Neuroprotective effect of A. gracillimum extract

AGE from *A. gracillimum* was prepared as described previously with some modifications (Mu et al., 1992). The consistency of the preparations was assured by their HPLC fingerprints (Fig. S1 and Table S1 of Supplementary information). The neuroprotective property of AGE against NMDA-induced cytotoxicity was investigated in cultured neurons. Quantitative analysis showed that AGE 10–50 μg/mL reduced cell death by ~60–80%, indicating AGE can significantly protect neurons from NMDA excitotoxicity (Fig. 1a). Memantine, which also exhibits neuroprotective effect (Lipton, 2006) was used as a positive control in this assay. In addition, AGE

Abbreviation: AGE, crude extract from *Adelostemma gracillimum*.

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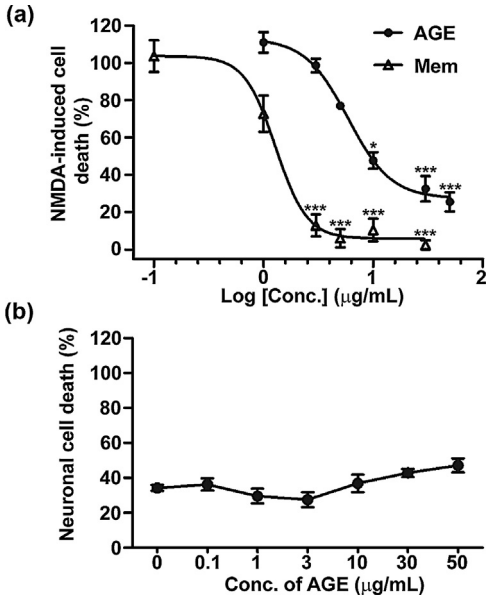

Fig. 1. Dose-dependent neuroprotective effect of extract from *Adelostemma gracillimum*. (a) Cell death in cultured rat cortical neurons was induced by 20 μM NMDA. Normalized cell death in the presence of various concentrations of AGE and memantine (control) are shown. Statistical analysis was performed by two-way ANOVA followed by Bonferroni post hoc tests, $^{*}p < 0.05$, $^{***}p < 0.005$. (b) AGE 0.1–50 μg/mL was not toxic to cortical neurons upon 24 h treatment as revealed by the relatively unchanged normalized cell death despite increasing concentration.

0.1–50 μg/mL did not induce any cytotoxic effect on cortical neurons compared to that of the vehicle control (Fig. 1b).

2.2. Structural elucidation

Four novel (**1–4**) and two known (**5**, **11**) lignans, and five known acetophenones (**6–10**) were isolated from AGE. The structural elucidations of the isolated compounds are described as follows.

Compound **1** was isolated as a light yellow oil, with a molecular formula of $C_{19}H_{22}O_5$ determined by the high-resolution mass spectrometry (HRMS) at *m/z* 353.1380 ($[M + Na]^+$) and *m/z* 313.1465 ($[M + H–H_2O]^+$). The ^1H and ^{13}C NMR spectra of **1** imply the presence of six aromatic protons belonging to two independent benzene rings (rings A, B). Careful analyses of 2D NMR spectra involving ^1H–^1H COSY, HSQC and HMBC (Fig. 3) successfully assign the six aromatic protons with signals at δ_H 7.05 (1H, s, H-2), 6.78 (1H, d, J = 8.0 Hz, H-5), and 6.84 (1H, d, J = 8.0 Hz, H-6) to ring A, and signals at δ_H 6.85 (1H, d, J = 1.5 Hz, H-2′), 6.83 (1H, d, J = 8.0 Hz, H-5′), and 6.72 (1H, dd, J = 8.0, 1.5 Hz, H-6′) to ring B, indicating the presence of two 1,3,4-trisubstituted aromatic rings in **1**. A methoxyl group at δ_H 3.85 (3H, s)/δ_C 56.4, attached to the C-3 position of ring A, is evidenced by the HMBC correlation between δ 3.85 and 148.8 (C-3), together with the NOESY spectrum showing correlation between the methoxyl group and H-2 (Fig. 4). The presence of a propylene glycol unit was deduced from the ^1H–^1H COSY spectrum displaying sequential cross peaks from H-7 (δ_H 4.88) to H-9 (δ_H 1.15) via H-8 (δ_H 4.43), and the linkage of propylene glycol unit at C-1 of ring A was indicated by the correlations of H-7 to both C-2 (δ_C 111.3)/C-6 (δ_C 120.4) in HMBC. Meanwhile, the linkage of a propenyl moiety at C-1′ of ring B is established by the HMBC correlations between H-7′ (δ_H 6.26) and C-6′ (δ_C 118.8)/C-2′ (δ_C 113.8), which is supported as well by the NOE correlations between H-7′ and H-2′ in the NOESY spectrum of **1**. The arrangement of an OH group at C-3′, suggested by the 1,3,4-trisubstituted pattern of aromatic ring B and the molecular formula of **1** being $C_{19}H_{22}O_5$, is clearly supported by the correlations of from H-2′ to C-3′ (δ_C 149.0)/C-6′/C-7′ (δ_C 131.9), along with H-5′ to C-3′ in HMBC. The NMR spectroscopic data of **1** resembles that of known lignan machilin C (Hada et al., 1988; Shimomura et al., 1987; Sung et al., 2001), with the major difference being that **1** has an OH group at C-3′, while a —OCH_3 at C-3′ in machilin C (Table 1). The *erythro*-configuration of protons H-7 and H-8 is suggested by both the small coupling constant of $J_{7,8}$ = 3.5 Hz (~3.0/8.0 for *erythro*/*threo*, respectively) and the carbon chemical shift of C-9 at δ_C 13.6 (~13.0/17.0 for *erythro*/*threo*, respectively) (Braga et al., 1984; Hada et al., 1988; Shimomura et al., 1987; Sung et al., 2001), being consistent with that of machilin C (Hada et al., 1988; Shimomura et al., 1987; Sung et al., 2001). Furthermore, the coupling constant between H-7′ and H-8′ (15.5 Hz) indicates the *trans* configuration of the olefinic double bond in the 1′-propenyl moiety. Therefore, the structure of **1** is elucidated to be *erythro*-3-methoxyl-3′,4,7-trihydroxyl-(7′*E*)-(8-*O*-4′)-neolignan-7′-en, as shown in Fig. 2.

Table 1
^1H (500 MHz) and ^{13}C NMR (75 MHz) data of compounds **1–4** in CD_3OD.

No	1		2		3		4	
	d_H (mult., J in Hz)	d_C	d_H (mult., J in Hz)	d_C	d_H (mult., J in Hz)	d_C	d_H (mult., J in Hz)	d_C
1	–	133.6	–	133.2	–	134.5	–	133.9
2	7.05 (s)	111.3	6.67 (s)	105.4	6.88 (s)	110.1	6.60 (s)	103.8
3	–	148.8	–	149.2	–	148.8	–	149.3
4	–	146.8	–	135.9	–	147.3	–	136.3
5	6.78 (d, 8.0)	115.8	–	149.2	6.74 (d, 8.0)	116	–	149.3
6	6.84 (d, 8.0)	120.4	6.67 (s)	105.4	6.75 (d, 8.0)	119.3	6.60 (s)	103.8
7	4.88 (d, 3.5)	76.2	4.58 (d, 8.0)	79.8	5.14 (d, 4.5)	92.6	5.16 (d, 5.0)	92.8
8	4.43 (m)	81.1	3.98 (m)	86.7	3.69 (m)	47.3	3.71 (m)	47.4
9	1.15 (d, 6.0)	13.6	1.13 (d, 6.5)	17.9	1.30 (d, 7.0)	20.7	1.31 (d, 6.5)	20.7
1′	–	133.9	–	132.2	–	123.8	–	124.1
2′	6.85 (d, 1.5)	113.8	6.50 (s)	102.5	–	132.4	–	132.5
3′	–	149	–	154.7	–	153.5	–	153.6
4′	–	145.6	–	135.8	–	153.4	–	153.6
5′	6.83 (d, 8.0)	117.2	–	152	6.85 (d, 8.0)	115	6.87 (d, 8.5)	115.1
6′	6.72 (dd, 8.0, 1.5)	118.8	6.51 (s)	107.9	6.83 (d, 8.0)	117.4	6.74 (d, 8.5)	117.6
7′	6.26 (d, 15.5)	131.9	6.28 (d, 16.0)	132.2		204.8	–	205.3
8′	6.08 (m)	124.5	6.16 (m)	125.7	2.60 (s)	32.1	2.60 (s)	32.2
9′	1.82 (d, 6.5)	18.5	1.84 (d, 7.5)	18.5	–	–	–	–
3-OCH_3	3.85 (s)	56.4	3.84 (s)	56.8	3.79 (s)	56.3	3.79 (s)	56.7
5-OCH_3	–	–	3.84 (s)	56.8	–	–	3.79 (s)	56.7
3′-OCH_3	–	–	3.80 (s)	56.3	–	–	–	–