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Lignans from the roots of *Acorus tatarinowii* Schott ameliorate β amyloid-induced toxicity in transgenic *Caenorhabditis elegans*

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ABSTRACT

A novel tetralignan, tatarinan T (**1**) with the rare C8-C7′ linkage pattern, along with a known monolignan (**2**) were isolated from the roots of *Acorus tatarinowii* Schott. Their chemical structures were elucidated on the basis of NMR and X-ray diffraction analysis. We evaluated the protective effects of two rare lignans against β-amyloid toxicity by using CL4176 transgenic *C. elegans* model for the first time, and found that they significantly delayed paralysis of worms at the concentration of 100 μM. Compound **2** exhibited the more potential protective effect against β-amyloid toxicity, its value of PT_{50} extended up to 62.3% at 100 μM compared with control, especially, it still has 30.8% extension at 10 μM.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder, and it is the most common cause of dementia in the aged population. Abnormal accumulation of β-amyloid peptide (Aβ) in the brain is regarded to be important in this disease. Thus, many efforts have been made to develop strategies targeting Aβ for the prevention and treatment of AD [1,2].

The roots of *Acorus tatarinowii* Schott (Araceae) are a well-known traditional Chinese medicine used in the improvement of memory and cognition [3]. It has reported that extract of *A. tatarinowii* can protect PC12 cells from amyloid-β induced neurotoxicity [4]. β-asarone, the major ingredient of *A. tatarinowii* Schott, has neuroprotective effects *in vitro* and *in vivo* [5,6,7].

In order to further study the effective material basis of title plant with anti-AD, we used the CL4176 transgenic *Caenorhabditis elegans* (*C. elegans*) as a model organism to examine the protective effects of isolated compounds *via* the potential reduction of Aβ toxicity. The CL4176 transgenic *C. elegans* strain was engineered to inducibly express human $Aβ_{1-42}$ peptide in muscle, and the expression and subsequent aggregation of Aβ in the muscle lead to progressive paralysis when temperature is raised [8,9]. This strain has already been employed as *in vivo* models of AD and used to demonstrate the effect of *Ginkgo biloba* extract EGb 761 [10], Liuwei Dihuang [11], and tetracycline [12], in counteracting the Aβ toxicity.

During our research periods, tatarinan T (**1**), a novel tetralignan with the rare C8-C7′ linkage pattern, along with a known monolignan (**2**) [13] were isolated. We individually investigated their anti-Aβ potential using CL4176 transgenic *C. elegans*, and found that these two compounds showed potential protective effect by delaying paralysis of worms. The present paper reported the isolation, structural elucidation, and anti-Aβ activity of these two lignans.

2. Experimental

2.1. General

Optical rotations were measured with a Rudolph autopol VI polarimeter. The IR spectra were recorded on a Nicolet Magna IR spectrophotometer. The NMR spectra were run on a Bruker AM-400 spectrometer with TMS as internal standard. HR-EI-MS spectra were carried out on a Bruker Apex IV FT-MS spectrometer. Column chromatographic separations were carried out on silica gel H-60 (Qingdao Haiyang Chemical Group Corporation, Qingdao, People's Republic of China), and LiChroprep RP-18 (40–63 μm, Merck). TLC was carried out on silica gel HSGF254 plates (Yantai Chemical Industrial Institute, Yantai, People's Republic of China), and spots were visualized by spraying with concentrated sulfuric acid-vanillin solution followed by heating.

2.2. Plant material

The roots of *A. tatarinowii* Schott, in dried form, were purchased from Kangqiao Pharmaceutical Co., Ltd., Shanghai, China, in 2010. A reference sample was deposited in the Tongji Herbarium.

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Table 1
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data for **1** in $CDCl_3$ (δ in ppm, *J* in Hz).

Position	δ_C	δ_H	Position	δ_C	δ_H
1	128.9		7″	40.4	2.58, m
2	152.4		8″	40.8	1.67, m
3	97.6	6.47, s	9″	12.5	0.85, d (6.62)
4	151.7		1‴	125.1	
5	140.4		2‴	151.5	
6	137.5		3‴	96.8	6.45, s
7	52.4	3.95, m	4‴	147.2	
8	42.4	1.25, m	5‴	141.1	
9	24.4	0.90, d (6.43)	6‴	114.4	6.27, s
1′	123.2		7‴	43.1	3.57, m
2′	152.7		8‴	17.8	1.80, m
					1.56, m
3′	98.5	6.41, s	9‴	12.8	0.56, t (7.08)
4′	147.3		OMe	55.5	3.47, s
5′	142.5		OMe	55.6	3.57, s
6′	113.4	6.69, s	OMe	55.7	3.57, s
7′	53.8	3.00, s	OMe	56.1	3.57, s
8′	35.8	2.76, m	OMe	56.1	3.64, s
9′	12.7	0.23, d (7.24)	OMe	56.4	3.80, s
1″	125.3		OMe	56.5	3.82, s
2″	152.4		OMe	56.8	3.82, s
3″	96.0	6.48, s	OMe	56.8	3.85, s
4″	147.1		OMe	57.2	3.85, s
5″	141.8		OMe	57.6	3.87, s
6″	114.8	6.56, s	OMe	60.2	3.92, s

2.3. Extraction and isolation

The air-dried roots of *A. tatarinowii* Schott (5.0 kg) were powdered and then extracted three times with 95% ethanol at room temperature. The combined ethanol extracts were concentrated under reduced pressure to give a residue (700 g), which was then partitioned successively with $CHCl_3$ and *n*-BuOH, respectively. The $CHCl_3$ extract (200 g) was subjected to silica gel column chromatography using a gradient solvent system of petroleum ether–acetone (50:1, 25:1, 15:1, 10:1, 5:1, 2:1, 1:1, v/v) to afford 6 fractions (Fr. 1–Fr. 6). Fr. 2 was subjected to silica gel column chromatography repeatedly and eluted with petroleum ether-EtOAc (15:1, 10:1, 5:1, 2:1, 1:1, v/v) to afford 6 fractions (Fr. 2a–Fr. 2f). Fr. 2a was subjected to repeated silica gel column chromatography with gradient elution with petroleum ether-acetone (20:1, 10:1, 5:1, 2:1, 1:1, v/v) to yield compound **2** (102.0 mg). Fr. 2d was chromatographed repeatedly on a RP-18 column eluting with acetone-H_2O (1:1, 2:1, 3:1, v/v), and a silica gel column eluting with a petroleum ether-EtOAc (5:1, 2:1, 1:1, v/v) to obtain compound **1** (10.6 mg).

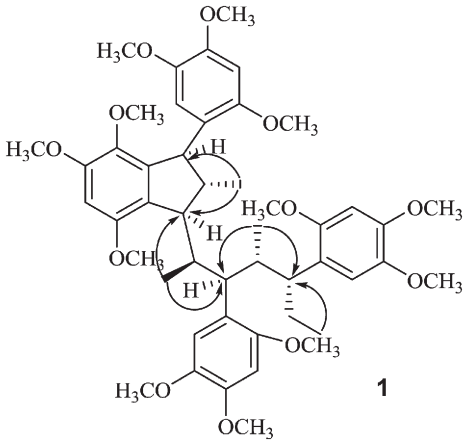

Fig. 2. Key HMBC correlations of compound **1**.

2.4. Spectral data of new compound

Colorless needles; $[\alpha]_D^{25}$-19.0 (c 0.05, $CHCl_3$); IR (KBr) ν_{max} 2963, 2952, 2936, 2833, 1608, 1509, 1465, 1325, 1232, 1204, 1177, 1039, 983, 813 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 1; HREIMS m/z 855.4282 $[M + Na]^+$ (calcd for $C_{48}H_{64}O_{12}Na$ 855.4290).

2.5. X-ray analysis

Crystal data for compound **1**: formula $C_{48}H_{64}O_{12}$; Mr. = 832.99; monoclinic crystalline system; space group Fdd2; unit cell dimensions a = 61.796 (5) Å, b = 10.2319 (9) Å, c = 30.175 (3) Å; V = 19,080 (3) Å3; Z = 16; Dx = 1.160 mg/m^3; absorption coefficient 0.082 mm^{-1}; F (000) = 7168; R (reflections) = 0.1175 (8867); wR2 (reflections) = 0.1890 (8867). Colorless crystals of **1** were obtained in a mixed solvent of petroleum ether and acetone.

Crystal data for compound **2**: formula $C_{24}H_{32}O_6$; Mr. = 416.49; monoclinic crystalline system; space group P21/n; unit cell dimensions a = 7.054 (2) Å, b = 11.912 (4) Å, c = 27.117 (8) Å; V = 2272.1 (12) Å3; Z = 4; Dx = 1.218 mg/m^3; absorption coefficient 0.086 mm^{-1}; F (000) = 896; R (reflections) = 0.0949 (4230); wR2 (reflections) = 0.2081 (4230). Colorless crystals of **2** were obtained in a mixed solvent of petroleum ether and acetone.

Crystal data were obtained on a Bruker Smart Apex CCD diffractometer, using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre, deposit No. CCDC 1425333 for **1**, and CCDC 1427221 for **2**. Copies of the data can be

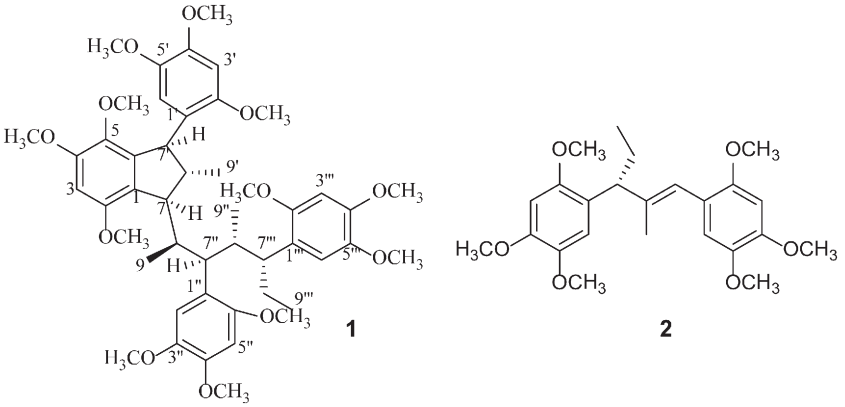

Fig. 1. Chemical structures of compounds **1** and **2**.