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Polyacetylenes from the florets of *Carthamus tinctorius* and their cytotoxicity

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ABSTRACT

Two new polyacetylenes, (*2R*,*11S*,*12S*)-(3*E*)-tridecene-5,7,9-triyne-1,2,11,12-tetraol (**1**) and (8*S*)-deca-4,6-diyne-1,8-diol-8-*O*-*β*-D-glucopyranoside (**2**), together with six known polyacetylenes (**3–8**), were isolated from the florets of *Carthamus tinctorius*. Their structures were elucidated on the basis of spectroscopic data (NMR and HRMS) and $Mo_2(OAc)_4$-induced circular dichroism. Compounds **4** and **5** were cytotoxic to the human cancer cell lines HL-60 (IC_{50} = 6.40, 3.66 μM), THP-1 (IC_{50} = 8.67, 6.69 μM) and PC-3 (IC_{50} = 11.30, 10.92 μM), comparable to the positive control, fluorouracil (IC_{50} = 2.58, 3.95 and 22.35 μM, respectively).

1. Introduction

Carthamus tinctorius L., commonly known as safflower, is an annual herbal plant in the family, Compositae. The plant is found in Xinjiang, Sichuan, and Henan Provinces of China. In traditional Uygur Medicine prescription, the florets of *C. tinctorius* are traditionally used to treat coronary heart and gynaecological diseases (Li et al., 2015; Zhang et al., 2016). Flavonoids (He et al., 2014; Yue et al., 2016), polyacetylenes (Kurimoto et al., 2010), lignans, alkaloids (Fan et al., 2009), and organic acids (Zhou et al., 2008) have been isolated from the florets of this plant. Among these classes of compounds, the polyacetylenes have been reported to show several pharmacological activities including antibiosis (Ayyad et al., 2015), anti-inflammatory (Zhang et al., 2013), neurotrophy (Yamazaki et al., 2001), and cytotoxicity (Mumm et al., 2004). While polyacetylenes are known to occur in several plant families including Compositae, Campanulaceae, Araliaceae and Pittosporaceae (Zhang et al., 2016), the Compositae is the richest source of this class of compounds. In our search for bioactive compounds from Xinjiang indigenous medicinal plants, two new (**1** and **2**) (Fig. 1), along with six known polyacetylenes (**3–8**), were isolated from the florets of *C. tinctorius*. Herein, the isolation, structure elucidation and cytotoxicity evaluation of these compounds are presented.

2. Results and discussion

Compound **1**, $[\alpha]_D^{25}$ +18 (*c* 0.050, MeOH), was isolated as a colourless amorphous solid. Its molecular formula was determined to be $C_{13}H_{14}O_4$ by HRESIMS which showed a $[M+COOH]^-$ at *m/z* 279.0840 (calcd for $C_{14}H_{15}O_6$, 279.0869). The IR spectrum of **1** exhibited absorption bands at 3398 and 2186 cm^{-1}, attributed to hydroxy and acetylene groups, respectively. In the ^1H NMR spectrum (Table 1), a secondary methyl signal at δ_H 1.23 (3H, d, *J* = 6.3 Hz, H-13) and the signals for three oxygenated methine groups at δ_H 3.78 (1H, m, H-12), 4.22 (1H, m, H-2), 4.24

(1H, d, *J* = 4.8 Hz, H-11), along with an oxygenated methylene group at δ_H 3.50 (2H, m, H-1), as well as a *trans*-oriented olefinic protons at δ_H 5.91 (1H, dd, *J* = 16.0, 1.5 Hz, H-4), 6.50 (1H, dd, *J* = 16.0, 5.0 Hz, H-3), were observed. The ^{13}C NMR and HSQC data showed the presence of 13 carbon atoms, comprising one methyl, one methylene, five methines, and six quaternary carbons (δ_C 81.2, 76.8, 74.9, 70.6, 66.4, and 63.2, suggesting the presence of three triple bonds). The aforementioned data suggested that compound **1** was a polyacetylene derivative (Ayyad et al., 2015; Liu et al., 2015).

Further analyses of the 1D and 2D NMR spectra data (including ^1H–^1H COSY, HSQC and HMBC) allowed for the elucidation of the structure of **1**. The ^1H–^1H COSY spectra displayed that compound **1** had two spin systems (C-1–C-4 and C-11–C-13, drawn with bold bonds in Fig. 2a). The two substructure units are linked with the polyethynyl (C-5–C-10) group as shown from the HMBC correlations. Thus correlations (Fig. 2a) from H-3 to C-5, and from H-4 to C-5, C-6, C-7, and C-8, allowed for the connection of C-4–C-5, while the attachment of C-10 and C-11 was determined by the HMBC correlations from H-12 to C-10, and from H-11 to C-10, C-9, C-8, and C-7. Thus, these data established the main carbon connectivity in **1**. The $Mo_2(OAc)_4$-induced circular dichroism (ICD) spectrum of **1** displayed a positive Cotton effect at 303 nm (see Supporting information), indicating that the 11,12-diol moiety has

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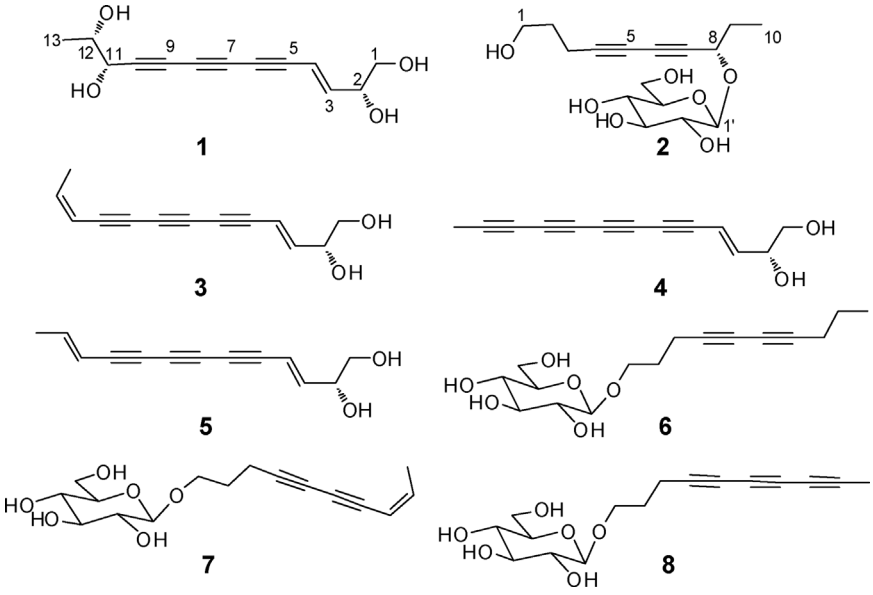

Fig. 1. The chemical structures of compounds **1–8**.

Table 1
^{1}H and ^{13}C NMR Data of **1–2** (in CD_3OD).[a]

No.	1		2	
	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C
1	3.50 (m)	66.5	3.60 (2H, t, 6.3)	61.3
2	4.22 (m)	73.5	1.71 (2H, m)	32.2
3	6.50 (dd, 16.0, 5.0)	150.9	2.38 (2H, t, 7.1)	16.3
4	5.91 (dd, 16.0, 1.5)	109.3		81.4
5		76.8		65.5
6		74.9		72.0
7		63.2		75.1
8		66.4	4.64 (t, 6.6)	69.6
9		70.6	1.76 (2H, m)	29.8
10		81.2	1.00 (3H, t, 7.4)	9.8
11	4.24 (d, 4.8)	68.5		
12	3.78 (m)	71.2		
13	1.23 (3H, d, 6.4)	18.9		
1′			4.54 (d, 7.8)	101.2
2′			3.16 (t, 7.8)	74.9
3′			3.36 (m)	78.0
4′			3.25 (m)	71.7
5′			3.26 (m)	78.1
6′			3.63 (dd, 12.1, 5.5)	62.8
			3.85 (dd, 12.1, 1.5)	

[a] Recorded at 400 and 100 MHz for ^{1}H and ^{13}C, resp.

(11*S*,12*S*) configuration based on the empirical rule proposed by Snatzke (Di Bari et al., 2001). Comparison of the chemical shift of H-2 (δ_H 4.22) with that of the known compound (2*R*)-(3*E*,11*Z*)-tridecadiene-5,7,9-triyne-1,2-diol (**3**) (δ_H 4.22–4.25) (Liu et al., 2015), tentatively assigned the remaining stereocenter C-2 as 2*R*, which is also identical to that of (2*E*,8*E*,10*E*)-tridecatriene-4,6-diyne-1,12,13-triol-1-*O*-β-D-glucopyranoside, previously isolated from the same species (He et al., 2011). Therefore, the structure of this new compound was elucidated as (*2R*,*11S*,*12S*)-(3*E*)-tridecene-5,7,9-triyne-1,2,11,12-tetraol (**1**).

Compound **2**, $[\alpha]_D^{25}$ −91.0 (*c* 0.200, MeOH), had a molecular formula of $C_{16}H_{24}O_7$, as indicated by HRESIMS at *m/z* 373.1489 $[M+COOH]^-$ (calcd for $C_{17}H_{25}O_9$, 373.1499). The IR spectrum of **2** showed absorption bands for hydroxy (3386 cm^{-1}), and triple-bond (2252 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of **2** (Table 1) displayed the presence of a glucopyranosly moiety [δ_H 4.54 (1H, d, *J* = 7.8 Hz); δ_C 101.2, 74.9, 78.0, 71.7, 78.1, and 62.8], and the coupling constant (7.8 Hz) of the anomeric proton indicated the β-linkage. In addition to the signals of the gluopyranosyl moiety, the ^{13}C NMR spectrum of **2** showed 10 carbon signals, which were attributed to one methyl, four methylene, one methine and four quaternary carbon atoms (δ_C 81.4, 75.1, 72.0, and 65.5, suggesting the presence of two triple bonds). This set of data indicated that compound **1** was a polyacetylenic glucoside. The ^{1}H–^{1}H COSY and HMBC spectra were then applied to establish the structure of **2**. The connections of C-1 to C-3 and C-8 to C-10 was determined by the ^{1}H–^{1}H COSY correlations (Fig. 2b) of H-1/H-2, H-2/H-3, H-8/H-9, and H-9/H-10. The HMBC correlations (Fig. 2b) from H-2 to C-4, from H-3 to C-4, C-5, and C-6, from H-9 to C-7, and from H-8 to C-7, C-6, and C-5, established the linkages of C-3–C-4 and C-7–C-8. The attachment of the glucopyranose moiety to C-8 was established from the HMBC correlation of H-1′ to C-8. Acid hydrolysis of compound **2** yielded D-glucose, which was identified by comparison with an authentic sample. Based on the Hudson's rules of isorotation (Hudson, 1909), the molecular rotation of the aglycone of **2** was calculated as a negative value from the measured specific rotation of **2** (Molecular rotation $[M]_D$ = MW × $[\alpha]_D$/100, where MW is the molecular weight and $[\alpha]_D$ is the specific rotation; thus, $[M]_{D(aglycone)}$ was calculated as a negative value based on the formula $[M]_{D(glycoside)} = [M]_{D(aglycone)} + [M]_{D(sugar)}$). Comparison of the molecular rotation of the aglycone to that of the known compound (*S*)-panaxjapyne A (Fang and Martin, 2014), indicated that the absolute configuration at C-8 is *S*. Therefore, the structure of compound **2** was elucidated as (8*S*)-deca-4,6-diyne-1,8-diol-8-*O*-β-D-glucopyranoside.

In addition six known compounds were isolated and identified on

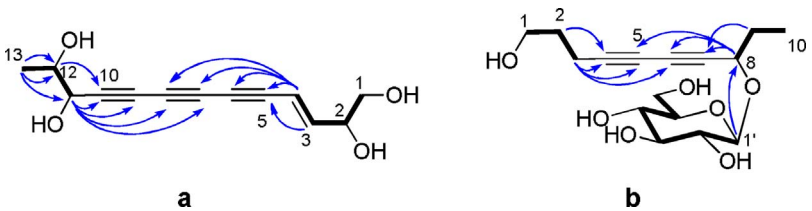

Fig. 2. Key ^{1}H–^{1}H COSY (▬) and selected HMBC correlations (H → C) of **1** (**a**) and **2** (**b**).