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Anti-inflammatory Inositol Derivatives from the Whole Plant of *Inula cappa*

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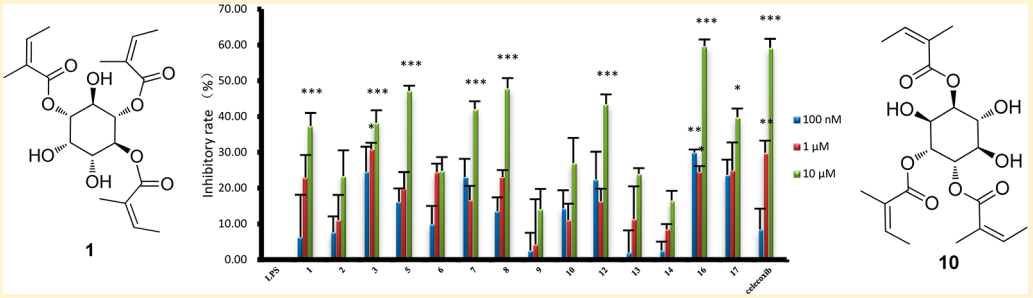
Supporting Information



ABSTRACT: Twelve new inositol derivatives, classified into myoinositol (**1**−**6**) and l-inositol (**10**−**15**) types, along with five known analogues were isolated from the whole plant of *Inula cappa*. The structures of the new compounds were established by extensive analysis of mass spectrometric and 1D and 2D NMR spectroscopic data. All the tested compounds showed anti-inflammatory activities against the production of NO in RAW264.7 macrophages stimulated by lipopolysaccharide, with IC_{50} values ranging from 7 to 23 μM.

In recent years, inositol derivatives have been studied extensively due to their various biological functions.[1−4] Inositol phosphates have been reported to possess significant health benefits, such as anticancer, kidney stone prevention, and serum cholesterol lowering effects.[5−7] Inositol nicotinate has been approved as a new drug for the treatment of coronary heart disease in the People's Republic of China. Inositol derivatives have attracted the interest of medicinal chemists, and many analogues have been prepared.[8−16] However, further investigations of inositol derivatives of natural origin, particularly those with promising biological activities, are needed.

Inula cappa (Buch.-Ham. ex D. Don) DC., belonging to the family Compositae, has long been used as a traditional Chinese medicinal herb to treat rheumatoid arthritis, malaria, dysentery, and hepatitis.[17] Known as "Yang Er Ju" in Chinese, this herb is distributed widely in the south of mainland China. As early as 1982, a phytochemical study of this plant resulted in the isolation of a new kind of inositol derivative, namely, inositol angelates.[8] In investigations following, sesquiterpene lactones,[18−20] inositol derivatives,[15] flavonoids,[21] and phenolic glycosides[22,23] were reported.

Herein, we report the isolation and structural elucidation of 12 new (**1**−**6**, **10**−**15**) and five known (**7**−**9**, **16**, **17**) inositol derivatives from the title plant and their anti-inflammatory activities against the production of NO in RAW264.7 stimulated by lipopolysaccharide (LPS).

RESULTS AND DISCUSSION

The whole plants of *I. cappa* were collected in Yulin County of Guangxi Province in September 2012. The material (30 kg) was air-dried, ground into a powder, and then extracted with 95% EtOH at room temperature. The crude extract was partitioned with CH_2Cl_2 and EtOAc, successively. The CH_2Cl_2 fraction was subjected to column chromatography over MCI gel, silica gel, and Sephadex LH-20 and then purified by preparative HPLC to yield 12 new inositol derivatives (**1**−**6**, **10**−**15**). Also obtained were five known compounds, which were identified as

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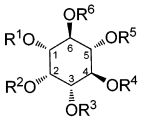
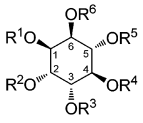
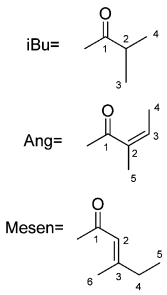


	R^1	R^2	R^3	R^4	R^5	R^6
1	Ang	H	H	Ang	Ang	H
2	Ang	Ac	H	Ang	Ang	H
3	Ang	H	Ac	Ang	Ang	H
4	H	Ac	Ang	H	Ang	Ang
5	Ang	iBu	H	Ang	Ang	H
6	Mesen	H	H	Ang	Ang	H
7	H	Ang	Ang	H	Ang	Ang
8	Ang	Ang	H	Ang	H	Ang
9	Ang	Ang	H	Ang	H	H



	R^1	R^2	R^3	R^4	R^5	R^6
10	H	Ang	Ang	H	H	Ang
11	Ang	H	Ang	Ang	H	H
12	H	Ac	Ang	Ang	H	Ang
13	Ac	Ac	Ang	Ang	H	Ang
14	Ang	H	Mesen	H	H	Ang
15	H	Ang	Ang	Ang	H	Mesen
16	Ang	H	Ang	H	Ang	Ang
17	H	Ang	Ang	H	Ang	Ang



myoinositol-1,3,4,6-tetraangelate (**7**),[8] myoinositol-2,4,5,6-tetraangelate (**8**),[8] *cis*-1,2,3,5-*trans*-4,6-inositol-2,3,6-triangelate (**9**),[15] l-inositol-1,2,3,5-tetraangelate (**16**),[15] and l-inositol-2,3,5,6-tetraangelate (**17**)[8] by comparison of their spectroscopic and MS data with values in the literature.

The ^{1}H NMR data (Tables 1 and 2) of compounds **1**−**6** and **10**−**15** showed six oxygenated proton signals appearing from δ_H 3.80 to 5.70. Correspondingly, their ^{13}C NMR spectra (Table 3) exhibited six oxygenated carbon resonances from δ_C 67.9 to 73.7. The ^{1}H−^{1}H COSY correlations between these oxygenated protons were observed for all these compounds. These data, together with the absence of any anomeric carbon signal, suggested the presence of a common oxygenated cyclohexane skeleton rather than a sugar moiety in all structures. Therefore, these compounds contained a basic skeleton of an inositol unit.

In general, the splitting patterns and coupling constants of the oxygenated protons of the inositol ring allowed for the distinction between axial and equatorial patterns of the substitutions on the ring. A splitting at about 10 Hz suggested an equatorial position of the substituent and therefore an axial position of the proton, whereas a splitting below 4 Hz suggested an axial position of the substituent and therefore an equatorial position of the proton or an equatorial position of the substituent with only equatorial protons adjacent. In particular, when an axial proton was adjacent to both an axial and an equatorial proton, its splitting pattern showed as a double doublet with J values of ∼4 and ∼10 Hz.[12]

Myoinositol Derivatives. An examination of the ^{1}H NMR spectra (Table 1) of compounds **1**−**6** revealed H-1 and H-3 in each molecule to be double doublets (J = ∼3 and ∼10 Hz), indicating these protons are axial with axial and equatorial protons adjacent. All H-4, H-5, and H-6 resonances appeared as triplets with a J value of ∼10 Hz, suggesting that they are axial and surrounded by axial protons. The H-2 proton appeared as a triplet (J = ∼3 Hz) in each case, indicative of an equatorial substitution. Consequently, compounds **1**−**6** were assigned as myoinositol derivatives.[12]

Compound **1** was obtained as a translucent colorless oil. The molecular formula $C_{21}H_{30}O_9$ was established by the ^{13}C NMR data and an HRESIMS ion at m/z 449.1779 $[M + Na]^+$ (calcd 449.1788), requiring seven indices of hydrogen deficiency. The IR spectrum suggested the presence of hydroxy groups (3450 cm^{-1}), carbonyl groups (1718 cm^{-1}), and double-bond groups (1646 cm^{-1}). The HMBC spectrum showed that the methyl at δ_H 1.94 correlated to the carbonyl carbon at δ_C 167.6 and the olefinic carbon at δ_C 139.4, and the methyl at δ_H 2.01 correlated to the olefinic carbon at δ_C 127.2, which indicated clearly the presence of an angelate moiety. Similarly, another two angeloyl groups were identified from the NMR data (Tables 1 and 3). These angeloyl groups were assigned to C-1, C-4, and C-5 as a result of three chemical shifts occurring downfield at δ_H 4.98, 5.47, and 5.22, respectively, and the HMBC correlations from

Table 1. ^{1}H NMR Data for Compounds 1−6 in $CDCl_3$ (δ in ppm, J in Hz)

position	**1**[c]	**2**[c]	**3**[d]	**4**[d]	**5**[c]	**6**[c]
1	4.98 dd (2.6, 9.9)	5.08 dd (3.0, 9.9)	5.05 dd (2.6, 10.0)	3.99 ddd (3.0, 6.9, 10.0)[a]	5.08 dd (2.9, 10.0)	4.93 dd (2.6, 9.9)
2	4.30 t (2.6)	5.66 t (3.0)	4.38 t (2.6)	5.68 t (3.0)	5.67 t (2.9)	4.27 t (2.6)
3	3.84 dd (2.6, 9.9)	4.02 ddd (3.0, 7.0, 9.9)[a]	5.15 dd (2.6, 10.0)	5.05 dd (3.0, 10.0)	4.02 dd (2.9, 10.0)	3.83 dd (2.6, 9.9)
4	5.47 t (9.9)	5.41 t (9.9)	5.76 t (10.0)	4.13 dt (6.0, 10.0)[a]	5.42 t (10.0)	5.45 t (9.9)
5	5.22 t (9.9)	5.26 t (9.9)	5.24 t (10.0)	5.25 t (10.0)	5.27 t (10.0)	5.21 t (9.9)
6	4.25 t (9.9)	4.10 dt (6.5, 9.9)[a]	4.29 dt (6.4, 10.0)[a]	5.42 t (10.0)	4.11 t (10.0)	4.20 dt (7.0, 9.9)[a]
	Ang-1	Ang-1	Ang-1	Ang-3	Ang-1	Ang-4
	6.16 m	6.10[b]	6.15 m	6.14[b]	6.12[b]	6.11 m
	2.01 m	1.96 m	2.01 m	1.97[b]	1.97 m	1.95 m
	1.94 m	1.84[b]	1.94[b]	1.86[b]	1.86 m	1.84[b]
	Ang-4	Ang-4	Ang-4	Ang-5	Ang-4	Ang-5
	6.11 m	6.10[b]	6.09 m	6.14[b]	6.12[b]	6.08 m
	1.95 m	1.93[b]	1.94[b]	1.97[b]	1.95[b]	1.93 m
	1.84[b]	1.84[b]	1.79 m	1.86[b]	1.84[b]	1.84[b]
	Ang-5	Ang-5	Ang-5	Ang-6	Ang-5	Mesen-1
	6.08 m	6.10[b]	6.04 m	6.14[b]	6.12[b]	5.78 m
	1.93 m	1.93[b]	1.90 m	1.97[b]	1.95[b]	2.19[b]
	1.84[b]	1.84[b]	1.84 m	1.86[b]	1.84[b]	1.08 t (7.4)
		Ac-2	Ac-3	Ac-2	iBu-2	2.18[b]
		2.17 s	2.06 s	2.19 s	2.71 hept (7.0)	
					1.24 d (7.0)	
					1.23 d (7.0)	

[a]Additional OH splitting J = 6−7 Hz. [b]Signals overlapped. [c]Data measured at 400 MHz. [d]Data measured at 600 MHz.

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Table 2. ^{1}H NMR Data for Compounds 10−15 in $CDCl_3$ (δ in ppm, J in Hz)

position	10[c]	11[c]	12[d]	13[d]	14[d]	15[c]
1	4.22 t (3.0)	5.47 t (3.0)	4.22 t (3.0)	5.45 t (2.8)	5.57 t (3.0)	4.24 t (3.0)
2	5.54 t (3.0)	4.25 t (3.0)	5.52 t (3.0)	5.45 t (2.8)	4.20 t (3.0)	5.62 t (3.0)
3	5.38 dd (3.0, 10.0)	5.30 dd (3.0, 10.0)	5.55 dd (3.0, 10.0)	5.40 dd (2.8, 10.0)	5.09 dd (3.0, 10.0)	5.59 dd (3.0, 10.0)
4	3.95 t (10.0)	5.54 t (10.0)	5.50 t (10.0)	5.52 t (10.0)	4.10 t (10.0)	5.53 t (10.0)
5	4.13 t (10.0)	3.89 t (10.0)	4.22 t (10.0)	4.10 t (10.0)	3.99 t (10.0)	4.21 dt (6.0, 10.0)[a]
6	5.10 dd (3.0, 10.0)	4.22 dd (3.0, 10.0)	5.23 dd (3.0, 10.0)	5.32 dd (2.8, 10.0)	5.41 dd (3.0, 10.0)	5.19 dd (3.0, 10.0)
	Ang-2	Ang-1	Ang-3	Ang-3	Ang-1	Ang-2
	6.12[b]	6.18 m	6.08[b]	6.08 m	6.16 m	6.17 m
	1.93 m	2.03 m	1.92 m	1.92 m	2.00 m	2.03 m
	1.81 m	1.95[b]	1.78 m	1.76 m	1.92 m	1.98 m
	Ang-3	Ang-3	Ang-4	Ang-4	Ang-6	Ang-3
	6.12[b]	6.11[b]	6.08[b]	6.10 m	6.11 m	6.06[b]
	1.99[b]	1.94[b]	1.94 m	1.94 m	1.97 m	1.92[b]
	1.91[b]	1.84[b]	1.84 m	1.84[b]	1.84 m	1.76 m
	Ang-6	Ang-4	Ang-6	Ang-6	Mesen-3	Ang-4
	6.12[b]	6.11[b]	6.17 m	6.13 m	5.77 m	6.06[b]
	1.99[b]	1.94[b]	2.01 m	1.97 m	2.19[b]	1.92[b]
	1.91[b]	1.84[b]	1.93 m	1.84[b]	1.08 t (7.4)	1.85 m
			Ac-2	Ac-1	2.18[b]	Mesen-6
			2.15 s	2.16 s		5.76 m
				Ac-2		2.20[b]
				2.14 s		1.08 t (7.4)
						2.18[b]

[a]Additional OH splitting J = 6−7 Hz. [b]Signals overlapped. [c]Data measured at 400 MHz. [d]Data measured at 500 MHz.

Table 3. ^{13}C NMR Data of Compounds 1−6 and 10−15 (δ in ppm)

position	1[a]	2[a]	3[c]	4[c]	5[a]	6[a]	10[a]	11[a]	12[b]	13[b]	14[b]	15[a]
1	73.5 d	71.3 d	73.1 d	69.9 d	71.4 d	72.7 d	68.4 d	71.7 d	68.5 d	68.0 d	70.0 d	68.6 d
2	70.7 d	71.3 d	68.9 d	71.1 d	70.9 d	70.8 d	70.1 d	68.6 d	70.1 d	67.9 d	68.8 d	69.6 d
3	71.4 d	69.6 d	71.4 d	71.3 d	70.0 d	71.4 d	71.0 d	71.3 d	68.4 d	68.6 d	72.6 d	68.7 d
4	72.8 d	72.2 d	68.8 d	70.6 d	72.4 d	72.9 d	72.7 d	71.9 d	72.3 d	71.9 d	71.8 d	72.4 d
5	72.8 d	72.6 d	73.1 d	72.5 d	72.7 d	72.7 d	71.5 d	73.0 d	70.4 d	70.7 d	72.7 d	70.4 d
6	70.1 d	70.5 d	70.0 d	72.3 d	70.7 d	70.0 d	73.2 d	70.2 d	73.7 d	71.2 d	70.9 d	73.1 d
	Ang-1	Ang-1	Ang-1	Ang-3	Ang-1	Ang-4	Ang-2	Ang-1	Ang-3	Ang-3	Ang-1	Ang-2
	167.6 s	167.1 s	167.4 s	167.0 s	167.1 s	168.6 s	166.3 s	167.0 s	166.6 s	166.4 s	166.1 s	166.2 s
	127.2 s	127.0 s	127.3 s	127.2 s	127.2 s	127.2 s	127.0 s	127.2 s	127.0 s	127.3 s	127.2 s	127.0 s
	139.4 d	139.4 d	139.9 d	140.4 d	140.3 d	140.1 d	139.5 d	140.2 d	139.9 d	139.4 d	140.3 d	140.4 d
	15.9 q	16.0 q	16.1 q	16.0 q	15.9 q	15.9 q	16.0 q	16.0 q	15.8 q	15.9 q	16.1 q	16.2 q
	20.5 q	20.5 q	20.7 q	20.5 q	20.5 q	20.6 q	20.5 q	20.6 q	20.4 q	20.4 q	20.8 q	20.9 q
	Ang-4	Ang-4	Ang-4	Ang-5	Ang-4	Ang-5	Ang-3	Ang-3	Ang-4	Ang-4	Ang-6	Ang-3
	168.6 s	168.2 s	166.8 s	167.7 s	168.3 s	167.8 s	167.7 s	166.6 s	168.0 s	167.8 s	167.6 s	166.5 s
	127.3 s	127.2 s	127.2 s	127.0 s	127.1 s	127.4 s	127.5 s	127.1 s	127.3 s	127.0 s	127.0 s	127.1 s
	140.0 d	140.2 d	139.7 d	140.4 d	140.3 d	139.2 d	140.4 d	139.3 d	139.2 d	140.2 d	140.0 d	138.8 d
	16.1 q	15.9 q	16.0 q	16.0 q	16.0 q	16.0 q	16.0 q	15.9 q	15.9 q	15.9 q	16.0 q	15.9 q
	20.7 q	20.5 q	20.5 q	20.5 q	20.5 q	20.5 q	20.7 q	20.6 q	20.5 q	20.4 q	20.5 q	20.4 q
	Ang-5	Ang-5	Ang-5	Ang-6	Ang-5	Mesen-1	Ang-6	Ang-4	Ang-6	Ang-6	Mesen-3	Ang-4
	167.8 s	167.8 s	167.7 s	168.3 s	167.8 s	166.3 s	167.6 s	168.3 s	167.5 s	167.1 s	166.2 s	167.9 s
	127.3 s	127.1 s	127.2 s	126.9 s	127.0 s	113.4 d	127.3 s	127.4 s	127.4 s	126.8 s	113.5 d	127.5 s
	139.8 d	140.0 d	138.9 d	139.7 d	139.4 d	165.0 s	140.0 d	140.4 d	140.0 d	140.3 d	164.8 s	138.9 d
	16.0 q	15.9 q	15.8 q	16.0 q	16.0 q	34.0 t	16.1 q	16.2 q	16.1 q	16.0 q	34.1 t	15.9 q
	20.6 q	20.5 q	20.5 q	20.5 q	20.5 q	12.0 q	20.7 q	20.8 q	20.7 q	20.5 q	12.0 q	20.6 q
		Ac-2	Ac-3	Ac-2	iBu-2	19.3 q			Ac-2	Ac-1	19.3 q	Mesen-6
		170.4 s	169.7 s	170.2 s	176.2 s				169.7 s	169.1 s		166.2 s
		21.0 q	20.9 q	21.0 q	34.2 d				21.0 q	20.8 q		113.4 d
					19.1 q					Ac-2		165.0 s
					19.3 q					169.0 s		34.1 t
										20.8 q		12.0 q
												19.3 q

[a]Data measured at 400 MHz. [b]Data measured at 500 MHz. [c]Data measured at 600 MHz.

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H-1, H-4, and H-5 to the corresponding carbonyls in the angeloyl groups. Thus, **1** was elucidated as myoinositol-1,4,5-triangelate.

Compound **2**, isolated as a translucent colorless oil, was found to possess a molecular formula of $C_{23}H_{32}O_{10}$ as determined from its ^{13}C NMR and HRESIMS data, indicating eight indices of hydrogen deficiency. The ^{1}H and ^{13}C NMR spectra of compound **2** (Tables 1 and 3) showed close similarities to those of **1**. The main difference observed was an additional acetyl group, which was identified from the NMR data (δ_C 170.4 and 21.0; δ_H 2.17, 3H, s). The acetyl group was placed at C-2, as inferred from the HMBC correlation from H-2 (δ_H 5.66) to the carbonyl carbon of the acetyl group. The downfield chemical shifts of H-1 (δ_H 5.08), H-4 (δ_H 5.41), and H-5 (δ_H 5.26) were in agreement with those of **1**, suggesting also the presence of three angeloyl groups at C-1, C-4, and C-5. Thus, **2** was elucidated as myoinositol-2-acetate-1,4,5-triangelate.

Compound **3** was obtained as a translucent colorless oil and gave a molecular formula of $C_{23}H_{32}O_{10}$ as established from its ^{13}C NMR and HRESIMS data, indicating eight indices of hydrogen deficiency. It shared the same molecular formula as compound **2**, and the same substituent groups were inferred from its NMR data (Tables 1 and 3). The differences between these two compounds resulted from the substituent pattern of the inositol ring. The substitution positions of three angelate residues of **3** were found to be identical to those of **2** on the basis of the downfield chemical shifts of H-1 (δ_H 5.05), H-4 (δ_H 5.76), and H-5 (δ_H 5.24) and their HMBC correlations to the corresponding carbonyl groups in the ester units. The acetoxy group in **3** was located at C-3 rather than at C-2 in **2** from the HMBC correlation from H-3 (δ_H 5.15) to the carbonyl carbon of the acetyl group. Thus, **3** was elucidated as myoinositol-3-acetate-1,4,5-triangelate.

Compound **4** gave a molecular formula of $C_{23}H_{32}O_{10}$, the same as those of compounds **2** and **3**. A similar analysis of its NMR data demonstrated that an acetoxy group is located at C-2, and three angeloyloxy groups occur at C-3, C-5, and C-6, respectively. Thus, **4** was elucidated as myoinositol-2-acetate-3,5,6-triangelate.

Compound **5** showed a molecular formula of $C_{25}H_{36}O_{10}$ as determined by its ^{13}C NMR and HRESIMS data, requiring eight indices of hydrogen deficiency. The ^{1}H NMR spectrum displayed signals at δ_H 2.71 (hept, J = 7.0 Hz) and two methyl doublets at δ_H 1.24 and δ_H 1.23 (J = 7.0 Hz), indicating the presence of an isobutyryl residue. The carbonyl carbon at δ_C 176.2 of the residue showed a HMBC correlation with H-2 (δ_H 5.67), suggesting its attachment to C-2. The ^{1}H and ^{13}C NMR spectra also showed the presence of three angeloyl moieties (Tables 1 and 3), which were deduced to be located at C-1, C-4, and C-5 from the observed HMBC correlations. Therefore, **5** was elucidated as myoinositol-2-isobutyryloxy-1,4,5-triangelate.

The molecular formula of compound **6** was established as $C_{22}H_{32}O_9$ with seven indices of hydrogen deficiency. The ^{1}H NMR spectrum showed signals at δ_H 1.08 (3H, t, J = 7.4 Hz) and 2.19 (2H, overlapped) and an olefinic signal at δ_H 5.78 (1H, m), indicating the presence of a 4-methylsenecioyl residue. This residue was assigned to C-1 from the HMBC correlation between its carbonyl carbon and H-1 (δ_H 4.93). Two angeloyloxy groups were identified from the ^{1}H and ^{13}C NMR data (Tables 1 and 3) and placed at C-4 and C-5, respectively, from the downfield chemical shifts of H-4 and H-5 at δ_H 5.45 and 5.21, and were supported by the HMBC correlations observed. Accordingly, **6** was established as myoinositol-1-(4-methylsenecioyloxy)-4,5-diangelate.

l-Inositol Derivatives. Comparison of ^{1}H NMR spectra (Table 2) of compounds **10**−**15** with those of **1**−**6** revealed in each case that H-6 appeared as a double doublet with J values of ∼10.0 and ∼3.0 Hz. This indicated that the C-1 oxygenation of the inositol skeletons in **10**−**15** changed from equatorial in **1**−**6** to axial in **10**−**15**. As C-2 is oxygenated axially, the equatorial orientation of H-2 would allow only a small splitting with either axial or equatorial protons at C-1 and C-3. Thus, the coupling pattern of H-2 remained unchanged, while the H-6 protons in **10**−**15** exhibited one large and one small coupling constant. Thus, compounds **10**−**15** were found to possess a different geometry of the inositol ring and were classified as l-inositol derivatives.[12]

Compound **10** was assigned a molecular formula of $C_{21}H_{30}O_9$ with seven indices of hydrogen deficiency according to the ^{13}C NMR and HRESIMS data. Analysis of its NMR spectra suggested the presence of three angeloyloxy groups (Tables 2 and 3), which were placed at C-2, C-3, and C-6 of the ring, as deduced from the downfield chemical shifts of H-2 (δ_H 5.54), H-3 (δ_H 5.38), and H-6 (δ_H 5.10) and from the HMBC correlations from H-2, H-3, and H-6 to the respective carbonyls in the ester units. Thus, **10** was established as l-inositol-2,3,6-triangelate.

Compound **11** shared the same molecular formula as **10** as determined by the ^{13}C NMR and HRESIMS data. Interpretation of its NMR data (Tables 2 and 3) revealed the structure of **11** to be very close to that of **10**, with the differences being the substituent pattern of the inositol ring. Three angeloyloxy groups were determined to be at C-1, C-3, and C-4 by the downfield chemical shifts of H-1 (δ_H 5.47), H-3 (δ_H 5.30), and H-4 (δ_H 5.54) and from the HMBC correlations. Therefore, **11** was constructed and named l-inositol-1,3,4-triangelate.

Compound **12** gave a molecular formula of $C_{23}H_{32}O_{10}$ on the basis of the ^{13}C NMR and HRESIMS data. The ^{1}H and ^{13}C NMR spectra of **12** (Tables 2 and 3) showed the presence of three angeloyl moieties and one acetyl group, similar to those of compounds **2**−**4**. The acetoxy group was deduced to be at C-2 in **12** by the HMBC correlation from H-2 (δ_H 5.52) to its carbonyl carbon. The downfield chemical shifts of H-3 (δ_H 5.55), H-4 (δ_H 5.50), and H-6 (δ_H 5.23) in the ^{1}H NMR spectrum were in agreement with the presence of three angeloyloxy groups located at C-3, C-4, and C-6. Thus, **12** was determined as l-inositol-2-acetate-3,4,6-triangelate.

Compound **13** was found to possess a molecular formula of $C_{25}H_{34}O_{11}$ as deduced from the ^{13}C NMR and HRESIMS data, suggesting nine indices of hydrogen deficiency. Its ^{1}H and ^{13}C NMR data (Tables 2 and 3) showed the presence of two acetyl groups and three angeloyl units. The two acetoxy groups were assigned to C-1 and C-2 by the HMBC correlations from H-1 (δ_H 5.45) and H-2 (δ_H 5.45) to the carbonyl carbons of the respective acetyls, while three angelates esterified hydroxy groups at C-3, C-4, and C-6 from the evidence of the downfield H-3 (δ_H 5.40), H-4 (δ_H 5.52), and H-6 (δ_H 5.32) chemical shifts and an HMBC experiment. Thus, **13** was elucidated as l-inositol-1,2-diacetate-3,4,6-triangelate.

Compound **14** shared the same molecular formula of $C_{22}H_{32}O_9$ as compound **6**, as established by the ^{13}C NMR and HRESIMS data. The NMR spectra (Tables 2 and 3) of **14** also resembled those of **6**, showing the presence of one 4-methylsenecioyloxy group and two angeloyloxy groups. The 4-methylsenecioyloxy unit was placed at C-3 and the two

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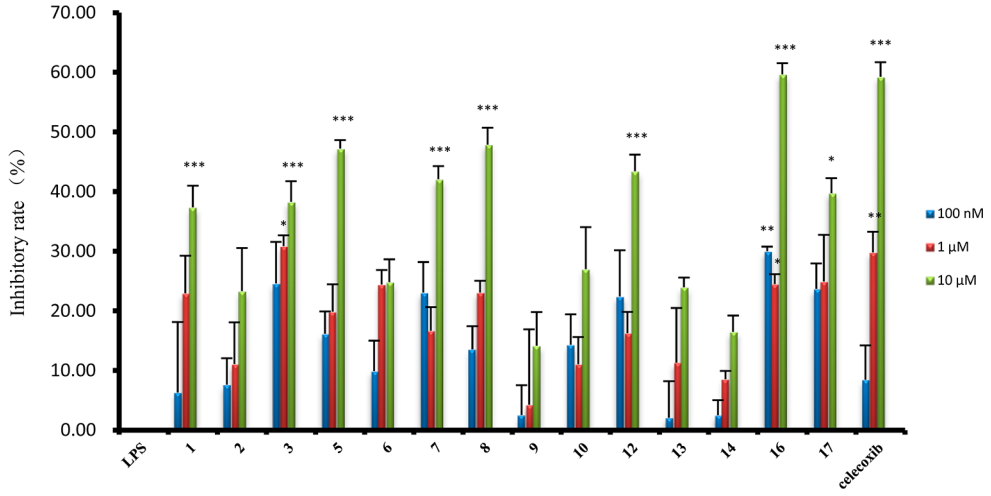

Figure 1. Effects of compounds tested on NO production in LPS-stimulated RAW 264.7 cells (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, when compared with the LPS group).

angeloyloxy groups were placed at C-1 and C-6 from the HMBC correlations observed. Thus, compound **14** was established as l-inositol-3-(4-methylsenecioyloxy)-1,6-diangelate.

Compound **15** was assigned a molecular formula of $C_{27}H_{38}O_{10}$ from the ^{13}C NMR and HRESIMS data, corresponding to nine indices of hydrogen deficiency. The ^{1}H NMR data at δ_H 1.08 (3H, t, J = 7.4 Hz), 2.20 (2H, overlapped), and 5.76 (1H, m) suggested the presence of a 4-methylsenecioyl residue, which was determined to be at C-6 by the HMBC correlation from H-6 (δ_H 5.19) to its carbonyl carbon. The ^{1}H and ^{13}C NMR spectra (Tables 2 and 3) indicated the presence of three angeloyloxy moieties, which were deduced to be at C-2, C-3, and C-4 from the observed HMBC correlations. Accordingly, **15** was determined as l-inositol-6-(4-methylsenecioyloxy)-2,3,4-triangelate.

Niric oxide (NO) plays an important role in the inflammatory process,[24] and an inhibitor of NO release may be considered as a potential therapeutic agent for inflammatory diseases.[25] In a previous study, various inositol angelates exhibited activity in this assay.[16] Thus, all the isolated compounds except for compounds **4**, **11**, and **15** were evaluated for their activities against the production of NO in RAW264.7 macrophages stimulated by lipopolysaccharide. Compound **16** showed the most potent activity, with an inhibitory rate of 59.6% at a concentration of 10 μM. The rate for the positive control celecoxib was 59.2% at the same concentration. Compounds **1**, **3**, **5**, **7**, **8**, **12**, and **17** showed moderate activities, with inhibitory rates of 37.4%, 38.3%, 47.2%, 42.1%, 47.9%, 43.4%, and 39.7%, respectively, at a concentration of 10 μM, whereas compounds **2**, **6**, **9**, **10**, **13**, and **14** exhibited less potent activities at the same concentration (Figure 1). In another experiment, the IC_{50} values of compounds **2**, **5**, **7**, **10**, **12**, **13**, **14**, **16**, and **17** were measured to be 9.6, 7.6, 9.8, 8.9, 11.9, 7.1, 8.4, 9.9, and 7.0 μM, respectively, while the data for compounds **1** and **8** were 23.4 and 19.0 μM. The IC_{50} value of the positive control celecoxib was 1.6 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin−Elmer 341 polarimeter. IR spectra were recorded on a Nicolet Magna FT-IR 750 spectrophotometer using KBr disks. NMR spectra were recorded on Bruker AM-400 and Innova-600 NMR spectrometers. The chemical shift (δ) values are given in ppm with TMS as internal standard, and coupling constants (J) are in Hz. ESIMS and HRESIMS data were recorded on Waters 2695−3100 LC-MS and Waters Xevo TOF mass spectrometers. Silica gel (Qingdao Marine Chemical Industrials, Qingdao, People's Republic of China) was used for flash chromatography. MCI gel CHP20P (75−150 μm, Mitsubishi Chemical Industries, Tokyo, Japan) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) were used for column chromatography (CC). TLC was carried out on precoated silica gel GF_{254} plates (Yantai Chemical Industrials, Yantai, People's Republic of China), and the TLC spots were viewed at 254 nm and visualized with 5% H_2SO_4 in EtOH containing 10 mg/mL vanillin. Analytical HPLC was performed on a Waters 2690 instrument with an Alltech ELSD 2000 detector. Preparative HPLC was performed on a Varian PrepStar system with an Alltech 3300 ELSD. Chromatographic separations were carried out on a Waters Sunfire RP C_{18}, 5 μm, 30 mm × 150 mm column and a Waters Sunfire RP C_{18}, 5 μm, 19 mm × 150 mm column, using a gradient solvent system composed of H_2O and CH_3CN, with a flow rate of 25.0 and 10.0 mL/min, respectively.

Plant Material. The whole plants of *I. cappa* were collected in Yulin County, Guangxi Province, People's Republic of China, in September 2012, and identified by Professor Jin-Gui Shen from the Shanghai Institute of Materia Medica. A voucher specimen (no. 20131020) was deposited at the Herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation. The dried and powdered whole plants of *I. cappa* (30 kg) were extracted with 95% EtOH (3 × 40 L, 7 days each) at room temperature. After evaporation of the solvent, the residue obtained was dissolved in water (10 L) and partitioned with CH_2Cl_2 (10 L × 3) and EtOAc (10 L × 3), successively. The CH_2Cl_2 fraction was concentrated and then dissolved with 80% MeOH in water (5 L × 3). The MeOH layer was concentrated and then subjected to CC over MCI gel (EtOH/H_2O, 50% to 95%) to yield three fractions (1−3). Fraction 1 was subjected to CC over MCI gel (EtOH/H_2O, 30% to 50%) to give four fractions (1a−1d). Fraction 1b was subjected to CC over silica gel eluting with petroleum ether/acetone (10:1 to 1:1) in a stepwise manner, affording three fractions (1b1−1b3). Fraction 1b2 was then subjected to CC over silica gel eluting with CH_2Cl_2/MeOH (50:1 to 10:1) to give two subfractions (1b2a and 1b2b). Subfractions 1b2a and 1b2b were purified further by preparative HPLC (CH_3CN/H_2O, 30% to 60%) to yield **1** (15 mg) and **11** (2 mg), respectively. Fraction 1b3 was subjected to CC over Sephadex LH-20 (MeOH) to yield two subfractions (1b3a and 1b3b), and subfraction 1b3a was purified further by preparative HPLC (CH_3CN/H_2O, 30% to 60%) to give **9** (11 mg) and **10** (35 mg).

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Fraction 1c was subjected to CC over silica gel to give four fractions (1c1−1c4). Fraction 1c1 was then purified by preparative HPLC (CH_3CN/H_2O, 60% to 80%) to afford **2** (16 mg), **3** (6 mg), and **4** (1 mg). Similarly, fractions 1c3 and 1c4 were purified by preparative HPLC (CH_3CN/H_2O, 60% to 80%) to afford **6** (7 mg) and **14** (8 mg), respectively. Fraction 1d was subjected to CC over silica gel (CH_2Cl_2/MeOH, 100:1, 50:1, 20:1, 10:1) to afford two fractions (1d1 and 1d2), and fraction 1d1 was then purified by preparative HPLC (CH_3CN/H_2O, 60% to 80%) to yield **12** (16 mg). Fraction 2 was subjected to CC over silica gel eluting with petroleum ether/acetone (10:1 to 1:1) in a stepwise manner, giving five fractions (2a−2e). Fraction 2c was subjected to CC over Sephadex LH-20 (MeOH) to give two subfractions (2c1 and 2c2). Fraction 2c2 was then subjected to CC over silica gel to yield two fractions (2c2a and 2c2b). Compounds **13** (24 mg), **15** (3 mg), and **16** (10 mg) were separated from fraction 2c2b by preparative HPLC (CH_3CN/H_2O, 60% to 80%). Fraction 2d was subjected to CC over silica gel (CH_2Cl_2/MeOH, 100:1, 50:1, 20:1, 10:1) to afford three fractions (2d1−2d3). Fraction 2d1 was purified further by preparative HPLC (CH_3CN/H_2O, 60% to 80%) to yield **8** (28 mg). Similarly, compounds **5** (15 mg), **7** (23 mg), and **17** (23 mg) were separated from fraction 2d3 by preparative HPLC (CH_3CN/H_2O, 60% to 80%).

*Myoinositol-1,4,5-triangelate (**1**):* translucent colorless oil; $[\alpha]^{20}_D$ −12.8 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3450, 2972, 2928, 1718, 1646, 1458, 1385, 1154, 1044 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; HRESIMS *m*/*z* 449.1779 $[M + Na]^+$ (calcd for $C_{21}H_{30}O_9Na$, 449.1788).

*Myoinositol-2-acetate-1,4,5-triangelate (**2**):* translucent colorless oil; $[\alpha]^{20}_D$ −1.2 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3447, 2974, 2930, 1753, 1727, 1646, 1458, 1384, 1229, 1149, 1044 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; HRESIMS *m*/*z* 491.1882 $[M + Na]^+$ (calcd for $C_{23}H_{32}O_{10}Na$, 491.1893).

*Myoinositol-3-acetate-1,4,5-triangelate (**3**):* translucent colorless oil; $[\alpha]^{20}_D$ +2.0 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3471, 2957, 2927, 1751, 1728, 1646, 1458, 1385, 1353, 1230, 1149, 1084, 1043 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; HRESIMS *m*/*z* 491.1882 $[M + Na]^+$ (calcd for $C_{23}H_{32}O_{10}Na$, 491.1893).

*Myoinositol-2-acetate-3,5,6-triangelate (**4**):* translucent colorless oil; $[\alpha]^{20}_D$ −3.3 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3450, 2957, 2928, 1753, 1727, 1646, 1384, 1354, 1229, 1149, 1084, 1044 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; HRESIMS *m*/*z* 491.1889 $[M + Na]^+$ (calcd for $C_{23}H_{32}O_{10}Na$, 491.1893).

*Myoinositol-2-isobutyryloxy-1,4,5-triangelate (**5**):* translucent colorless oil; $[\alpha]^{20}_D$ +1.0 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3451, 2973, 2927, 1721, 1646, 1392, 1230, 1150, 1045 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; HRESIMS *m*/*z* 519.2195 $[M + Na]^+$ (calcd for $C_{25}H_{36}O_{10}Na$, 519.2206).

*Myoinositol-1-(4-methylsenecioyloxy)-4,5-diangelate (**6**):* translucent colorless oil; $[\alpha]^{20}_D$ −3.0 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3433, 2961, 2924, 1719, 1645, 1458, 1384, 1354, 1229, 1149, 1042 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 3; HRESIMS *m*/*z* 463.1932 $[M + Na]^+$ (calcd for $C_{22}H_{32}O_9Na$, 463.1944).

*l-Inositol-2,3,6-triangelate (**10**):* translucent colorless oil; $[\alpha]^{20}_D$ −32.0 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3447, 2971, 2927, 1723, 1646, 1458, 1384, 1356, 1231, 1148, 1085, 1044 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 449.1778 $[M + Na]^+$ (calcd for $C_{21}H_{30}O_9Na$, 449.1788).

*l-Inositol-1,3,4-triangelate (**11**):* translucent colorless oil; $[\alpha]^{20}_D$ −36.2 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3512, 3424, 2971, 2927, 1715, 1646, 1459, 1384, 1263, 1236, 1160, 1087, 1042 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 449.1777 $[M + Na]^+$ (calcd for $C_{21}H_{30}O_9Na$, 449.1788).

*l-Inositol-2-acetate-3,4,6-triangelate (**12**):* translucent colorless oil; $[\alpha]^{20}_D$ −12.8 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3447, 2971, 2927, 1754, 1726, 1646, 1379, 1231, 1149, 1045 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 491.1894 $[M + Na]^+$ (calcd for $C_{23}H_{32}O_{10}Na$, 491.1893).

*l-Inositol-1,2-diacetate-3,4,6-triangelate (**13**):* translucent colorless oil; $[\alpha]^{20}_D$ 0 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3445, 2973, 2929, 1761, 1728, 1646, 1458, 1372, 1229, 1146, 1048 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 533.2010 $[M + Na]^+$ (calcd for $C_{25}H_{34}O_{11}Na$, 533.1999).

*l-Inositol-3-(4-methylsenecioyloxy)-1,6-diangelate (**14**):* translucent colorless oil; $[\alpha]^{20}_D$ −23.0 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3440, 2970, 2929, 1720, 1646, 1458, 1383, 1228, 1146, 1047 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 463.1946 $[M + Na]^+$ (calcd for $C_{22}H_{32}O_9Na$, 463.1944).

*l-Inositol-6-(4-methylsenecioyloxy)-2,3,4-triangelate (**15**):* translucent colorless oil; $[\alpha]^{20}_D$ −45.0 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3446, 2962, 2926, 1728, 1646, 1458, 1383, 1228, 1144, 1042 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 545.2369 $[M + Na]^+$ (calcd for $C_{27}H_{38}O_{10}Na$, 545.2363).

RAW264.7 Macrophage Assay. The assay was carried out as previously described[16] with minor modifications. Briefly, RAW264.7 macrophages were harvested and seeded in 96-well plates (2.0×10^5 cells/well) for NO production. The plates were pretreated with various concentrations of samples for 30 min and incubated with LPS (1 μg/mL) for 24 h. The amount of NO was determined by the nitrite concentration in the cultured RAW264.7 macrophage supernatants with the Griess reagent. The absorbance was measured using a microplate reader (NovoStar, BMG) at 490 nm. Inhibition activity (%) = $[1 - (B - C)/(A - C)] \times 100\%$. [*A*: LPS (+), sample (−); *B*: LPS (+), sample (+); *C*: LPS (−), sample (−)]. Celecoxib was used as the positive control.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00135.

IR, HRESIMS, ^1H and ^{13}C NMR, $^1H-^1H$ COSY, HSQC, HMBC, and ROESY spectra of compounds **1**−**6**, **10**−**15** (PDF)

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Notes

The authors declare no competing financial interest.

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