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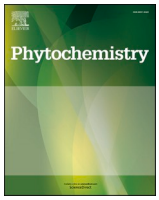

Polyacetylenes from the roots of *Cirsium japonicum* var. *ussuriense*

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ABSTRACT

Eight previously undescribed polyacetylenes, cirussurynes A-H, were isolated from the methanolic extract of the roots of *Cirsium japonicum* var. *ussuriense*. Their structures were elucidated by interpretation of extensive 1D and 2D NMR spectroscopy and HRESIMS spectrometry data. The configuration of triols in cirussurynes A, B, and E-G was deduced by the *J*-value based configuration analysis together with specific rotation values. All compounds were evaluated for their inhibitory effects on nitric oxide production against LPS-induced RAW 264.7 macrophages, and exhibited IC_{50} values ranging from 5.5 to 68.7 μM.

1. Introduction

A perennial herbaceous plant that belongs to the family Asteraceae, *Cirsium japonicum* var. *ussuriense* (Regel) Kitam. ex Ohwi (synonym *Cirsium japonicum* var. *maackii* (Maxim.) Matsum.), is widely distributed in Korea, mainland China, and Japan. The roots of this plant exhibit a variety of biological activities, including anti-inflammatory, analgesic, and antipyretic properties (Kim, 1989; Bae, 2000; Park et al., 2004). Despite the ethnopharmacological significance of *C. japonicum* var. *ussuriense*, its chemical composition has rarely been investigated. Previous phytochemical investigations have revealed the presence of flavonoids and polyacetylenes in this plant. (Jeong et al., 2008; Kang et al., 2011; Thao et al., 2011; Shim et al., 2012; Park et al., 2017; Han et al., 2018).

Macrophages are known as the major cells involved in the inflammatory response by regulating various inflammatory mediators, such as pro-inflammatory cytokines, nitric oxide (NO), and tumor necrosis factor-α. Among these mediators, NO is a highly reactive substance, which is synthesized from L-arginine by NO synthase (NOS). NOS is classified as constitutive NOS (cNOS) and inducible NOS (iNOS). In particular, the expression of iNOS in various cells occurs upon stimulation by inflammatory cytokines and leads to the production of a large amount of NO (Cinelli et al., 2020). Therefore, the discovery of NO production inhibitors is expected to contribute to the treatment of inflammatory diseases (Kumar et al., 2017).

In a continuing search for the discovery of new anti-inflammatory constituents in traditional Korean medicinal plants, we found that *n*-hexane and CH_2Cl_2-soluble fractions from the MeOH extract of the roots of *C. japonicum* var. *ussuriense* possess inhibitory effects against LPS-induced NO production. Bioassay-guided separation of the *n*-hexane and CH_2Cl_2-soluble fractions led to the isolation of eight previously undescribed polyacetylenes (Fig. 1). Herein, details of the isolation and structure determination of the new compounds (**1**–**8**) as well as their inhibitory effects against NO production in LPS-induced RAW 264.7 macrophages are described.

2. Results and discussion

2.1. Structure determination of the previously undescribed polyacetylenes

Compound **1** was obtained as a brown syrup. Its molecular formula was established as $C_{17}H_{24}O_3$ based on the ^{13}C NMR and HRESIMS data (*m/z* 299.1617 $[M + Na]^+$, calcd 299.1618), indicating six degrees of unsaturation. The IR spectrum exhibited the characteristic absorptions of hydroxy group (3370 cm^{-1}), conjugated acetylene (2296 cm^{-1}), and terminal vinylic (1641, 912 cm^{-1}) functionalities. The UV absorptions at 213, 266, and 283 nm suggested the presence of a polyacetylene moiety (Tsukamoto et al., 1997). The ^{13}C NMR and HSQC spectra of compound **1** showed 17 carbon signals, comprising a methyl group, five methylene carbons, three oxymethine carbons, two olefinic carbons at δ_C 144.0

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(C-16) and 108.6 (C-15), a terminal vinylic group at δ_C 139.0 (C-2) and 114.3 (C-1), and four non-protonated alkyne carbons at δ_C 80.0 (C-11), 77.2 (C-13), 76.5 (C-14), and 72.0 (C-12) (Table 1). The ^{1}H NMR spectrum of compound **1** revealed the presence of a *cis*-olefinic group at δ_H 6.16 (1H, dq, J = 10.8, 7.0 Hz, H-16) and 5.51 (1H, d, J = 10.8 Hz, H-15) and three oxygenated methine protons at δ_H 4.65 (1H, d, J = 3.8 Hz, H-10), 4.05 (1H, m, H-8), and 3.56 (1H, dd, J = 3.5, 2.5 Hz, H-9) (Table 1). The HMBC correlations of H-8 (δ_H 4.05)/C-6 (δ_C 25.3), C-7 (δ_C 33.7), C-9 (δ_C 74.3), C-10 (δ_C 66.3), H-9 (δ_H 3.56)/C-11 (δ_C 80.0), and H-10 (δ_H 4.65)/C-8 (δ_C 71.5), C-9 (δ_C 74.3), C-11 (δ_C 80.0), C-12 (δ_C 72.0) suggested the presence of triol groups at C-8, C-9, and C-10 (Fig. 2). This was further confirmed by the COSY correlations of H-8, H-9, and H-10 (Fig. 2). In addition, the HMBC correlations of H-15/C-13, C-14, C-16, C-17; and H-16/C-14, C-15, C-17 suggested the presence of a diyne-ene unit between C-11 and C-16 (Fig. 2). The relative configuration of compound **1** was assigned by Murata's method of *J*-value based configuration analysis (Matsumori et al., 1999). The most important part of this method is obtaining the precise values of the heteronuclear coupling constant between proton and carbon, therefore, the HETLOC, and HSQC-HECADE experiments were conducted to get the accurate measurement of coupling constants (Kozmiński and Nanz, 2000; Menche, 2008). The measured values of ^3J (H-8, H-9), ^3J (H-8, C-10), ^3J (C-7, H-9), ^2J (C-8, H-9), and ^2J (C-9, H-8) matched well with the A-1 form among six staggered rotamers in Murata's method (Matsumori et al., 1999). Additionally, the set of *J*-values between C-9 and C-10 matched well with the B-2 form (Table 2). To clarify the absolute configuration of **1**, ECD calculation was performed using the same method used in a previous study (Lai et al., 2014); however, the calculated data were insufficient to determine the absolute configuration (Wavefunction Inc., 2013). Therefore, the absolute configuration of C-8/C-9/C-10 can be assigned as 8*R*/9*S*/10*R* or 8*S*/9*R*/10*S*, and these two forms revealed the opposite specific rotation values in previous reports (Takaishi et al., 1990, 1991; Baek et al., 1995). Consequently, the absolute configuration of triol was assigned as 8*S*/9*R*/10*S* from the observed positive specific rotation value ($[\alpha]_D^{25}$ = +4, *c* 0.1, in MeOH). From these, the structure of compound **1** was determined as (+)-(8*S*,9*R*,10*S*,*Z*)-heptadeca-1, 15-diene-11,13-diyne-8,9,10-triol, and named as cirussuryne A.

Compound **2** was obtained as a brown syrup. Its molecular formula was established as $C_{17}H_{28}O_3$ based on the ^{13}C NMR and HRESIMS data (*m/z* 303.1931 $[M + Na]^+$, calcd 303.1931), indicating four degrees of unsaturation. The ^{1}H and ^{13}C NMR data were similar to those of compound **1**, except for the large coupling constant of the *trans*-olefinic group [δ_H 6.15 (1H, dt, J = 15.9, 7.2 Hz, H-14), 5.47 (1H, d, J = 15.9 Hz, H-13)], and two non-protonated alkyne carbons [δ_C 86.4 (C-12) and 84.8 (C-11)] (Table 1). The COSY correlations of the oxymethine protons revealed the same spin system of 8, 9, 10-triol (Fig. 2). The HMBC correlations of H-9 (δ_H 3.52)/C-11 (δ_C 84.8), H-10 (δ_H 4.63)/C-11 (δ_C 84.8), C-12 (δ_C 86.4), H-13 (δ_H 5.47)/C-11 (δ_C 84.8), C-12 (δ_C 86.4), C-14 (δ_C 146.4), C-15 (δ_C 35.1), and H-14 (δ_H 6.15)/C-12 (δ_C 86.4), C-15 (δ_C 35.1), C-16 (δ_C 21.8) suggested that the yne-ene (alkyne C≡C triple bond and alkene C=C double bond) unit is placed between C-11 and C-14 (Fig. 2). The *J*-value based configuration analysis was applied to compound **2** as well, and the same sets of $^3J_{H,H}$, $^3J_{C,H}$, $^2J_{C,H}$ matched well with the A-1 form between C-8 and C-9, and the B-2 form between C-9

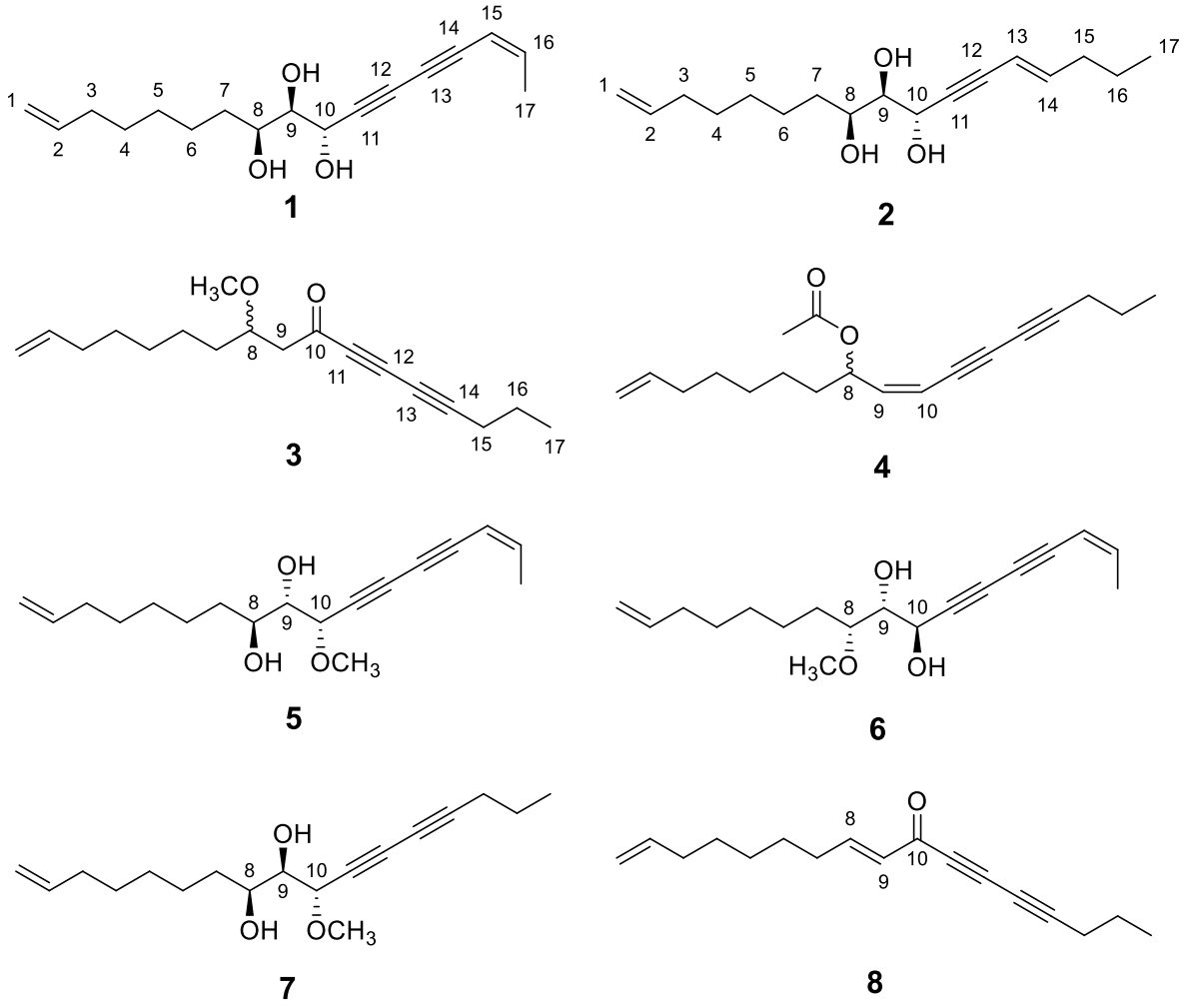


Fig. 1. Structures of compounds **1**–**8**.

Table 1
^{1}H and ^{13}C NMR spectroscopic data for **1**–**4** in $CDCl_3$[a].

Carbon No.	1[b]		2[c]		3[d]		4[e]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1a	4.97 dd (17.1, 1.8)	114.3	4.97 dd (17.2, 2.0)	108.5	4.98 dd (17.1, 1.8)	114.3	4.98 dd (17.2, 1.7)	114.3
1b	4.92 dd (10.2, 1.8)		4.92 dd (10.2, 2.0)		4.93 dd (10.2, 1.9)		4.93 dd (10.2, 1.7)	
2	5.76 ddt (17.1, 10.2, 6.8)	139.0	5.77 ddt (17.1, 10.2, 6.8)	139.0	5.76 ddt (17.1, 10.2, 6.8)	139.0	5.78 ddt (17.1, 10.2, 6.8)	139.0
3	2.03 q (7.0)	33.7	2.03 q (6.9)	33.7	2.03 q (6.9)	33.7	2.03 q (6.9)	33.7
4	1.3–1.7 m	28.9	1.3–1.7 m	33.7	1.2–1.7 m	28.8	1.2–1.6 m	28.7
5	1.3–1.7 m	28.8	1.3–1.7 m	29.0	1.2–1.7 m	29.1	1.2–1.6 m	28.8
6	1.3–1.7 m	25.3	1.3–1.7 m	28.8	1.2–1.7 m	24.9	1.2–1.6 m	24.6
7	1.3–1.7 m	33.7	1.3–1.7 m	25.3	1.2–1.7 m	33.8	1.2–1.6 m	34.1
8	4.05 m	71.5	4.03 m	71.2	3.75 m	77.0	5.63 q (7.5)	72.8
9	3.56 dd (3.5, 2.5)	74.3	3.52 dd (3.9, 2.4)	74.5	2.61 dd (15,7, 7.5) 2.79 dd (15,7, 7.5)	50.1	5.90 dd (11.1, 7.5)	143.9
10	4.65 d (3.8)	66.3	4.63 d (3.8)	66.1		185.4	5.60 d (11.1)	110.3
11		80.0		84.8		72.4		70.3
12		72.0		86.4		76.7		80.5
13		77.2	5.47 d (15.9)	114.3		64.0		65.1
14		76.5	6.15 dt (15.9, 7.2)	146.4		91.0		86.1
15	5.51 d (10.8)	108.6	2.09 q (7.1)	35.1	2.35 t (6.9)	21.6	2.30 t (7.0)	21.6
16	6.16 dq (10.8, 7.0)	144.0	1.36 m	21.8	1.60 m	21.4	1.60 m	21.7
17	1.92 dd (6.9)	16.6	0.89 t (7.4)	13.6	1.01 t (7.4)	13.4	0.99 t (7.4)	13.5
OCH_3					3.34 s	57.1		
Acetyl CH_3							2.05 s	21.2
Acetyl CO								170.2

[a] The assignments were supported by HSQC and HMBC experiments.
[b] ^{1}H-NMR, 400 MHz; ^{13}C-NMR, 100 MHz.
[c] ^{1}H-NMR, 400 MHz; ^{13}C-NMR, 100 MHz.
[d] ^{1}H-NMR, 900 MHz; ^{13}C-NMR, 225 MHz.
[e] ^{1}H-NMR, 800 MHz; ^{13}C-NMR, 200 MHz.

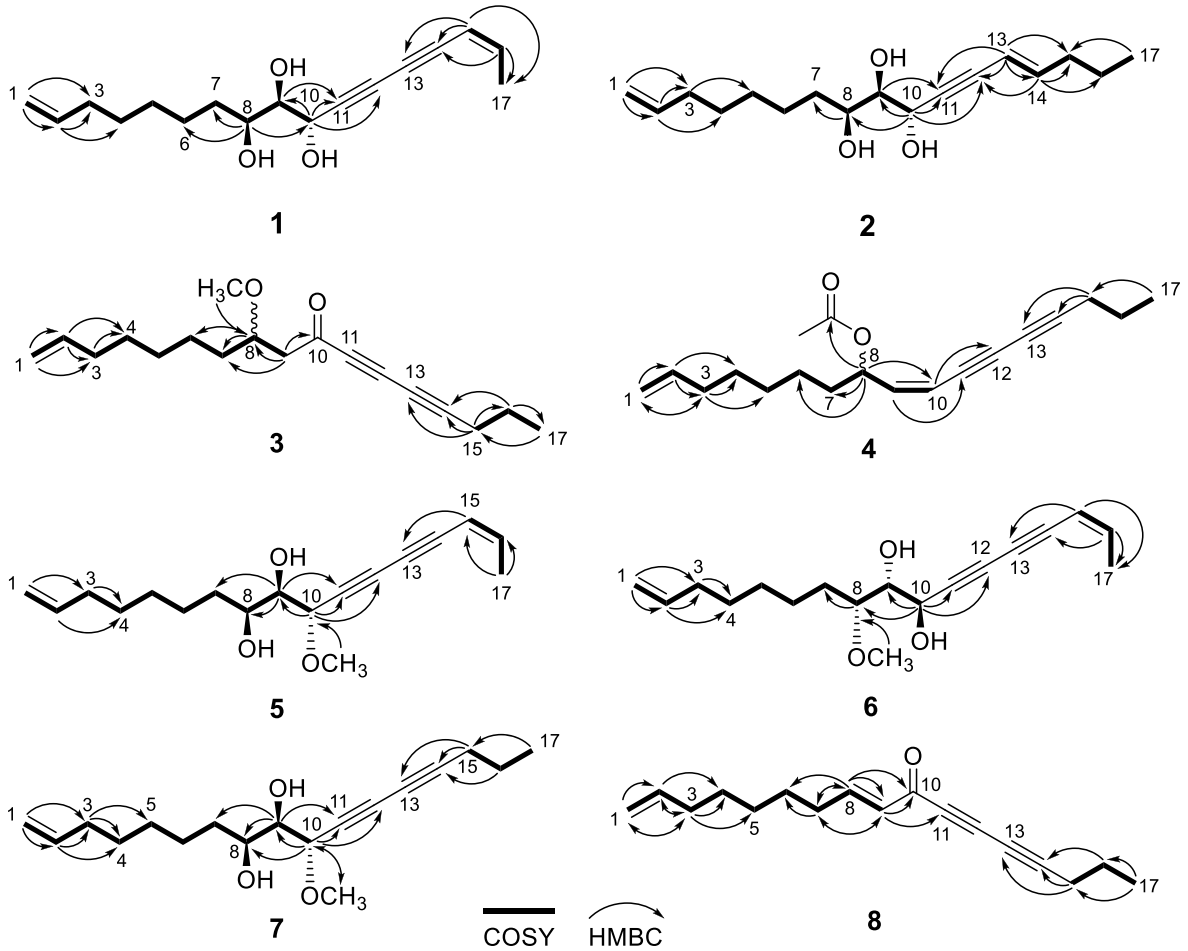
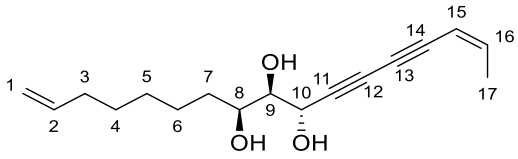
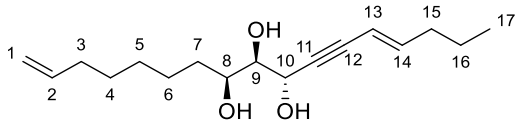


Fig. 2. Key HMBC and COSY correlations of compounds **1**–**8**.

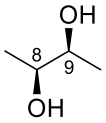
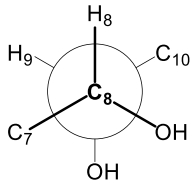
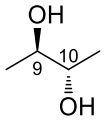
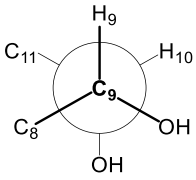
Table 2
J-value based configuration analysis of the C8–C9 and C9–C10 segments of compounds **1** and **2**.



1



2

segment	$^{2,3}J_{HX}$	nuclei	value (Hz)[a]		magnitude estimation	rotamer (configuration)
			1	2		
 C8-C9	$^{3}J_{HH}$	H8–H9	2.5	2.4	small	 A-1
	$^{3}J_{HC}$	H8–C10	2.2	1.8	small	
		C7–H9	2.5	2.1	small	
	$^{2}J_{HC}$	C8–H9	0.8	0.3	small	
		C9–H8	0.8	1.1	small	
 C9-C10	$^{3}J_{HH}$	H9–H10	3.8	3.8	small	 B-2
	$^{3}J_{HC}$	H9–C11[b]	–	–	–	
		C8–H10	3.8	4.5	large	
	$^{2}J_{HC}$	C9–H10	4.8	5.0	large	
		C10–H9	1.0	1.0	small	

[a] The values of the heteronuclear coupling constant were obtained by HETLOC and HSQC-HECADE experiments.
[b] $^{3}J_{(H\text{-}9,C\text{-}11)}$ could not be obtained.

and C-10, implying the configuration of 8*R*/9*S*/10*R* or 8*S*/9*R*/10*S* (Table 2). As in compound **1**, the absolute configuration of triol in compound **2** was determined as 8*S*/9*R*/10*S* from the observed positive specific rotation value ($[\alpha]_D^{25} = +3$, *c* 0.1, in MeOH). Thus, the structure of compound **2** was determined as (+)-(8*S*,9*R*,10*S*,*E*)-heptadeca-1,13-diene-11-yne-8,9,10-triol, and named as cirussuryne B.

Compounds **3** was obtained as a brown syrup. Its molecular formula was established as $C_{18}H_{26}O_2$ based on the ^{13}C NMR and HRESIMS data (*m/z* 297.1828 $[M + Na]^+$, calcd 297.1825), indicating six degrees of unsaturation. The IR spectrum exhibited the characteristic absorptions of carbonyl (1745 cm^{-1}), conjugated acetylene (2298 cm^{-1}), and terminal vinylic (1643, 904 cm^{-1}) functionalities. The ^{13}C NMR and HSQC spectra of **3** showed 18 carbon signals assigned to a methyl, eight methylenes, an oxymethine, a terminal vinylic group [δ_C 139.0 (C-2) and 114.3 (C-1)], four non-protonated alkynes [δ_C 91.0 (C-14), 76.7 (C-12), 72.4 (C-11), and 64.0 (C-13)], a carbonyl carbon [δ_C 185.4 (C-10)], and a methoxy carbon [δ_C 57.1 (8-OCH$_3$)] (Table 1). The ^1H NMR spectrum of **3** revealed the presence of an oxygenated methine proton at δ_H 3.75 (H-8) and a downfield-shifted methylene [δ_H 2.79 (H-9a) and 2.61 (H-9b)] (Table 1). The HMBC correlations of H-8 (δ_H 3.75)/C-6 (δ_C 24.9), C-7 (δ_C 33.8), C-9 (δ_C 50.1), C-10 (δ_C 185.4), 8-OCH$_3$ (δ_C 57.1), and H-9 (δ_H 2.61 and 2.79)/C-7 (δ_H 33.8), C-8 (δ_C 77.0), C-10 (δ_C 185.4), C-11 (δ_C 72.4) confirmed that a methoxy group is connected to C-8 and a carbonyl group is located at C-10 (Fig. 2). Based on the COSY correlations of H-1a (δ_H 4.98) and H-1b (δ_H 4.93)/H-2 (δ_H 5.76), H-2 (δ_H 5.76)/H-3 (δ_H 2.03), H-8 (δ_H 3.75)/H-9 (δ_H 2.61 and 2.79), H-15 (δ_H 2.35)/H-16 (δ_H 1.60), and H-16 (δ_H 1.60)/H-17 (δ_H 1.01), the carbonyl group was verified to be located at C-10 and two alkyne groups were assigned to C-11 through C-14 (Fig. 2). Thus, the structure of **3** was determined as (−)-heptadeca-1-ene-11,13-diyne-8-methoxy-10-one, and named as cirussuryne C.

Compound **4** was isolated as a brown syrup. Its molecular formula was established as $C_{19}H_{26}O_2$ from the ^{13}C NMR and HRESIMS data (*m/z* 309.1823 $[M + Na]^+$, calcd 309.1825), indicating seven degrees of unsaturation. The 1D NMR data of **4** were similar to those of **3**, except for the absence of a carbonyl and methoxy group, and the presence of olefinic [δ_C 143.9 and 110.3; δ_H 5.90 (1H, dd, $J = 11.1$ and 7.5 Hz, H-9) and 5.60 (1H, d, $J = 11.1$ Hz, H-10)] and acetyl group [δ_C 170.2 (C=O) and 21.2 (O=C–CH$_3$); δ_H 2.05 (3H, s, O=C–CH$_3$)]. The HMBC correlations of H-8 (δ_H 5.63)/C-6 (δ_C 24.6), C-7 (δ_C 34.1), C-9 (δ_C 143.9), C-10 (δ_C 110.3), and 8-acetyl C=O (δ_C 170.2) confirmed that the acetyl group is located at C-8. The COSY correlations of H-1a (δ_H 4.98) and H-1b (δ_H 4.93)/H-2 (δ_H 5.78), H-2 (δ_H 5.78)/H-3 (δ_H 2.03), H-7 (δ_H 1.2,1.6)/H-8 (δ_H 5.63), H-8 (δ_H 5.63)/H-9 (δ_H 5.90), H-9 (δ_H 5.90)/H-10 (δ_H 5.60), H-15 (δ_H 2.30)/H-16 (δ_H 1.60), and H-16 (δ_H 1.60)/H-17 (δ_H 0.99), and the HMBC correlations of H-9 (δ_H 5.90)/C-7 (δ_C 34.1), C-10 (δ_C 110.3), C-11 (δ_C 70.3) and H-10 (δ_H 5.60)/C-8 (δ_C 72.8), C-9 (δ_C 143.9), C-12 (δ_C 80.5) indicated that the olefinic bond was located at C-9 and C-10 (Fig. 2). Additionally, the olefinic group was assigned as the *Z* configuration based on the vicinal coupling constant ($J = 11.1$ Hz). Thus, the structure of **4** was determined as (+)-8-acetyloxy-(*Z*)-heptadeca-1,9-diene-11,13-diyne, and named as cirussuryne D.

Compounds **5** was obtained as a brown syrup. Its molecular formula was established as $C_{18}H_{26}O_3$ based on the ^{13}C NMR and HRESIMS data (*m/z* 313.1772 $[M + Na]^+$, calcd 313.1774). Its molecular weight was 14 Da higher than that of **1**, indicating the presence of a methoxy group rather than that of a hydroxy group. The 1D NMR data of compound **5** was also similar to that of **1**, except for the presence of a methoxy group at C-10 [δ_H 3.49 (3H, s); δ_C 57.2] (Table 3). Furthermore, the HMBC correlations of H-9 (δ_H 3.68)/C-7 (δ_C 32.5), C-8 (δ_C 72.1), C-11 (δ_C 78.4), H-10 (δ_H 4.24)/C-9 (δ_C 75.6), C-11 (δ_C 78.4), C-12 (δ_C 72.7), 10-OCH$_3$ (δ_C 57.2), and 10-OCH$_3$ (δ_H 3.49)/C-10 (δ_C 72.6) confirmed that the methoxy group is attached to C-10 (Fig. 2). The configuration of triol was determined by *J*-value based configuration analysis, as in compounds **1** and **2**. In compound **5**, the coupling constants of ^3J (H-8, H-9), ^3J (H-8, C-10), ^3J (C-7, H-9), ^2J (C-8, H-9), and ^2J (C-9, H-8) indicated

Table 3
^{1}H and ^{13}C NMR spectroscopic data for **5**–**8** in $CDCl_3$[a].

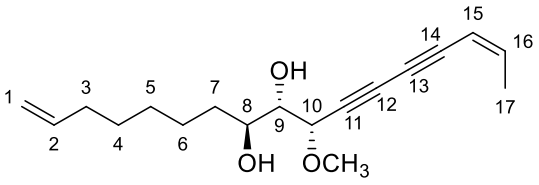
Carbon No.	5[b]		6[b]		7[c]		8[c]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1a	4.98 dd (17.1, 2.0)	114.3	4.99 dd (17.1, 1.8)	114.4	4.98 dd (17.1, 1.6)	114.2	4.99 dd (17.1, 1.7)	114.5
1b	4.93 dd (10.2, 2.0)		4.94 dd (10.2, 1.8)		4.92 dd (10.2, 1.6)		4.94 dd (10.2, 1.7)	
2	5.79 ddt (17.0, 10.3, 6.8)	139.1	5.79 ddt (17.0, 10.2, 6.7)	138.9	5.78 ddt (17.1, 10.1, 6.8)	139.1	5.77 ddt (17.1, 10.2, 6.6)	138.8
3	2.04 q (7.0)	33.7	2.05 q (7.0)	33.7	2.04 q (6.9)	33.8	2.04 q (6.8)	33.6
4	1.2–1.7 m	28.8	1.2–1.7 m	28.8	1.3–1.6 m	28.8	1.2–1.6 m	28.6
5	1.2–1.7 m	29.0	1.2–1.7 m	29.3	1.3–1.6 m	29.0	1.2–1.6 m	28.6
6	1.2–1.7 m	25.6	1.2–1.7 m	24.9	1.3–1.6 m	25.6	1.2–1.6 m	27.7
7	1.2–1.7 m	32.5	1.2–1.7 m	29.2	1.3–1.6 m	32.5	2.28 q (7.0)	32.7
8	3.79 m	72.1	3.65 m	81.5	3.78 m	72.1	7.14 dt (15.8, 7.0)	155.2
9	3.68 t (5.3)	75.6	3.62 m (7.8, 4.4)	73.0	3.64 dd (5.1)	72.5	6.15 d (15.8)	132.3
10	4.24 d (5.5)	72.6	4.56 q (4.5)	65.7	4.15 d (5.6)	75.6		177.6
11		78.4		80.7		71.2		70.9
12		72.7		71.3		73.1		76.5
13		77.2		77.2		64.4		64.1
14		76.0		76.0		81.9		89.6
15	5.52 d (10.9)	108.6	5.52 d (10.8)	108.7	2.26 t (7.0)	21.2	2.35 t (7.0)	21.6
16	6.19 dq (10.9, 6.9)	143.9	6.17 dq (10.8, 7.0)	143.7	1.56 m	21.6	1.59 m	21.4
17	1.93 d (6.9)	16.9	1.92 d (7.0)	16.5	0.99 t (7.4)	13.5	1.01 t (7.5)	13.5
OCH_3	3.49 s	57.2	3.44 s	57.4	3.47 s	57.0		

[a] The assignments were supported by HSQC and HMBC experiments.
[b] ^{1}H-NMR, 900 MHz; ^{13}C-NMR, 225 MHz.
[c] ^{1}H-NMR, 700 MHz; ^{13}C-NMR, 175 MHz.

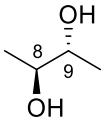
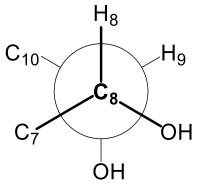
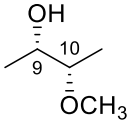
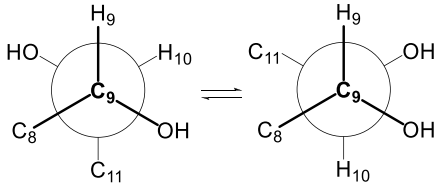
that the conformation of C-8 and C-9 matched well with the B-2 form, while ^{3}J (H-9, H-10), and ^{3}J (C-8, H-10) showed medium values, suggesting the presence of A-2 and A-3 transition conformers between C-9 and C-10. This analysis indicated that the configuration of triol can be 8*R*/9*R*/10*R* or 8*S*/9*S*/10*S* (Table 4), and it was assigned as 8*S*/9*S*/10*S* by positive specific rotation value ($[\alpha]_D^{25} = +6$, *c* 0.1, in MeOH). Thus, the structure of compound **5** was determined as (+)-(8*S*,9*S*,10*S*,*Z*)-10-methoxy-heptadeca-1,15-diene-11,13-diyne-8,9-diol, and named as cirussuryne E.

Compounds **6** was obtained as a brown syrup. Its molecular formula was established as $C_{18}H_{26}O_3$, the same as that of **5**, based on the ^{13}C NMR and HRESIMS data (*m/z* 313.1773 $[M + Na]^+$, calcd 313.1774). The ^{1}H, ^{13}C NMR, COSY, and HSQC spectra of **6** were similar to that of **5**, except for the position of the methoxy group (Table 3). The HMBC correlations of H-8 (δ_H 3.65)/C-7 (δ_C 29.2), C-10 (δ_C 65.7), 8-O$\underline{C}H_3$ (δ_C 57.4), 8-OC$\underline{H}_3$ (δ_H 3.44)/C-8 (δ_C 81.5), and H-10 (δ_H 4.56)/C-8 (δ_C 81.5),

Table 4
J-value based configuration analysis of the C8–C9 and C9–C10 segments of compound **5**.



5

segment	$^{2,3}J_{HX}$	nuclei	value (Hz)[a]	magnitude estimation	rotamer (configuration)
	$^{3}J_{HH}$	H8–H9	3.8	small	
	$^{3}J_{HC}$	H8–C10	3.6	small	
		C7–H9	10.9	large	
	$^{2}J_{HC}$	C8–H9	5.0	large	
		C9–H8	2.5	small	
C8-C9					B-2
	$^{3}J_{HH}$	H9–H10	5.5	medium	
	$^{3}J_{HC}$	H9–C11[b]	–	–	
		C8–H10	2.6	medium	
	$^{2}J_{HC}$	C9–H10	4.7	large	
		C10–H9	5.6	large	
C9-C10					A-2 ↔ A-3

[a] The values of the heteronuclear coupling constant were obtained by HETLOC and HSQC-HECADE experiments.
[b] $^{3}J_{(H\text{-}9,C\text{-}11)}$ could not be obtained.

C-9 (δ_C 73.0), C-11 (δ_C 80.7), C-12 (δ_C 71.3) confirmed that the methoxy group is connected to C-8 (Fig. 2). The absolute configuration of compound **6** was determined in the same manner as that of compound **5**. The *J*-value based configuration analysis obtained by the HETLOC and HSQC-HECADE experiments indicated A-1 conformation for C-8 and C-9 and the mixed conformation of B-2 and B-3 for C-9 and C-10, implying the configuration of either 8*R*/9*R*/10*R* or 8*S*/9*S*/10*S* (Table 5). The absolute configuration of **6** was determined as 8*R*/9*R*/10*R* by the observed negative specific rotation value ($[\alpha]_D^{25} = -12$, *c* 0.1, in MeOH). Thus, the structure of compound **6** was determined as (−)-(8*R*,9*R*,10*R*,*Z*)-8-methoxy-heptadeca-1,15-diene-11,13-diyne-9,10-diol, and named as cirussuryne F.

Compound **7** was obtained as a brown syrup. Its molecular formula was established as $C_{18}H_{28}O_3$ based on the ^{13}C NMR and HRESIMS data (*m/z* 315.1928 $[M + Na]^+$, calcd 315.1931). The ^1H and ^{13}C NMR data of compound **7** were similar to those of **5**, except for the presence of a methylene group [δ_H 2.26 (2H, t, *J* = 7.0 Hz, H-15) and 1.56 (2H, m, H-16); δ_C 21.2 and 21.6] instead of a *cis*-olefinic group in **5** (Table 3). The *J*-value based configuration analysis of **7** indicated A-1 conformation for C-8 and C-9 and the mixed conformation of B-2 and B-3 for C-9 and C-10, suggesting the configuration of either 8*R*/9*S*/10*R* or 8*S*/9*R*/10*S* (Table 5). The positive specific rotation value of **7** ($[\alpha]_D^{25} = +6$, *c* 0.1, in MeOH) confirmed the absolute configuration of 8*S*/9*R*/10*S*. Thus, the structure of **7** was determined as (+)-(8*S*,9*R*,10*S*)-10-methoxy-heptadeca-1-ene-11,13-diyne-8,9-diol, and named as cirussuryne G.

Compound **8** was obtained as a brown syrup. Its molecular formula was established as $C_{17}H_{22}O$ based on the ^{13}C NMR and HRESIMS data (*m/z* 265.1562 $[M + Na]^+$, calcd 265.1563), indicating the presence of 7 degrees of unsaturation. The ^1H and ^{13}C NMR data of compound **8** were similar to those of **3**, except for the presence of a *trans*-olefinic group [δ_H 7.14 (1H, dt, *J* = 15.8, 7.0 Hz, H-8), 6.15 (1H, d, *J* = 15.8 Hz, H-9); δ_C 155.2 and 132.3] instead of a methoxy group in **3** (Table 3). The HMBC correlations of H-7 (δ_H 2.28)/C-6 (δ_C 27.7), C-8 (δ_C 155.2), C-9 (δ_C 132.3), H-8 (δ_H 7.14)/C-6 (δ_C 27.7), C-7 (δ_C 32.7), C-9 (δ_C 132.3), C-10 (δ_C 177.6), and H-9 (δ_H 6.15)/C-7 (δ_C 32.7), C-10 (δ_C 177.6), C-11 (δ_C 70.9) confirmed that a carbonyl group is located at C-10 and the *trans*-olefinic proton was positioned between C-8 and C-9 (Fig. 2). Thus, the structure of **8** was determined as (*E*)-heptadeca-1,8-diene-11,13-diyne-10-one, and named as cirussuryne H.

2.2. Inhibitory effects on nitric oxide production

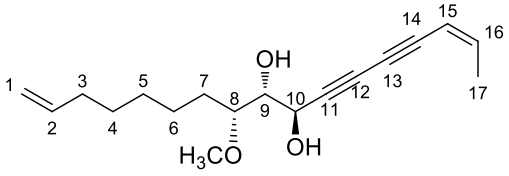
All compounds were evaluated for their inhibitory effects on LPS-induced NO production in RAW 264.7 macrophages using the Griess reaction. Aminoguanidine was used as a positive control (IC_{50} = 20.6 μM) (Table 6). Cell viability was evaluated by an MTT assay, indicating

Table 6
Inhibitory effects of compounds **1**–**8** on LPS-induced NO production in RAW 264.7 cells[a].

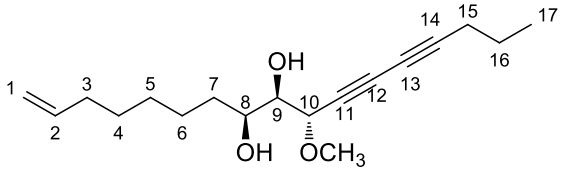
Compound	IC_{50} (μM)[a]	Compound	IC_{50} (μM)[a]
1	13.4	**5**	7.6
2	44.0	**6**	9.7
3	5.5	**7**	68.7
4	17.8	**8**	6.1

[a] The results are expressed as mean IC_{50} values from triplicate experiments. Aminoguanidine was used as the positive control (IC_{50} = 20.6 μM).

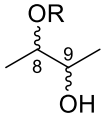
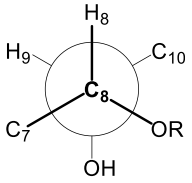
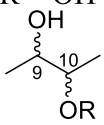
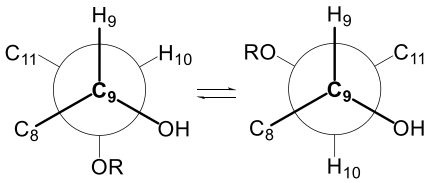
Table 5
J-value based configuration analysis of the C8–C9 and C9–C10 segments of compounds **6** and **7**.



6



7

segment	$^{2,3}J_{HX}$	nuclei	value (Hz)[a]		magnitude estimation	rotamer (configuration)
			6	7		
	$^3J_{HH}$	H8–H9	3.6	1.0	small	
	$^3J_{HC}$	H8–C10	1.1	3.5	small	
		C7–H9	1.2	2.3	small	
	$^2J_{HC}$	C8–H9	1.4	1.7	small	
		C9–H8	1.3	2.1	small	
C8-C9						A-1
6 R = OCH_3						
7 R = OH						
	$^3J_{HH}$	H9–H10	4.4	5.1	medium	
	$^3J_{HC}$	H9–C11[b]	–	–	–	
		C8–H10	5.5	4.2	medium	
	$^2J_{HC}$	C9–H10	5.0	4.7	medium	
		C10–H9	1.9	2.7	large	
C9-C10						B-2 ↔ B-3
6 R = OH						
7 R = OCH_3						

[a] The values of the heteronuclear coupling constant were obtained by HETLOC and HSQC-HECADE experiments.
[b] $^3J_{(H\text{-}9,C\text{-}11)}$ could not be obtained.

that all test compounds showed no cytotoxicity to RAW 264.7 cells at their effective concentration range (data not shown). All polyacetylenes (**1**–**8**) suppressed NO production in LPS-induced RAW 264.7 cells with IC_{50} values ranging from 5.5 to 68.7 μM. Compounds **3** and **8** exhibited potent inhibitory effects, with IC_{50} values of 5.5 and 6.1 μM, respectively, suggesting that the presence of a carbonyl group adjacent to the diyne moiety seems to be crucial for NO inhibition. Compounds **1**, **4**, **5**, and **6**, which contain diene and diyne moieties, showed significant inhibitory effects, with IC_{50} values of 13.4, 17.8, 7.6, and 9.7 μM, respectively. However, compounds **2** and **7** showed weak inhibitory effects with IC_{50} values of 44.0 and 68.7 μM, respectively. These results implied that the conjugated diyne-ene moiety had a significant role in the NO inhibitory activity. Polyacetylenes, a class of secondary metabolites characterized by the presence of one or more carbon-carbon triple bonds in their structures, are commonly discovered in the members of Apiaceae, Asteraceae, Araliaceae, and Campanulaceae families (Chen et al., 2015; Negri, 2015). Polyacetylenes are well known to exhibit cytotoxic, antimicrobial, antifungal, and anti-inflammatory activities (Christensen, 2020). Some polyacetylenes from the Asteraceae family have been shown to inhibit NO production (Yao and Yang, 2014; Jeong et al., 2019; Li et al., 2021). Recently, 1-heptadecene-11,13-diyne-8,9, 10-triol from the root of *C. japonicum* var. *ussuriense* inhibited LPS-induced NO, TNF-α, and IL-1β production in RAW 264.7 cells by inhibiting NF-κB activation (Kang et al., 2011).

3. Conclusions

In our current study, eight previously undescribed polyacetylenes (**1**–**8**) were obtained from the roots of *C. japonicum* var. *ussuriense*, and their chemical structures were elucidated using 1D and 2D NMR spectroscopic and the *J*-value based configuration analyses. All isolates showed inhibitory effects on LPS-induced NO production in RAW264.7 cells. The present study provides insights into the phytochemical composition of the non-polar *n*-hexane-soluble fraction of *C. japonicum* var. *ussuriense*, which is a rich source of polyacetylenes. Accordingly, polyacetylenes from *C. japonicum* var. *ussuriense* might be worthy of further investigation for their potential as anti-inflammatory agents.

4. Experimental

4.1. General experimental procedures

Optical rotations were determined using a JASCO DIP-1000 polarimeter (Jasco Co., Tokyo, Japan). UV spectra were recorded on a JASCO UV-550 spectrophotometer, and IR spectra were measured on a JASCO FT-IR 4100 spectrometer (Jasco Co., Tokyo, Japan, respectively). NMR spectra were recorded on Bruker AVANCE 400, 500, 700, 800, and 900 MHz spectrometers. HRESIMS spectra were measured on maXis 4G mass spectrometer. Column chromatography was performed using silica gel (70–230 and 230–400 mesh, Merck, Darmstadt, Germany) and Lichroprep RP-18 (40–63 μm, Merck, Darmstadt, Germany). MPLC was performed on a Biotage Isolera Prime chromatography system. Preparative HPLC was performed using a system equipped with a Waters 515 pump and 2996 photodiode-array detector and a YMC J'sphere ODS-H80 column (4 μm, 150 × 20 mm, i.d., flow rate 6 mL/min). TLC was performed using precoated silica gel 60 F_{254} (0.25 mm, Merck, Darmstadt, Germany) plates, and the spots were detected by a 10% vanillin-H_2SO_4 in water spray reagent.

4.2. Plant material

The dried roots of *Cirsium japonicum* var. *ussuriense* (Regel) Kitam. ex Ohwi (Asteraceae) (3.0 kg) were purchased from Kyungdong herbal market, Seoul, Korea, in May 2018. A voucher specimen (CBNU-2018-05-CJ) was authenticated by B. Y. Hwang and deposited at the Herbarium of the College of Pharmacy, Chungbuk National University, Republic of Korea.

4.3. Extraction and isolation

The dried and powdered roots of *C. japonicum* var. *ussuriense* (3.0 kg) were extracted with MeOH (3 x 18 L) at room temperature for 3 days and then filtered. The extract was evaporated under reduced pressure, and the residue (155.0 g) was suspended in water and successively partitioned with *n*-hexane (2 x 2 L), CH_2Cl_2 (2 x 2 L), EtOAc (2 x 2 L), and *n*-BuOH (2 x 2L). The *n*-hexane-soluble fraction (74.0 g) was chromatographed on a silica gel column (230–400 mesh) and eluted with an *n*-hexane-EtOAc gradient system (200:1–1:1) to afford 11 fractions (CJH1 – CJH11). Fraction CJH4 (588.2 mg) was separated by MPLC using a Lichroprep RP-18 column and eluted with a MeOH–H_2O gradient system (70:30–100:0) to afford 8 sub-fractions (CJH4-1 – CJH4-8). Fraction CJH4-5 (57.7 mg) was further purified by preparative HPLC (MeCN–H_2O, 85:15, isocratic) to obtain compound **4** (t_R = 30.0 min, 1.7 mg). Fraction CJH5 (1.8 g) was separated by MPLC using a Lichroprep RP-18 column and eluted with a MeOH–H_2O gradient system (70:30–100:0) to provide 6 sub-fractions (CJH5-1 – CJH5-6). Fraction CJH5-2 was further purified by preparative HPLC (MeCN–H_2O, 80:20, isocratic) to obtain compound **3** (t_R = 40.0 min, 1.8 mg) and compound **8** (t_R = 43.0 min, 2.3 mg). Fraction CJH6 (12.2 g) was chromatographed on a silica gel column (230–400 mesh) and eluted with an *n*-hexane-EtOAc gradient system (30:1 to 1:1) to afford 7 sub-fractions (CJH6-1 – CJH6-7). Fraction CJH6-6 was separated by RP-MPLC with a MeOH–H_2O gradient system (60:40–100:0) to afford 10 sub-fractions (CJH6-6-1 – CJH6-6-10). Fraction CJH6-6-2 was purified by preparative HPLC (MeCN–H_2O, 60:40, isocratic) to obtain compound **5** (t_R = 36.0 min, 0.6 mg) and compound **6** (t_R = 40.0 min, 2.3 mg). Fraction CJH6-6-3 was further purified by preparative HPLC (MeCN–H_2O, 60:40, isocratic) to obtain compound **7** (t_R = 40.0 min, 6.3 mg). The CH_2Cl_2-soluble fraction (23.5 g) was chromatographed on a silica gel column (230–400 mesh) and eluted with an *n*-hexane-EtOAc gradient system (30:1–1:1) to afford 9 fractions (CJC1 – CJC9). Fraction CJC8 (6.0 g) was chromatographed on a silica gel column (230–400 mesh) and eluted with a CH_2Cl_2–MeOH gradient system (100:1–1:1) to afford 6 sub-fractions (CJC8-1 – CJC8-6). Fraction CJC8-4 (3.0 g) was further purified by preparative HPLC (MeCN–H_2O, 55:45, isocratic) to obtain compound **1** (t_R = 30.0 min, 2.4 mg) and compound **2** (t_R = 32.0 min, 1.2 mg).

4.3.1. Cirussuryne A (1)

Brown syrup; $[\alpha]_D^{25}$ +4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 213, 266, 283 nm; IR ν_{max} (film) 3370, 2296, 1641, 912 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HRESIMS *m/z* 299.1617 $[M + Na]^+$ (calcd for $C_{17}H_{24}NaO_3$, 299.1618).

4.3.2. Cirussuryne B (2)

Brown syrup; $[\alpha]_D^{25}$ +3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 227, 289 nm; IR ν_{max} (film) 3370, 2298, 1643, 910 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HRESIMS *m/z* 303.1931 $[M + Na]^+$ (calcd for $C_{17}H_{28}NaO_3$, 303.1931).

4.3.3. Cirussuryne C (3)

Brown syrup; $[\alpha]_D^{25}$ -4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 211, 270 nm; IR ν_{max} (film) 2298, 1745, 1643, 904 cm^{-1}; ^1H NMR (900 MHz, $CDCl_3$) and ^{13}C NMR (225 MHz, $CDCl_3$), see Table 1; HRESIMS *m/z* 297.1828 $[M + Na]^+$ (calcd for $C_{18}H_{26}NaO_2$, 297.1825).

4.3.4. Cirussuryne D (4)

Brown syrup; $[\alpha]_D^{25}$ +2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 214, 268, 284 nm; IR ν_{max} (film) 2296, 1643, 914 cm^{-1}; ^1H NMR (800 MHz, $CDCl_3$) and ^{13}C NMR (200 MHz, $CDCl_3$), see Table 1; HRESIMS *m/z* 309.1823 $[M + Na]^+$ (calcd for $C_{19}H_{26}NaO_2$, 309.1825).

4.3.5. Cirussuryne E (5)

Brown syrup; $[\alpha]_D^{25}$ +6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 215, 268, 283 nm; IR ν_{max} (film) 3342, 2298, 1643, 896 cm^{-1}; ^1H NMR (900 MHz, $CDCl_3$) and ^{13}C NMR (225 MHz, $CDCl_3$), see Table 3; HRESIMS *m/z* 313.1772 $[M + Na]^+$ (calcd for $C_{18}H_{26}NaO_3$, 313.1774).

4.3.6. Cirussuryne F (6)

Brown syrup; $[\alpha]_D^{25}$ -12 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 215, 268, 283 nm; IR ν_{max} (film) 3388, 2300, 1641, 910 cm^{-1}; ^1H NMR (900 MHz, $CDCl_3$) and ^{13}C NMR (225 MHz, $CDCl_3$), see Table 3; HRESIMS *m/z* 313.1773 $[M + Na]^+$ (calcd for $C_{18}H_{26}NaO_3$, 313.1774).

4.3.7. Cirussuryne G (7)

Brown syrup; $[\alpha]_D^{25}$ +6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 242 nm; IR ν_{max} (film) 3415, 2298, 1641, 910 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$) and ^{13}C NMR (175 MHz, $CDCl_3$), see Table 3; HRESIMS *m/z* 315.1928 $[M + Na]^+$ (calcd for $C_{18}H_{28}NaO_3$, 315.1931).

4.3.8. Cirussuryne H (8)

Brown syrup; $[\alpha]_D^{25}$ +3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 215, 247, 276 nm; IR ν_{max} (film) 2296, 1747, 1643, 912 cm^{-1}; ^1H NMR (700 MHz, $CDCl_3$) and ^{13}C NMR (175 MHz, $CDCl_3$), see Table 3; HRESIMS *m/z* 265.1562 $[M + Na]^+$ (calcd for $C_{17}H_{22}NaO$, 265.1563).

4.4. Measurement of LPS-induced NO production and cell viability

The RAW 264.7 macrophage cell line was obtained from ATCC (Rockville, MD, USA) and cultured in DMEM containing 5% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin and grown at 37 °C in a humidified atmosphere containing 5% CO_2. RAW 264.7 cells were seeded into 96-well tissue culture plates at 2 x 10^5 cells/mL and stimulated with 1 μg/mL of LPS in the presence or absence of test compounds. After incubation at 37 °C for 24 h, 100 μL of cell-free supernatant was mixed with 100 μL of Griess reagent containing equal volumes of 2% (w/v) sulfanilamide in 5% (w/v) phosphoric acid and 0.2% (w/v) of *N*-(1-naphthyl) ethylenediamine solution to determine nitrite production. Absorbance was measured at 550 nm against a calibration curve with sodium nitrite standards. Cell viability of the remaining cells was determined by an MTT (Sigma Chemical Co., St. Louis, MO)-based colorimetric assay (Kim et al., 2021).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.phytochem.2022.113319.

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