Triterpenoids and Flavonoids from Celery (*Apium graveolens*)

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Three new triterpenoids, 11,21-dioxo-2β,3β,15α-trihydroxyurs-12-ene-2-*O*-β-D-glucopyranoside (**1**), 11,21-dioxo-3β,15α,24-trihydroxyurs-12-ene-24-*O*-β-D-glucopyranoside (**2**), and 11,21-dioxo-3β,15α,24-trihydroxyolean-12-ene-24-*O*-β-D-glucopyranoside (**3**), and two new flavonoids, apigenin-7-*O*-[2″-*O*-(5‴-*O*-feruloyl)-β-D-apiofuranosyl]-β-D-glucopyranoside (**4**) and chrysoeriol-7-*O*-[2″-*O*-(5‴-*O*-feruloyl)-β-D-apiofuranosyl]-β-D-glucopyranoside (**5**), were isolated from the whole plant of fresh celery (*Apium graveolens*), together with 10 known flavonoids. The structures of the new compounds were elucidated by analysis of spectroscopic data. The inhibitory effects of the compounds isolated on nitric oxide production in lipopolysaccaride-activated macrophages were evaluated.

Celery (*Apium graveolens* L., Umbelliferae) is a common annual herb widely cultivated in temperate zones, with its leaf stalks consumed as a popular vegetable. The stalks of celery have shown effects on bioassays related to epilepsy, hyperlipemia, hypertension, and memory enhancement,[1] and the seeds are used for treating bronchitis, asthma, and liver and spleen diseases.[2] The seeds of celery contain a variety of bioactive constituents such as phthalides,[3−6] coumarins,[7−10] flavonoids,[11] sesquiterpenoids,[12] and aromatic glucosides.[12] However, to date, the chemical composition from the aerial parts of celery has not yet been reported. This paper describes the isolation and structure elucidation of three new triterpenoids (**1**−**3**) and two new flavonoids (**4** and **5**) from fresh celery and the inhibitory effect of the isolated compounds on nitric oxide production in lipopolysaccaride-activated macrophages.

Results and Discussion

A 70% aqueous ethanol extract of fresh celery was partitioned with petroleum ether and EtOAc, in turn. The EtOAc portion was subjected to silica gel and Sephadex LH-20 column chromatography and RP-HPLC to afford three new triterpenoids (**1**−**3**) and two new flavonoids (**4** and **5**).

Compound **1** was obtained as a white, amorphous powder, and its molecular formula, $C_{36}H_{56}O_{10}$, was inferred from the positive-ion HRESIMS (*m*/*z* 671.3764 $[M + Na]^+$). The ^1H NMR spectrum taken in C_5D_5N showed six methyls resonating as singlets and two as doublets, which signified that compound **1** is a pentacyclic triterpenoid with an ursane skeleton.[13] The ^{13}C NMR spectrum exhibited 36 carbon signals that were sorted by a DEPT experiment as eight methyls, six methylenes, 14 methines, and eight quarternary carbons, including three oxygenated methines and two carbonyl groups. The ^1H and ^{13}C NMR data were assigned from the $^1H-^1H$COSY, HSQC, HMBC, and NOESY spectra (see Tables 1 and 2). The absorption maximum at 247 nm in the UV spectrum, along with the appearance of a carbonyl group at δ 199.8 and two olefinic carbon signals at δ 164.2 and 132.3 in the ^{13}C NMR spectrum, indicated the presence of an 11-oxo, $\Delta^{12,13}$ functionality.[14] The carbonyl carbon signal at δ 211.1 was assigned to C-21 due to the HMBC correlations from H-22 (δ 2.31 and 2.43), H-19 (δ 2.02), H-20 (δ 2.16), H-28 (δ 1.01), and H-30 (δ 1.09) to this carbonyl carbon. The HMBC correlation from H-3 (δ 3.50) to C-23 (δ 30.4) and C-24 (δ 18.6) and from H-15 (δ 4.48) to C-8 (δ 47.3), C-14 (δ 50.2), C-16 (δ 40.8), and C-27 (δ 16.3) supported the two oxygenated carbon signals being assigned to C-3 and C-15, respectively. The $^1H-^1H$ COSY correlation between H-3 (δ 3.50) and H-2 (δ 4.41), along with HMBC correlations from H-2 (δ 4.41) to C-4 (δ 39.5) and C-10 (δ 37.8), indicated an oxygenated carbon signal at δ 83.6 that was assigned to C-2. Acid hydrolysis of **1** with 1 N HCl yielded D-glucose (see Experimental Section), and the β-configuration of the glycosidic linkage was established from the coupling constant of its anomeric proton at δ 5.10 (1H, d, J = 7.8 Hz). The sugar unit was linked to C-2 on the basis of the HMBC correlation between H-1′ (δ 5.10) and C-2 (δ 83.6). In the NOESY spectrum, H-15 at δ 4.48 showed cross-peaks with CH_3-26 at δ 1.33 and CH_3-28 at δ 1.01, which indicated that H-15 is β-oriented. The presence of correlations between H-3/H-5, H-3/CH_3-23, H-2/H-5, and H-2/H-9 and the absence of any correlation between H-2/CH_3-25 and H-3/CH_3-25 implied the α-orientations of H-3 and H-2. Thus, the structure of compound **1** was established as 11,21-dioxo-2β,3β,15α-trihydroxyurs-12-ene-2-*O*-β-D-glucopyranoside.

Compound **2** was obtained as a white, amorphous powder. The positive-ion HRESIMS showed a quasimolecular ion at *m*/*z* 671.3763 $[M + Na]^+$, corresponding to the molecular formula $C_{36}H_{56}O_{10}$. The ^1H NMR spectrum exhibited five methyl signals that resonated as singlets and two as doublets, which suggested that compound **2** is also a pentacyclic triterpenoid with an ursane skeleton.[13] The ^{13}C NMR spectrum gave 36 carbon signals as seven methyls, eight methylenes, 13 methines, and eight quarternary carbons, including three oxygenated carbons and two carbonyl groups. In the HMBC spectrum, H-23 (δ 1.61), H-3 (δ 3.64), and H-5 (δ 1.08) showed correlations with the carbon signal resonating at δ 74.0, indicating the carbon at C-24 to be oxygenated. The sugar unit was determined as D-glucose by the same method as described for compound **1**, and the β-configuration of the glycosidic linkage was established from the coupling constant of its anomeric proton at δ 5.03 (1H, d, J = 7.8 Hz). The sugar unit was linked to C-24 on the basis of the HMBC correlations from H-24 (δ 4.37 and δ 4.44) to C-1′ (δ 106.5) and from H-1′ (δ 5.03) to C-24 (δ 74.0). Except for the A-ring signals, the ^1H and ^{13}C NMR data and the HMBC and NOE correlations of compound **2** were similar to those of compound **1**. In the NOESY spectrum, H-24 (δ 4.37 and δ 4.44) showed correlations with CH_3-25 (δ 1.38) and CH_3-26 (δ 1.28), which confirmed that CH_3-24 is β-oriented and CH_3-23 α-oriented. The NOESY correlations of H-3/H-5 and H-3/CH_3-23 indicated the β-orientation of OH-3. Therefore, compound **2** was identified as 11,21-dioxo-3β,15α,24-trihydroxyurs-12-ene-24-*O*-β-D-glucopyranoside.

Compound **3** was obtained as a white, amorphous powder. The positive-ion HRESIMS (*m*/*z* 671.3758 $[M + Na]^+$) was supportive of the molecular formula being $C_{36}H_{56}O_{10}$. The ^1H NMR spectrum

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Table 1. ^{1}H NMR Data (600 MHz) for Compounds **1**−**3** (δ_H, C_5D_5N, *J* in Hz)

position	1	2	3
1	(α) 1.45 dd (17.4, 3.0)	(α) 1.23 dd (13.1, 4.7)	(α) 1.31 br d (13.3)
	(β) 3.93 dd (17.4, 1.5)	(β) 3.15 br d (13.3)	(β) 3.23 br d (13.3)
2	4.41 dd (3.6, 4.2)	(α) 2.03 m	(α) 2.00 m
		(β) 2.22 m	(β) 2.20 m
3	3.50 d (4.2)	3.64 dd (11.8, 3.9)	3.63 m
5	1.04 m	1.08 m	1.08 m
6	(α) 1.70 m	(α) 1.75 m	(α) 1.76 br d (13.7)
	(β) 1.64 m	(β) 1.50 m	(β) 1.57 m
7	(α) 2.26 m	(α) 2.09 m	(α) 2.07 m
	(β) 2.14 m	(β) 2.17 m	(β) 2.19 m
9	2.59 s	2.65 s	2.65 s
12	5.95 s	5.96 s	5.99 s
15	4.48 dd (10.8, 5.4)	4.46 br d (10.1)	4.42 m
16	(α) 2.07 m	(α) 2.10 m	(α) 2.14 m
	(β) 1.77 dd (13.2,4.2)	(β) 1.77 m	(β) 1.72 m
18	2.12 m	2.08 m	2.63 dd (3.7, 14.2)
19	2.02 m	2.01 m	(α) 3.23 dd (13.3, 14.2)
			(β) 1.31 dd (13.3, 3.7)
20	2.16 m	2.13 m	
22	(α) 2.31 d (12.6)	(α) 2.30 d (12.8)	(α) 2.21 d (14.2)
	(β) 2.43 d (12.6)	(β) 2.42 d (12.8)	(β) 2.58 d (14.2)
23	1.25 s	1.61 s	1.60 s
24	1.39 s	4.37 br d (10.1)	4.36 br d (10.0)
		4.44 m	4.44 m
25	1.86 s	1.38 s	1.36 s
26	1.33 s	1.28 s	1.25 s
27	1.57 s	1.57 s	1.61 s
28	1.01 s	0.99 s	1.00 s
29	0.99 d (6.6)	0.95 d (6.4)	1.20 s
30	1.09 d (6.6)	1.08 d (6.4)	1.11 s
1′	5.10 d (7.8)	5.03 d (7.8)	5.04 d (7.5)
2′	4.09 m	4.04 m	4.04 m
3′	4.22 t (9.0)	4.28 m	4.21 m
4′	4.15 t (9.0)	4.27 m	4.27 m
5′	3.97 m	4.01 m	4.02 m
6′	(a) 4.35 dd (12.0, 6.0)	(a) 4.44 m	(a) 4.33 m
6′	(b) 4.57 dd (11.4, 2.4)	(b) 4.58 dd (11.9, 2.2)	(b) 4.57 dd (12.0, 2.4)

showed seven methyl singlet resonances, which suggested compound **3** to be a pentacyclic triterpenoid with an oleanane skeleton.[15] The ^{13}C NMR spectrum revealed 36 carbon signals, as seven methyls, nine methylenes, 11 methines, and nine quarternary carbons. Except for the E ring due to the location of CH_3-29 and CH_3-30, the 1D and 2D NMR data of compound **3** were very close to those of compound **2** (Tables 1 and 2). The HMBC spectrum exhibited correlations from both CH_3-29 (δ 1.20) and CH_3-30 (δ 1.11) to C-21 (δ 214.4) and C-19 (δ 46.6), suggesting that both CH_3-29 and CH_3-30 are linked to C-20. The sugar unit was determined to be D-glucose by the same method as used for compound **1**. The anomeric proton signal resonating at δ 5.04 (1H, d, $J = 7.5$ Hz) confirmed the β-orientation of the glycosidic linkage. The HMBC correlation from H-24 (δ 4.36 and 4.44) to C-1′ (δ 106.5) suggested that the sugar unit is attached to C-24. Therefore, compound **3** was established as 11,21-dioxo-3β,15α,24-trihydroxy-olean-12-ene-24-*O*-β-D-glucopyranoside.

Table 2. ^{13}C NMR Data (150 MHz) for Compounds **1**−**3** (δ_C, C_5D_5N)

position	1	2	3	position	1	2	3
1	45.7	40.5	40.6	19	41.9	41.9	46.6
2	83.6	29.1	29.2	20	50.8	50.8	45.8
3	78.7	79.8	79.8	21	211.1	211.0	214.4
4	39.5	44.0	44.1	22	55.4	55.5	51.5
5	55.1	56.5	56.6	23	30.4	24.0	24.0
6	18.5	19.3	19.3	24	18.6	74.0	74.0
7	36.7	37.1	37.3	25	18.3	17.8	17.7
8	47.3	47.3	47.6	26	19.7	19.5	19.7
9	62.9	62.6	62.8	27	16.3	16.2	18.9
10	37.8	37.6	38.1	28	28.8	28.8	28.9
11	199.8	199.7	200.1	29	18.7	18.7	24.8
12	132.3	132.2	130.3	30	13.2	13.2	25.8
13	164.2	164.0	168.2	1′	108.3	106.5	106.5
14	50.2	50.0	49.8	2′	76.3	75.7	75.7
15	67.1	67.2	66.7	3′	79.3	79.1	79.1
16	40.8	40.8	40.4	4′	72.3	72.0	72.0
17	39.8	39.9	39.5	5′	79.0	79.1	79.1
18	59.0	59.0	48.1	6′	63.6	63.2	63.2

Compound **4** was obtained as a yellow, amorphous powder. The positive-mode HRESIMS exhibited a quasimolecular ion peak at *m*/*z* 763.1861, consistent with the molecular formula, $C_{36}H_{36}O_{17}Na$. The UV spectrum showed absorption maxima at 267 and 334 nm, characteristic of a flavone skeleton.[16] Resonances at δ 6.34 (1H, d, $J = 2.1$ Hz), 6.68 (1H, d, $J = 2.1$ Hz), 6.62 (1H, s), 7.81 (2H, d, $J = 8.8$ Hz), and 6.86 (2H, d, $J = 8.8$ Hz) in the ^{1}H NMR spectrum indicated the presence of a 5,7,4′-tri-*O*-substituted flavone. Signals at δ 6.14 (1H, d, $J = 15.8$ Hz) and 7.25 (1H, d, $J = 15.8$ Hz), along with those at δ 7.03 (1H, d, $J = 1.8$ Hz), 6.64 (1H, d, $J = 8.0$ Hz), 6.81 (1H, dd, $J = 1.8$, 8.0 Hz), and 3.68 (3H, s), suggested the presence of a feruloyl unit. A doublet at δ 5.17 (1H, d, $J = 7.4$ Hz) and a broad singlet at δ 5.35 (1H, br s) in the ^{1}H NMR spectrum revealed the presence of two sugar units, which were assigned as anomeric protons of glucose and apiose, respectively, by comparing the NMR data with those of apiin obtained in a previous investigation.[17] The β-D-glucopyranose moiety was determined by the same method as described for compound **1**. It should be noted that there was no apiose authentic sample at hand, and this branched-chain sugar can occur in four isomeric forms.[18] A β-D-apiofuranose moiety was characterized on the basis of the carbon chemical shift and the broad singlet of the anomeric proton, in combination with the NOE correlation between H-2-Api and H-5-Api.[18−20] The HMBC correlations from H-1″ (δ 5.17) to C-7 (δ 162.4) and from H-1‴ (δ 5.35) to C-2″ (δ 75.5) demonstrated the glucose moiety to be linked to C-7 of the aglycon, with the apiose moiety connected to C-2″ of glucose. The HMBC spectrum also showed a correlation from H-5‴ (δ 4.02) to the carbonyl signal at δ 166.3, indicating the esterified position is at C-5‴. According to the above evidence, compound **4** was identified as apigenin-7-*O*-[2″-*O*-(5‴-*O*-feruloyl)-β-D-apiofuranosyl]- β-D-glucopyranoside.

Compound **5** was also obtained as a yellow, amorphous powder, with the molecular formula, $C_{37}H_{38}O_{18}$, determined from the positive-ion HRESIMS (*m*/*z* 793.1951 $[M + Na]^+$). The UV spectrum showed absorption maxima at 249 and 326 nm. Except for the signals of the B ring in the aglycon moiety, the ^{1}H and ^{13}C NMR data and HMBC and NOESY correlations of compound **5** were found to be very similar to those of compound **4** (Table 3), including the signals for the A ring, the C ring, the two sugar units, and the feruloyl moiety. The ^{1}H NMR spectrum revealed signals at δ 7.42 (1H, d, $J = 2.0$ Hz), 6.88 (1H, d, $J = 8.2$ Hz), and 7.46 (1H, dd, $J = 8.2$, 2.0 Hz), which were assigned to H-2′, H-5′, and H-6′, respectively. The NOESY correlation between the methoxy proton signal at δ 3.84 and H-2′ (δ 7.42) implied that this methoxy group is linked to C-3′. Therefore, the structure of compound **5** was established as chrysoeriol-7-*O*-[2″-*O*-(5‴-*O*-feruloyl)-β-D-apiofuranosyl]-β-D-glucopyranoside.

Table 3. NMR Data (600 MHz for ^{1}H and 150 MHz for ^{13}C) of Compounds **4** and **5** (in DMSO-d_6)

	4		**5**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
2		164.1		164.1
3	6.62 s	103.0	6.73 s	103.4
4		181.8		181.8
5		161.2		161.2
6	6.34 d (2.1)	97.7	6.34 d (2.1)	97.7
7		162.4		162.4
8	6.68 d (2.1)	94.5	6.70 d (2.1)	94.7
9		156.9		156.9
10		105.3		105.3
1′		121.0		121.3
2′	7.81 d (8.8)	128.5	7.42 d (2.0 Hz)	110.2
3′	6.86 d (8.8)	115.9		148.0
4′		161.3		150.9
5′	6.86 d (8.8)	115.9	6.88 d (8.2)	115.7
6′	7.81 d (8.8)	128.5	7.46 dd (8.2, 2.0)	120.4
1″	5.17 d (7.4)	99.0	5.17 d (7.4)	99.0
2″	3.51 m	75.5	3.51 m	75.5
3″	3.71 m	76.6	3.71 m	76.6
4″	3.15 m	69.8	3.15 m	69.8
5″	3.42 m	77.1	3.42 m	77.1
6″	3.66 dd (2.0, 10.0), 3.42 m	60.2	3.66 dd (2.0, 10.0), 3.42 m	60.2
1‴	5.35 br s	108.2	5.35 br s	108.2
2‴	3.49 m	76.8	3.49 m	76.8
3‴		77.5		77.5
4‴	4.02 m, 3.74 m	73.8	4.02 m, 3.74 m	73.8
5‴	4.02 m	66.7	4.02 m	66.7
1⁗		125.3		125.3
2⁗	7.03 d (1.8)	110.7	7.03 d (1.8)	110.7
3⁗		147.7		147.7
4⁗		149.3		149.3
5⁗	6.64 d (8.0)	115.3	6.64 d (8.0)	115.3
6⁗	6.81 dd (1.8, 8.0)	122.9	6.81 dd (1.8, 8.0)	122.9
C=O		166.3		166.3
α	6.14 d (15.8)	113.7	6.14 d (15.8)	113.7
β	7.25 d (15.8)	144.9	7.25 d (15.8)	144.9
−OCH_3-3⁗	3.68 s	55.5	3.68 s	55.5
−OCH_3-3′			3.84 s	55.9
−OH-5	12.88		12.88	

In addition, 10 known flavonoids, luteolin-7-*O*-[2″-*O*-(5‴-*O*-feruloyl)-*β*-D-apiofuranosyl]-*β*-D-glucopyranoside,[21] naringenin-7-*O*-(2-*O*-*β*-D-apiofuranosyl)-*β*-D-glucopyranoside,[22] apiin,[17] luteolin-7-*O*-*β*-D-glucopyranoside,[23] chrysoeriol-7-*O*-*β*-D-glucopyranoside,[23] apigenin-7-*O*-*β*- D-glucopyranoside,[24] luteolin (**6**),[25] chrysoeriol (**7**),[25] apigenin (**8**),[25] and quercetin (**9**),[25] were also isolated and identified by comparison of their spectroscopic data with those reported in the literature (Figure 1).

All compounds isolated were examined for their inhibitory effects on NO production induced by LPS in macrophages (Table 4). Cell viability in the present experiment was determined by the MTT method to find whether inhibition of NO production was due to the cytotoxicity of tested compounds. In this experiment, curcumin (IC_{50} 8.3 ± 1.8 μM) was used as a positive control. Compounds **6**−**9** showed strong inhibition of NO production induced by LPS in macrophages with IC_{50} values of 7.5, 14.2, 6.7, and 11.4 μM, respectively. From the structural features of the skeleton, it was found that only flavone aglycons exhibited inhibitory activity, and introduction of a OCH_3 group led to an increase of activity (e.g., **7**).

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. UV spectra were recorded with a Shimadzu UV 2201 spectrophotometer. IR spectra were conducted on a Bruker IFS 55 spectrometer. 1D NMR (^{1}H, ^{13}C, and DEPT) and 2D (HSQC, HMBC, ^{1}H−^{1}H COSY, and NOESY) spectra were recorded on Bruker ARX-300 and 600 spectrometers. Chemical shifts are stated relative to TMS and expressed in δ values (ppm), with coupling constants reported in Hz. HRESIMS were obtained on an Agilent 6210 TOF mass spectrometer, and ESIMS were recorded on an Agilent 1100-LC/MSD TrapSL mass spectrometer. Silica gel GF_{254} prepared for TLC and silica gel (200−300 mesh) for column chromatography were obtained from Qingdao Marine Chemical Factory (Qingdao, People's Republic of China). Macroporous resin D101 was a product of Chemical Plant of NanKai University (Tianjin, China). Sephadex LH-20 was a product of Pharmacia. Polyamide (80−140 mesh) was supplied by Taizhou Luqiao Sijia Biochemical Company (Zhejiang, People's Republic of China). Octadecyl silica gel was purchased from Merck Chemical Company Ltd. RP-HPLC separations were conducted using a Waters 600 series pumping system equipped with a Waters 490 UV detector and performed with a C_{18} column (250 mm × 20 mm, 10 μm; GL Science Inc.). GC was carried out on a Shimadzu GC-2010 series system and performed with a DB-1701 column (30 m × 0.25 mm × 0.25 μm; Agilent, Santa Clara, CA). All the reagents were HPLC grade or analytical grade and purchased from Tianjin Damao Chemical Company.

Plant Material. The fresh herb of celery (*Apium graveolens*) was purchased in July 2007 from Shenyang Nanta vegetable market, Shenyang, People's Republic of China, and identified by Professor Qishi Sun of the School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University. A voucher specimen (AG-20070716) has been deposited in the herbarium of the Department of Natural Products Chemistry, Shenyang Pharmaceutical University.

Extraction and Isolation. The whole plant of fresh celery (100 kg) was cut into approximately 2 cm pieces and extracted with 70% EtOH (120 L) for 2 h. A portion of the residue after removing EtOH was suspended in water and partitioned with petroleum ether and EtOAc, successively. The EtOAc extract (70 g) was subjected to silica gel column chromatography with a $CHCl_3$−MeOH gradient solvent system (100:0 to 0:100) to obtain 10 fractions (E1−E10), which were combined according to TLC analysis. Fraction E4 (5 g) was subjected to Sephadex LH-20 column chromatography [$CHCl_3$−CH_3OH (1:1)] to yield fractions E41−E44. Fraction E43 (350 mg) was purified by ODS open column chromatography [MeOH−H_2O (1:9 to 7:3)], and resolution of fraction E432 (120 mg) by preparative TLC (cyclohexane−acetone, 5:1) yielded **7** (11.5 mg) and **8** (10.5 mg). Fractions E5 (1.1 g) and E6 (1 g) were recrystallized from $CHCl_3$−MeOH (1:1) to yield **6** (30.5 mg) and **9** (95.5 mg), respectively. Fraction E7 (9 g) [$CHCl_3$−MeOH (10:1)] was passaged over Sephadex LH-20 [$CHCl_3$−CH_3OH (1:1)], to yield fractions E71−E73. Fraction E72 was subjected to ODS column chromatography [MeOH−H_2O (1:9 to 7:3)], and subfraction E723 (200 mg) was separated by RP-HPLC with MeOH−H_2O (2:3) to afford compounds **1** (26.3 mg, t_R 67.6 min), **2** (16.5 mg, t_R 38.2 min), and **3** (20.5 mg, t_R 54.8 min). Fraction E8 (13 g) was chromatographed on polyamide (13−40 mesh), eluted with MeOH−H_2O (0:100 to 100:0). The 50:50 eluent (fraction E83) was concentrated and then separated on polyamide (200−300 mesh) eluted with MeOH−H_2O (0:100 to 100:0) to yield fractions E831−E835. Fraction E832 was subjected to ODS open column chromatography [MeOH−H_2O (1:9 to 8:2)] to yield subfractions E8321−E8325. Fraction E8323 was separated by RP-HPLC with MeOH−H_2O (45:55) to afford luteolin-7-*O*-*β*-D-glucopyranoside (24.6 mg, t_R 31.4 min), chrysoeriol-7-*O*-*β*-D-glucopyranoside (7.6 mg, t_R 37.3 min), and apigenin-7-*O*-*β*-D- glucopyranoside (9.4 mg, t_R 53.4 min). Fraction E833 was subjected to HPLC, using MeOH−H_2O (35:65), to give naringenin-7-*O*-(2-*O*-*β*-D-apiofuranosyl)-*β*-D-glucopyranoside (11.7 mg, t_R 28.7 min) and luteolin-7-*O*-[2″-*O*-(5‴-*O*-feruloyl)-*β*-D-apiofuranosyl]-*β*-D-glucopyranoside (4.3 mg, t_R 41.8 min). Fraction E834 was separated using HPLC with MeOH−H_2O (35:65) to give a mixture of compounds **4** and **5** (16 mg), which was further separated

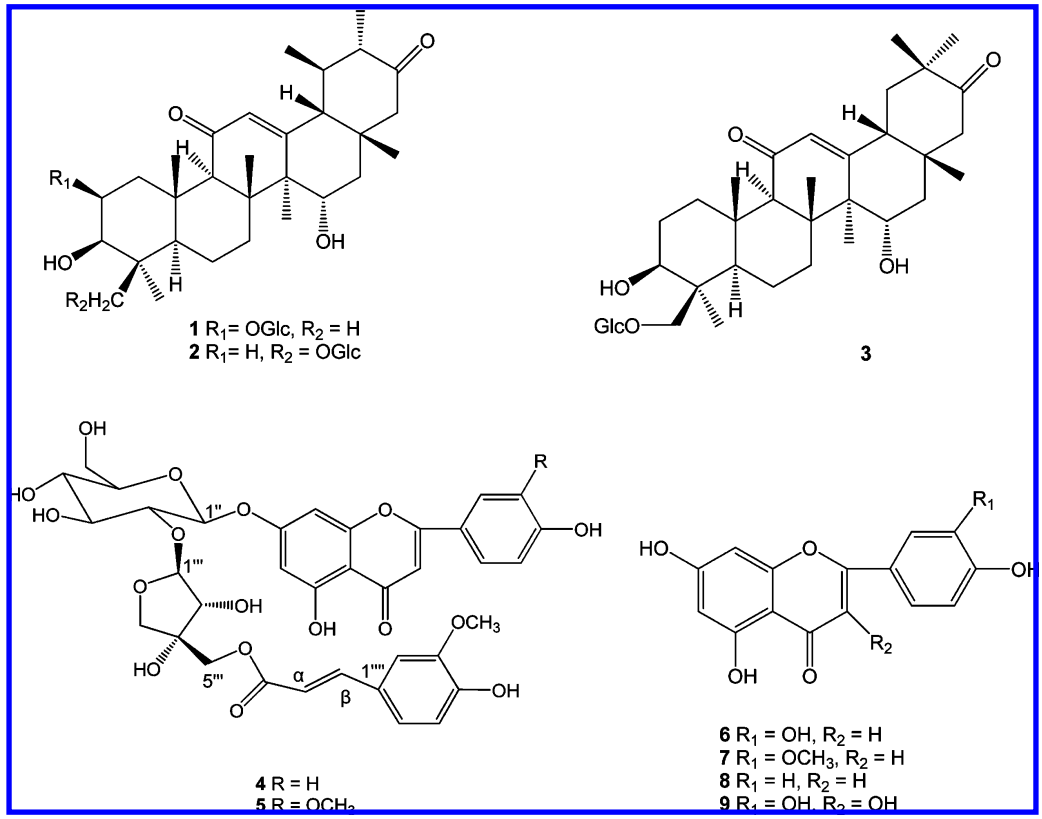

Figure 1

Table 4. Inhibitory Effect of Compounds Isolated from *Apium graveolens* on NO Production Induced by LPS in Macrophages[a,b]

compound	$IC_{50} \pm SD$ (μM)
6	7.5 ± 1.3
7	14.2 ± 2.3
8	6.7 ± 1.4
9	11.4 ± 1.6
curcumin[c]	8.3 ± 1.8

[a] NO concentration of control group, 3.6 ± 0.3 μM; NO concentration of LPS-treated group, 35.7 ± 2.1 μM. [b] Compounds **1**−**5** and luteolin-7-*O*-[2″-*O*-(5‴-*O*-feruloyl)-β-D-apiofuranosyl]-β-D-glucopyranoside, naringenin-7-*O*-(2-*O*-β-D-apiofuranosyl)-β-D-glucopyranoside, apiin, luteolin-7-*O*-β-D-glucopyranoside, chrysoeriol-7-*O*-β-D-glucopyranoside, and apigenin-7-*O*-β-D-glucopyranoside were inactive (IC_{50} >20 μM). [c] Positive control.

by HPLC with MeOH−THF−H_2O (20:13:67) to yield compounds **4** (5.5 mg, t_R 40.2 min) and **5** (6.2 mg, t_R 53.9 min).

The water extract (8 g) was chromatographed on D101 (100 mesh), eluted with a gradient of EtOH−H_2O (0:100 to 95:5), to obtain five fractions (W1−W5). Fraction 3 (50:50 eluent) was concentrated and subjected to polyamide column chromatography (13−40 mesh), eluted with MeOH−H_2O (0:100 to 100:0). The 50:50 eluent was concentrated and crystallized from water to yield apiin (200 mg).

Compound 1: white, amorphous powder (MeOH); $[\alpha]^{25}_D$ +38.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 247 (4.0) nm; IR ν_{max} (KBr) 3418, 2961, 2840, 1658, 1384, 1076 cm^{-1}; ^{1}H NMR (C_5D_5N, 600 MHz), see Table 1, and ^{13}C NMR (C_5D_5N, 150 MHz), see Table 2; ESIMS *m/z* 671 $[M + Na]^+$, 647 $[M - H]^-$, 683 $[M + Cl]^-$, HRESIMS *m/z* 671.3764 $[M + Na]^+$ (calcd for $C_{36}H_{56}O_{10}Na$, 671.3771).

Compound 2: white, amorphous powder (MeOH); $[\alpha]^{25}_D$ +29.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 246 (4.0) nm; IR ν_{max} (KBr) 3387, 2948, 2840, 1652, 1453, 1031 cm^{-1}; ^{1}H NMR (C_5D_5N, 600 MHz), see Table 1, and ^{13}C NMR (C_5D_5N, 150 MHz), see Table 2; ESIMS *m/z* 671 $[M + Na]^+$, 647 $[M - H]^-$, 683 $[M + Cl]^-$, HRESIMS *m/z* 671.3763 $[M + Na]^+$ (calcd for $C_{36}H_{56}O_{10}Na$, 671.3771).

Compound 3: white, amorphous powder (MeOH); $[\alpha]^{25}_D$ +31.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 247 (4.1) nm; IR ν_{max} (KBr) 3400, 2948, 2840, 1649, 1452, 1018 cm^{-1}; ^{1}H NMR (C_5D_5N, 600 MHz), see Table 1, and ^{13}C NMR (C_5D_5N, 150 MHz), see Table 2; ESIMS *m/z* 671 $[M + Na]^+$, 647 $[M - H]^-$, 683 $[M + Cl]^-$, HRESIMS *m/z* 671.3758 $[M + Na]^+$ (calcd for $C_{36}H_{56}O_{10}Na$, 671.3771).

Compound 4: yellow, amorphous powder (MeOH); $[\alpha]^{25}_D$ −28.5 (*c* 0.1, EtOH); UV (MeOH) λ_{max} (log ε) 267 (3.9), 334 (4.2) nm; IR ν_{max} (KBr) 3361, 2946, 2833, 1656, 1451, 1030, 670 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) see Table 3; ESIMS *m/z* 763 $[M + Na]^+$, 739 $[M - H]^-$; HRESIMS *m/z* 763.1861 $[M + Na]^+$ (calcd for $C_{36}H_{36}O_{17}Na$, 763.1850).

Compound 5: yellow, amorphous powder (MeOH); $[\alpha]^{25}_D$ −25.6 (*c* 0.1, EtOH); UV (MeOH) λ_{max} (log ε) 249 (3.6), 334 (4.1) nm; IR ν_{max} (KBr) 3358, 2946, 2833, 1657, 1451, 1030, 668 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz), see Table 3; ESIMS *m/z* 793 $[M + Na]^+$, 769 $[M - H]^-$; HRESIMS *m/z* 793.1951 $[M + Na]^+$ (calcd for $C_{37}H_{38}O_{18}Na$, 793.1956).

Acid Hydrolysis of Compounds 1−5. A solution of each compound (2.0 mg) in 1 N HCl (1 mL) was stirred at 90 °C in a stoppered vial for 2 h. The solution after cooling was evaporated under a stream of N_2. Anhydrous pyridine solutions (0.1 mL) of each residue and L-cysteine methyl ester hydrochloride (0.06 N) were mixed and warmed at 60 °C for 1 h. Then, trimethylsilyl imidazole (0.15 mL) was added, and the mixture was warmed at 60 °C for another 30 min. After drying the solution, the residue was partitioned between H_2O and cyclohexane. The cyclohexane layer was concentrated, then dissolved in 200 μL of acetone, and analyzed by GC using a DB-1701 column. Temperatures of the injector and detector were 270 and 280 °C, respectively. A temperature gradient system was used for the oven, starting at 160 °C for 1 min and increasing up to 230 °C at a rate of 5 °C/min. The peaks of authentic samples of D-glucose and L-glucose after treatment in the same manner were detected at 24.08 and 25.50 min.

Bioassay for NO Production. Mouse monocyte-macrophage RAW 264.7 cells (ATCC TIB-71) were purchased from the Chinese Academy of Science, People's Republic of China. RPMI 1640 medium, penicillin, streptomycin, and fetal bovine serum were purchased from Invitrogen (New York). Lipopolysaccharide (LPS), dimethylsufoxide (DMSO), 3-(4,5-dimethyl-2-thiazolyl)- 2,5-diphenyltetrazolium bromide (MTT), and curcumin were obtained from Sigma Chemical Co. (St. Louis, MO). RAW 264.7 cells were suspended in RPMI 1640 medium supplemented

with penicillin (100 U/mL), streptomycin (100 μg/mL), and 10% heat-inactivated fetal bovine serum. The cells were harvested with trypsin and diluted to a suspension in fresh medium. The cells were seeded in 96-well plates with 1×10^5 cells/well and allowed to adhere for 2 h at 37 °C in 5% CO_2 in air. Then, the cells were treated with 1 μg/mL of LPS for 24 h with or without various concentrations of test compounds. DMSO was used as a solvent for the test compounds, which were applied at a final concentration of 0.2% (v/v) in cell culture supernatants. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using Griess reagent.[26] Briefly, 100 μL of the culture supernatants from incubates was mixed with an equal volume of Griess reagent (0.1% *N*-[1-naphthyl]ethylenediamine and 1% sulfanilamide in 5% H_3PO_4). Cytotoxicity was determined by the MTT colorimetric assay, after 24 h incubation with test compounds. The concentration of NO_2^- was calculated by using 0, 1, 2, 5, 10, 20, 50, and 100 μM sodium nitrite solutions, and the inhibitory rate on NO production induced by LPS was calculated by the NO_2^- levels as follows:

$$\text{Inhibitory rate (\%)} = 100 \times \frac{[NO_2^-]_{LPS} - [NO_2^-]_{LPS+sample}}{[NO_2^-]_{LPS} - [NO_2^-]_{untreated}}$$

Experiments were performed in triplicate, and data are expressed as the mean ± SD of three independent experiments.

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