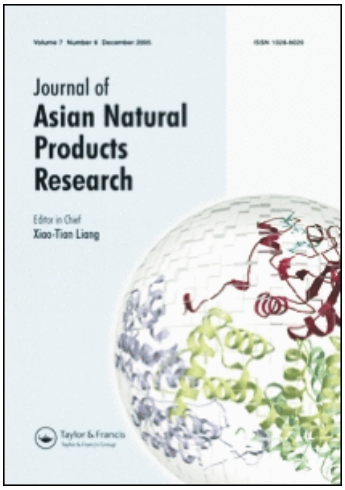

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A novel analgesic pyrazine derivative from the leaves of *Croton tiglium* L.

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A novel analgesic pyrazine derivative from the leaves of *Croton tiglium* L.

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A novel analgesic pyrazine derivative, named crotonine, was isolated from the leaves of *Croton tiglium* L. The structure was elucidated as 2-(furan-2-yl)-5-(2,3,4-trihydroxy-butyl)-1,4-diazine by spectroscopic analysis. Crotonine inhibited remarkably the acetic acid-induced writhing in mice.

Keywords: *Croton tiglium* L; Euphoriaceae; 2-(Furan-2-yl)-5-(2,3,4-trihydroxy-butyl)-1,4-diazine; Crotonine analgesic effects

1. Introduction

Croton tiglium L. (Euphoriaceae) is a shrub or small arbour, which grows widely in China, particularly in Sichuan province. The seeds of *C. tiglium* are used as a traditional medicine for wound healing and the treatment of diarrhoea, or as insecticide and antimicrobial agent. Some diterpenoids have been isolated from *C. tiglium* previously [1].

As part of our continuing investigation on anti-inflammatory and analgesic plants and their active constituents of medicinal interest, we have examined the extracts of the leaves of *C. tiglium*. A novel pyrazine derivative, 2-(furan-2-yl)-5-(2,3,4-trihydroxy-butyl)-1,4-diazine, named crotonine, was isolated from the EtOH extract with some known compounds: β-sitosterol, stigmasterol, 1-octacosanol, 24-ethyl-5,22-cholest-dien-3-ol, 12-*O*-(2-methyl)-butyrylphorbol-13-acetate, 12-*O*-tigloylphorbol-13-(2-methylpropionate), 12-*O*-tigloyl-phorbol-13-(2-methylbutyrate).

Pyrazines are heterocyclic nitrogen-containing compounds found mainly in processed food, where they are created chemically in a dry heating process [2]. They are also found naturally in plants, such as *Ligusticum wallichil* Franchat., *Ephedra sinica* Stapf in China and *Jatropha podagrica* in Africa [3–6], and some micro-organisms are known to produce pyrazines during their primary or secondary metabolism [7,8]. Pyrazine derivatives showed good medicinal interest, such as relaxing cardiovascular and uterine smooth muscle [9–11], anti-thrombotic [12], anti-aggregation [5], COX-2 inhibiting and analgesic effects [13].

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We report here the isolation and structural elucidation of crotonine, as well as its analgesic effect.

2. Results and discussion

Crotonine (**1**) was isolated from the 95% EtOH extract of dried leaves of *C. tiglium* L., as yellow solid. HREI-MS gave a $[M]^+$ peak at *m/z* 250.0954, corresponding to the molecular formula $C_{12}H_{14}N_2O_4$. A strong and broad band at 1560 cm^{-1} in the IR spectrum, together with the band at 875 cm^{-1}, indicated the existence of a furan moiety [14]. The UV absorptions at 205, 280 and 335 nm suggested the presence of furan and pyrazine moieties [15].

The ^1H NMR signals at δ_H 7.69 (1H, dd, $J = 0.6$ Hz, 1.7 Hz, H-5′), 7.15 (1H, dd, $J = 0.6$ Hz, 3.4 Hz, H-3′) and 6.62 (1H, dd, $J = 1.7$ Hz, 3.4 Hz, H-4′) indicated an α-substituted furan moiety [16–18]. The proton signals at δ_H 8.88 (1H, d, $J = 1.4$ Hz, H-3) and 8.49 (1H, d, $J = 1.4$ Hz, H-6) were ascribable to a pyrazine moiety with two different substituents attached to C-2 and C-3, or C-2 and C-5 [18,19]. The ^{13}C NMR spectrum showed eight aromatic carbon signals (δ_C 154.8, 152.3, 145.9, 145.7, 143.9, 140.1, 113.3, 111.2), six of them were evidently attached to heteroatom, provided therefore further proof for the existence of furan and pyrazine moieties. The remaining part of crotonine was determined to be a 2,3,4-trihydroxybutyl by detailed analysis of ^1H NMR, ^{13}C NMR, ^1H—^1H COSY, HSQC and HMBC data. The ^1H NMR spectra demonstrated signals of two methylenes, both in the form of AB system, and two methines. The ^{13}C NMR signals at δ_C 76.1, 72.9, 64.6 and 39.6 and the corresponding HSQC signals indicated that a methylene and the two methines were linked to hydroxyl group. The ^1H—^1H COSY correlations between H-1″ at δ_H 2.91 (1H, dd, $J = 14.2$ Hz, 9.5 Hz,), 3.21 (1H, dd, $J = 14.2$ Hz, 3.1 Hz) and H-2″ at δ_H 3.95 (1H, m), H-2″ and H-3″ at δ_H 3.56 (1H, m), H-3″ and H-4″ at δ_H 3.62 (1H, dd, $J = 11.2$ Hz, 6.4 Hz), 3.77 (1H, dd, $J = 11.2$ Hz, 3.8 Hz) were observed, indicating the presence of a —CH_2—CHOH—CHOH—CH_2OH moiety.

The positions of the furan-2-yl and the 2,3,4-trihydroxybutyl side chain linkages were deduced from the HMBC experiment (table 1). The HMBC spectrum showed cross-peaks between H-3′ (δ 7.15) of the furan-2-yl and C-2 (δ 143.9) of the pyrazine moiety, H-3 (δ 8.88)

Table 1. ^1H (400 MHz) and ^{13}C NMR (100 MHz) data of **1** in CD_3OD.

Position	δ_H *(J in Hz)*	δ_C	*HMBC*
2		143.9	
3	8.88 (d, 1.4)	140.1	C-2, 2′
5		154.8	
6	8.49 (d, 1.4)	145.7	C-5, 1″
2′		152.3	
3′	7.15 (dd, 0.6, 3.4)	111.2	C-2′, 2, 4′, 5′
4′	6.61 (dd, 1.7, 3.4)	113.3	C-5′, 3′, 2′
5′	7.69 (dd, 0.6, 1.7)	145.9	C-4′, 3′
1″	2.91 (dd, 14.2, 9.5)	39.6	C-5, 6, 2″, 3″
	3.21 (dd, 14.2, 3.1)		
2″	3.95 (m)	72.9	C-5, 1″, 3″, 4″
3″	3.56 (m)	76.1	C-1″, 2″, 4″
4″	3.62 (dd, 11.2, 6.4)	64.6	C-2″, 3″
	3.77 (dd, 11.2, 3.8)		

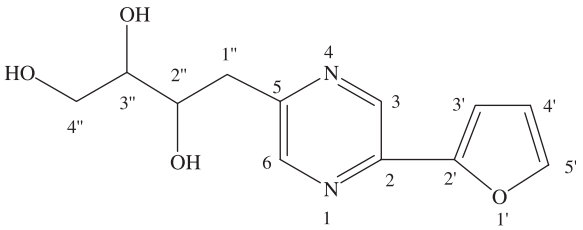

Figure 1. The structure of **1**.

of the pyrazine ring and C-2′ (δ 152.3) of the furan-2-yl, indicating that the furan-2-yl fragment was linked to C-2 of the pyrazine ring. Moreover, long-range correlations were also observed between H-6 (δ 8.49) and C-1″ (δ 39.6); H-1″ (δ 2.91, 3.21) and C-5 (δ 154.8), C-6 (δ 145.7); H-2″ (δ 3.6) and C-5 (δ 154.8), indicating that the trihydroxybutyl moiety was connected with C-5 of the pyrazine.

Based on the evidence mentioned above, the structure of crotonine was determined as 2-(furan-2-yl)-5-(2,3,4-trihydroxybutyl)-1,4-diazine (**1**) (figure 1).

Bioactivity tests showed that intraperitoneal administration of crotonine could inhibit the acetic acid induced writhing in mice. The inhibitory rates of crotonine at 5 mg/kg and morphine at 2 mg/kg, as positive control, were 72.0% and 89.1%, respectively. But the other compounds showed a weak analgesic effect; their inhibitory rates were lower than 30%.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a PE-243 spectrometer. UV spectra were measured with a Cintro-20 spectrometer (Australia). IR spectra were measured with Nicolet Manga spectrometer (American Micronicolet). NMR spectra were obtained with a JNM-ECA-400 (Japan) spectrometer (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR). ESI-MS spectra were obtained with a API3000 spectrometer (Applied Biosystems, USA). HREI-MS was obtained with Micromass ZabSpec spectrometer (70 eV). HP-20 macroreticular resin (Mitsubishi Chemical Corp., Japan), silica gel (Qingdao Haiyang Chemical Industry Co.) and Sephadex LH-20 (Pharmacia, Sweden) were used for column chromatography (MeOH/H_2O). TLC analysis was performed on silica gel plates (Qingdao Haiyang Chemical Industry Co.; $CHCl_3$/MeOH 10:1).

3.2 Plant material

The leaves of *Croton tiglium* L. (Euphoriaceae) were collected from Jiangjin District in Sichuan Province of China, in September 2003, and authenticated by Ma Qiyun. A voucher specimen has been deposited at the herbarium of Beijing Institute of Pharmacology and Toxicology.

3.3 Animal material

KM mice (18–22 g) were used in the analgesic tests. The mice were housed in groups of five animals in polypropylene cages under standard environmental conditions (kept at 25 ± 2°C,

60–70% humidity and in a 12:12-h dark/light cycle). The animals were maintained with free access to a standard commercial diet and water *ad libitum*, but supplied only with water in the last 24 h before the analgesic experiment.

3.4 Acetic acid induced writhing in mice

This test was done using the method described by Koster *et al.* [20]. Groups of 10 male mice were treated i.p. with crotonine (dissolved in H_2O) at dose of 5 mg/kg, 30 min before the intraperitoneal injection of a 0.6% aqueous acetic acid solution (0.4 ml/mouse). Each mouse was kept in an individual box during the analgesic effect observation. The numbers of muscular contractions were counted for 15 min, starting at the 5th minute after the acetic acid injection. Water and morphine (2 mg/kg) were used as negative and positive control, respectively.

3.5 Extraction and isolation

Powder of the air-dried plant material (3 kg) was refluxed by 95% EtOH. A dark-brown mass (380 g) was obtained after removal of the solvent under reduced pressure. This residue was suspended with water and successively extracted with petroleum ether (bp 60–90°C) and EtOAc. The remaining aqueous fraction (178 g) was concentrated with a rotary evaporator beneath 40°C, and applied to a HP-20 resin column. The column was eluted with EtOH/H_2O (0:100, 30:70, 50:50, 5:95), successively. Crotonine (**1**) (26 mg) was isolated from the 30% EtOH eluate (10 g) by repeated chromatography on silica gel with $CHCl_3$/MeOH (10:1), and further purification with Sephadex LH-20, eluting with MeOH.

3.5.1 Crotonine. Yellow solid, $[\alpha]_D^{20}$ −6.8 (*c* 0.25, MeOH). UVλ_{max}^{MeOH} nm: 205, 280, 335. IRν_{max}^{KBr}cm^{-1}: 1560, 875. The ^{1}H NMR and ^{13}C NMR data are given in table 1. EI-MS *m*/*z* 250, 232, 219, 189, 160, 220, 215, 201, 173, 133, 105, 92, 93. HREI-MS *m*/*z* 250.0954 [M$^+$] (calcd for $C_{12}H_{14}N_2O_4$, 250.0953).

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