Pyridinium Alkaloid-Coupled Secoiridoids from the Flower Buds of *Lonicera japonica*

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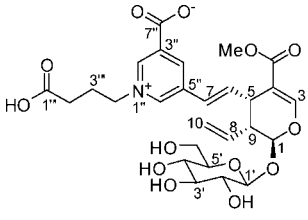
Three pyridinium inner salt alkaloids, lonijaposides A−C (**1**−**3**), based on an unprecedented skeleton of an *N*-substituted nicotinic acid nucleus coupled through C-5 with C-7 of a secoiridoid, together with seven known iridoids, have been isolated from the flower buds of *Lonicera japonica*. Their structures were elucidated by spectroscopic and chemical analyses. Lonijaposide C (**3**) showed activity against the release of glucuronidase in rat polymorphonuclear leukocytes induced by the platelet-activating factor.

The flower buds of *Lonicera japonica* Thunb. (Caprifoliaceae) represent one of the most common traditional Chinese medicines and are used for the treatment of various diseases including arthritis, diabetes mellitus, fever, infections, sores, and swelling.[1] Pharmacological studies indicated that extracts of the flower buds of *L. japonica* have a broad spectrum of biological activities, such as antibacterial, anti-inflammatory, antioxidant, antipyretic, antiviral, and hepato-protective effects.[1,2] A number of chemical constituents with diverse structures including cerebrosides, flavonoids, iridoids, polyphenols, and saponins have been reported from different parts of this plant.[3] As part of a program to assess the chemical and biological diversity of several traditional Chinese medicines,[4] three pyridinium inner salt alkaloids based on an unprecedented skeleton, designated lonijaposides A (**1**), B (**2**), and C (**3**), together with loganin, kingiside, morroniside, secologanin, sweroside, secologanoside, and secoxyloganin,[3] have been isolated from an aqueous extract of the flower buds of *L. japonica.* This extract showed activity in a preliminary anti-inflammatory assay against the release of glucuronidase in rat polymorphonuclear leukocytes induced by the platelet-activating factor with a 42% inhibition rate at 25 μg/mL. We report herein the isolation, structural elucidation, and biological test results of **1**−**3**.



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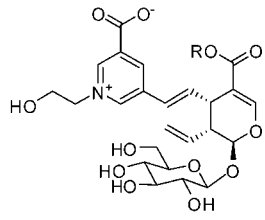

2 R = Me
3 R = H

Compound **1** was obtained as a beige amorphous solid with $[\alpha]^{20}_{D}$ −167.1 (*c* 0.40, H_2O). Its IR spectrum showed absorption bands for hydroxy (3362 cm^{-1}) and α,β-unsaturated ester carbonyl (1699 cm^{-1}) groups. The (+)- and (−)-ESIMS of **1** exhibited quasimolecular ion peaks at *m/z* 580 $[M + H]^+$ and 602 $[M + Na]^+$ and at *m/z* 578 $[M - H]^-$, respectively. (+)-HRESIMS at *m/z* 580.2071 $[M + H]^+$ indicated the molecular formula of **1** to be $C_{27}H_{33}NO_{13}$ (calcd for $C_{27}H_{34}NO_{13}$, 580.2030). In the deshielded region, the ^1H NMR spectrum of **1** in D_2O showed three singlets at δ 9.02 (1H, s, H-2″), 8.83 (1H, s, H-4″), and 8.85 (1H, s, H-6″), indicating the presence of a 3,5-disubstituted pyridine moiety in **1**. The ^1H NMR spectrum also displayed a singlet attributed to a deshielded trisubstituted olefinic proton at δ 7.77 (1H, s, H-3) and signals due to a *trans*-double bond substituted at both ends with a methine and a quaternary carbon, respectively, at δ 6.72 (1H, dd, J = 16.0, 7.5 Hz, H-6) and δ 6.64 (1H, d, J = 16.0 Hz, H-7). In addition, a group of signals was observed, attributed to a vinyl group attached to another methine at δ 5.81 (1H, ddd, J = 17.5, 10.5, 7.0 Hz, H-8), 5.34 (1H, d, J = 10.5 Hz, H-10b), and 5.39 (1H, d, J = 17.5 Hz, H-10a). The protons of the two methines resonated at δ 3.74 (1H, dd, J = 7.5, 7.5 Hz, H-5) and 2.87 (1H, ddd, J = 7.5, 7.5, 7.0 Hz, H-9), respectively. The ^1H NMR spectrum showed also a doublet assignable to an acetal proton at δ 5.59 (1H, d, J = 7.5 Hz, H-1), a methoxy singlet at δ 3.74 (3H, s, O*Me*), two methylene triplets at δ 4.66 (2H, t, J = 7.0 Hz, H_2-4‴) and 2.37 (2H, t, J = 7.0 Hz, H_2-2‴), and a methylene multiplet at δ 2.31 (2H, m, H_2-3‴). Characteristic signals due to a β-glucopyranosyl unit at δ 4.91 (1H, d, J = 8.0 Hz, H-1′), 3.35 (1H, dd, J = 9.0, 8.0 Hz, H-2′), 3.54 (1H, dd, J = 9.0, 9.0 Hz, H-3′), 3.41 (1H, dd, J = 9.0, 9.0 Hz, H-4′), 3.50 (1H, ddd, J = 9.0, 6.0, 2.0 Hz, H-5′), 3.91 (1H, dd, J = 12.0, 2.0 Hz, H-6′a), and 3.71 (1H, dd, J = 12.0, 6.0 Hz, H-6′b) were also observed. The presence of a β-glucopyranosyl unit was confirmed by enzymatic hydrolysis of **1** with β-glucosidase, which produced glucose identified on the basis of TLC by comparing with an authentic sugar sample. The glucose isolated from the hydrolysate gave a positive optical rotation, $[\alpha]^{20}_{D}$ +45.1 (*c* 0.37, H_2O), indicating that it was the β-D-glucose.[5] The ^{13}C NMR and DEPT spectra of **1** showed carbon resonances corresponding to the above structural units and an ester carbonyl (δ > 170 ppm) (Table 1). These data indicated that **1** is an unusual alkaloid β-D-glucoside possessing a 3,5-disubstituted pyridine moiety.

The structure of **1** was finally determined by a comprehensive analysis of its 2D NMR spectroscopic data. The proton and protonated carbon signals in the NMR spectra of **1** were assigned unequivocally on the basis of heteronuclear correlations in the gHMQC spectrum (Table 1). In the $^1H-^1H$ COSY spectrum of **1**, homonuclear vicinal coupling correlations (Figure 1, thick lines) from H-1 through H-9, H-5, and H-6 to H-7, in turn, of which H-9 correlated through H-8 with H_2-10, and a *W*-type homonuclear correlation between H-3 and H-5, in combination with chemical shifts and coupling constants of these protons, demonstrated unambiguously the presence of a secoiridoid moiety with the *trans* double bond between C-6 and C-7 and the vinyl group at C-9. Also, correlations from H-1′ through H-2′, H-3′, H-4′, and H-5′ to H_2-6′ confirmed the presence of the β-D-glucopyranosyl unit. In addition, vicinal coupling correlations between H_2-3‴ with both H_2-2‴ and H_2-4‴ indicated that there is a 3-substituted propyl group in **1**. In the HMBC spectrum, two- and three-bond correlations (Figure 1, arrows) from H-1 to C-3, C-8, and C-9, from H-5 to C-1, C-3, C-4, C-6, C-7, C-9, and C-11, from H-7 to C-5, from H-8 to C-1,

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Table 1. NMR Spectroscopic Data (δ) for Lonijaposides A (**1**), B (**2**), and C (**3**)[a]

	1		**2**		**3**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	5.59 d (7.5)	100.1	5.59 d (7.5)	100.1	4.46 d (7.5)	100.0
3	7.77 s	157.2	7.78 s	157.2	7.34 s	154.3
4		109.8		109.8		110.8[b]
5	3.74 dd (7.5, 7.5)	40.8	3.76 dd (7.5, 7.5)	40.8	3.72 dd (7.5, 7.5)	41.0
6	6.72 dd (16.0, 7.5)	140.3	6.73 dd (16.0, 7.5)	140.4	6.72 dd (16.0, 7.5)	141.4
7	6.64 d (16.0)	128.3	6.65 d (16.0)	128.3	6.58 d (16.0)	128.1
8	5.81 ddd (17.5, 10.5, 7.0)	136.0	5.81 ddd (17.5, 10.5, 7.0)	136.0	5.81 ddd (17.5, 10.5, 7.0)	136.5
9	2.87 ddd (7.5, 7.5, 7.0)	47.3	2.87 ddd (7.5, 7.5, 7.0)	47.3	2.85 ddd (7.5, 7.5, 7.0)	47.7
10a	5.39 d (17.5)	122.7	5.39 d (17.5)	122.7	5.36 d (17.5)	122.6
10b	5.34 d (10.5)		5.34 d (10.5)		5.31 d (10.5)	
11		172.1		172.1		175.9
1′	4.91 d (8.0)	102.0	4.91 d (8.0)	102.0	4.88 d (8.0)	102.0
2′	3.35 dd (9.0, 8.0)	75.3	3.35 dd (9.0, 8.0)	75.3	3.34 dd (9.0, 8.0)	75.5
3′	3.54 dd (9.0, 9.0)	78.4	3.54 dd (9.0, 9.0)	78.4	3.53 dd (9.0, 9.0)	78.6
4′	3.41 dd (9.0, 9.0)	72.3	3.41 dd (9.0, 9.0)	72.3	3.41 dd (9.0, 9.0)	72.5
5′	3.50 ddd (9.0, 6.0, 2.0)	79.1	3.50 ddd (9.0, 6.0, 2.0)	79.1	3.50 ddd (9.0, 6.0, 2.0)	79.3
6′a	3.91 dd (12.0, 2.0)	63.3	3.91 dd (12.0, 2.0)	63.4	3.91 dd (12.0, 2.0)	63.6
6′b	3.71 dd (12.0, 6.0)		3.71 dd (12.0, 6.0)		3.71 dd (12.0, 6.0)	
2″	9.02 s	145.6	9.01 s	145.8	8.98 s	145.9
3″		139.8		139.7		140.8
4″	8.83 s	143.9	8.88 s	144.1	8.87 s	144.3
5″		140.6		140.4		140.8
6″	8.85 s	145.4	8.84 s	145.7	8.84 s	145.9
7″		170.5[b]		170.6		170.8
1‴		182.1[b]	4.11 t (5.0)	63.0	4.10 t (5.0)	63.2
2‴	2.37 t (7.0)	35.2	4.76 t (5.0)	66.4	4.74 t (5.0)	66.6
3‴	2.31 m	29.6				
4‴	4.66 t (7.0)	64.2				
OMe	3.74 s	54.7	3.74 s	54.7		

[a] NMR data (δ) were measured in D_2O at 500 MHz for proton and at 125 MHz for carbon. The assignments were based on DEPT, $^1H-^1H$ COSY, HSQC, HMQC, and HMBC experiments. [b] Data obtained from the HMBC spectra of **1** and **3**.

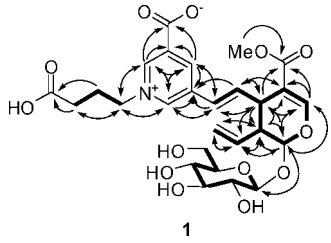

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Figure 1. Main $^1H-^1H$ COSY (thick lines) and HMBC (arrows from proton to carbon) correlations of lonijaposide A (**1**).

C-9, and C10, from H_2-10 to C-8 and C-9, from O*Me* to C-11, from H-1 to C-1′, and from H-1′ to C-1, together with chemical shift values of these protons and carbons, confirmed that **1** possesses a secologan-6-en-7-yl moiety similar to the co-occurring secologanin.[3b] HMBC correlations from H-2″ to C-3″, C-4″, C-6″, and C-7″ (δ 170.5, not observed in the ^{13}C NMR spectrum), from H-4″ to C-2″, C-3″, C-6″, C-7″, and C-7, from H-6″ to C-2″, C-4″, C-5″, and C-7, from H-6 to C-5″, and from H-7 to C-4″, C-5″, and C-6″, together with chemical shift values of these protons and carbons, demonstrated the presence of a structure of the 5-(secologan-6-en-7-yl)-3-carboxypyridine unit in **1**. In addition, HMBC correlations from H_2-2‴ to C-1‴ (δ 182.1, not seen in the ^{13}C NMR spectrum), C-3‴, and C-4‴, from H_2-3‴ to C-1‴, C-2‴, and C-4‴, from H_2-4‴ to C-3‴, from both H-2″ and H-6″ to C-4‴, and from H_2-4‴ to both C-2″ and C-6″ indicated the presence of a 3-carboxypropyl unit at the nitrogen of the pyridine unit to form a pyridinium moiety in **1**. Taking into account the molecular composition, **1** was assigned as an inner salt form. Although both C-1‴ and C-7″ were not observable in the ^{13}C NMR spectrum of **1**, their chemical shifts were accurately assigned from the heteronuclear correlations in the HMBC spectrum (Supporting Information), indicating that C-7″ was shielded 11.6 ppm from C-1‴. This suggested that C-7″ and C-1‴ were in the anionic and carboxylic acid forms, respectively. Therefore, the planar structure of **1** was elucidated as an unusual pyridinium inner salt alkaloid possessing a nicotinic acid nucleus substituted at *N*-1 and C-5 with 3-carboxypropyl and secologan-6-en-7-yl, respectively.

The relative configuration of **1** was determined on the basis of a NMR NOE-difference experiment and by biogenetic inferences.[6] In the NOE difference spectrum of **1**, irradiation of H-1 enhanced the H-6, H-7, and H-8 resonances, while irradiation of H-9 enhanced H-5. These data indicated that **1** has a relative stereochemistry identical to the co-occurring secologanin. From biosynthetic precedents, the 1*S*,9*R*-configuration of the secologan-6-en-7-yl moiety could be proposed.[7,8] Therefore, the structure **1** was proposed, and this compound was given the trivial name, lonijaposide A.

Compound **2** was obtained as a beige amorphous solid, with $[\alpha]^{20}_D$ –171.1 (*c* 0.09, H_2O). The (+)- and (−)-ESIMS of **2** exhibited quasimolecular ion peaks at *m*/*z* 538 $[M + H]^+$ and 560 $[M + Na]^+$ and at *m*/*z* 536 $[M - H]^-$, respectively. The molecular formula of **2**, $C_{25}H_{31}NO_{12}$ (calcd for $C_{25}H_{32}NO_{12}$, 538.1925), was indicated from the HRESIMS at *m*/*z* 538.1908 $[M + H]^+$. The IR and NMR spectroscopic features of **2** showed some similarities to those of **1**, except that the NMR signals due to the 3-carboxypropyl of **1** were replaced by signals attributable to an ethanol-2-yl unit [δ_H 4.11 (2H, t, J = 5.0 Hz, H-1‴) and 4.76 (2H, t, J = 5.0 Hz, H-2‴), and δ_C 63.0 and 66.4] in the NMR spectra of **2**. This suggested that **2** is a derivative of **1** containing an ethanol-2-yl unit at *N*-1 replacing the 3-carboxylpropyl substituent of **1**. This was confirmed by 2D NMR experiments of **2** that enabled the unambiguous assignment of its NMR data (Table 1). In particular, in the HMBC spectrum of **2**, long-range heteronuclear correlations from H-2‴ to both C-2″ and C-6″ and from both H-2″ and H-6″ to C-2‴ proved the location of the ethanol-2-yl unit in **2**. Therefore, the structure **2** was determined for lonijaposide B.

Compound **3** was obtained as a beige amorphous solid, with $[\alpha]^{20}_D$ −81.0 (*c* 0.32, H_2O). The (+)- and (−)-ESIMS of **3** exhibited quasimolecular ion peaks at *m*/*z* 524 $[M + H]^+$, 546 $[M + Na]^+$, and 562 $[M + K]^+$, and at *m*/*z* 522 $[M - H]^-$, respectively.

HRESIMS at *m/z* 524.1729 $[M + H]^+$ indicated the molecular formula of **3** as $C_{24}H_{29}NO_{12}$ (calcd for $C_{24}H_{30}NO_{12}$, 524.1768), which represented a CH_2 unit less than that of **2**. The IR and NMR spectra of **3** closely resembled those of **2** except for the absence of signals for a methoxy group. A comparison of the NMR data between **2** and **3** (Table 1) indicated that H-1, H-3, and H-7 of **3** were shielded significantly by $\Delta\delta_H$ 1.13, 0.44, and 0.07 ppm, respectively, and that C-11 was deshielded by $\Delta\delta_C$ 3.8 ppm. These data suggested that **3** is the demethyl analogue of **2**. This was verified by the 2D NMR spectroscopic data of **3** (Supporting Information). Although the resonance of C-4 was not clearly observable in the ^{13}C NMR spectrum of **3**, the HMBC spectrum indicated that there was a carbon resonance at δ_C 110.8 that correlated with H-3, H-5, H-6, and H-9. Thus, the structure of **3** was determined, and the compound was named as lonijaposide C.

On a biosynthetic basis, **1** may be derived from an enzymatic-catalyzed coupling between nicotinic acid, γ-hydroxy- or γ-aminobutyric acid, and the co-occurring secologanin, and **2** and **3** from coupling among nicotinic acid, 2-aminoethanol (or 1,2-ethandiol), and the co-occurring secologanin (for **2**) or secologanic acid (for **3**). Compound **2** may also result from an enzymatic-catalyzed decarboxylation of **1** or methylation of **3**, and **3** from demethylation of **2**.[9] Although pyridinium inner salt alkaliods are not rare in marine sponges and terrestrial plants, their structural diversity is somewhat limited. Pyridinebetaine A, possessing a nicotinic acid nucleus substituted at *N*-1 with an ethanol-2-yl group, as well as its pyrrol-2-carboxylate (daminin) and 4-bromopyrrol-2-carboxylate (agelongine) were isolated from the marine sponges *Agelas dispa*[10] and *Axinella damicornis*,[11] while *N*-(3-amino-3-carboxypropyl)-nicotinic acid was isolated from a fungus *Lentinus edodes*[12] and tobaco leaves.[13] In addition, tripterospermumcin A, a secologanol nicotinic acid ester, was reported from *Tripterospermum chinensis*.[14] Compounds **1**−**3** are unusual examples of pyridinium inner salt alkaloids possessing the nicotinic acid nucleus substituted at *N*-1 and C-5 with 3-carboxypropyl (for **1**) or ethanol-2-yl (for **2** and **3**) and a complex secoiridoid glucoside derivative moiety, respectively.

In an in vitro assay, lonijaposide C (**3**) showed inhibitory activity against the release of glucuronidase in rat polymorphonuclear leukocytes (PMNs) induced by the platelet-activating factor (PAF) with an inhibition rate of 69.5% at 10 μM, while compounds lonijaposides A and B (**1** and **2**) give inhibitory activities with 11.0% and 35.8% inhibition rates at the same concentration, respectively.[15] This suggested that the ethanol-2-yl unit at *N*-1 and the acid form of C-11 may increase the activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a PE model 343. UV and CD spectra were measured on a JASCO J-810 spectropolarimeter. IR spectra were recorded on a Nicolet 5700 FT-IR Microscope spectrometer (FT-IR Microscope Transmission). 1D- and 2D-NMR spectra were obtained at 500 MHz for ^1H and at 125 MHz for ^{13}C, respectively, on an INOVA 500 MHz spectrometer in D_2O with TMS as references. ESIMS data were measured with a Q-Trap LC/MS/MS (Turbo Ionspray source) spectrometer. HRESIMS data were, in turn, measured on an AccuToFCS JMS-T100CS spectrometer. Column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, People's Republic of China) and Pharmadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). HPLC separation was performed on an instrument consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual λ absorbance detector with a Prevail (250 × 10 mm i.d.) semipreparative column packed with C_{18} (5 μm). TLC was carried out with glass precoated silica gel GF254 plates. Spots were visualized under UV light or by spraying with 7% H_2SO_4 in 95% EtOH followed by heating.

Plant Material. The air-dried flower buds of *Lonicera japonica* were purchased in Beijing, People's Republic of China, which were collected at Henan province, in May 2005, People's Republic of China. The plant identification was verified by Professor Lin Ma (Institute of Materia Medica). A voucher specimen (no. 20050509) was deposited at the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Beijing, People's Republic of China.

Extraction and Isolation. The air-dried flower buds of *L. japonica* (12.0 kg) were extracted with H_2O (15.0 L) for 3 × 12 h at room temperature. The combined extract was evaporated to 2.0 L under reduced pressure and then subjected to column chromatography over macroporous adsorbent resin (SP-700, 5 kg, dried weight). The column was eluted successively with H_2O, 20% EtOH, 50% EtOH, and 95% EtOH (5000 mL each), to yield four fractions (A−D) after removing the solvents. Fraction B (350 g) was subjected to chromatography over Pharmadex LH-20 with H_2O as the mobile phase to give subfractions B_1−B_8 by TLC analysis. Subfraction B_3 (135 g) was chromatographed over a normal-phase silica gel column, eluting with a gradient of MeOH (0–100%) in $CHCl_3$, to yield subfractions B_3-1−B_3-15. Of these, subfraction B_3-13 (4.8 g) was further separated by flash chromatography over reversed-phase silica gel, eluting with a gradient of EtOH (0–30%) in H_2O to give subfractions (B_3-13-1− B_3-13-15). B_3-13-10 (527 mg) and B_3-13-5 (60 mg) were separately subjected to reversed-phase semipreparative HPLC, for B_3-13-10 using CH_3CN-H_2O (11:89) containing 0.5% HOAc as the mobile phase, to afford **1** (65 mg, 0.000541%) and **2** (3.1 mg, 0.000026%) and for B_3-13-5 using CH_3CN−H_2O (5:95) with 0.5% HOAc as the mobile phase, to afford **3** (6.3 mg, 0.000053%).

Lonijaposide A (1). Beige amorphous solid; $[\alpha]^{20}_D$ –167.1 (*c* 0.40, H_2O); UV (H_2O) λ_{max} (log ϵ) 232 (5.04), 265 (4.84), 309 (4.25) nm; CD (H_2O) λ_{max} (*c* 0.000581 mol/L, $\Delta\epsilon$) 212 (+0.23), 232 (−2.82), 244 (−2.53), 265 (−6.15) nm; IR ν_{max} 3362, 2932, 1699, 1636, 1589, 1438, 1389, 1304, 1166, 1077, 939 cm^{-1}; ^1H NMR (D_2O, 500 MHz) and ^{13}C NMR (D_2O, 125 MHz) data, see Table 1; positive-mode ESIMS *m/z* 580 $[M + H]^+$ and 602 $[M + Na]^+$; negative-mode ESIMS *m/z* 578 $[M - H]^-$; HRESIMS at *m/z* 580.2071 $[M + H]^+$ (calcd for $C_{27}H_{34}NO_{13}$, 580.2030).

Lonijaposide B (2). Beige amorphous solid; $[\alpha]^{20}_D$ −171.1 (*c* 0.09, H_2O); UV (H_2O) λ_{max} (log ϵ) 231 (5.03), 266 (4.80), 308 (4.33) nm; CD (H_2O) λ_{max} (*c* 0.000388 mol/L, $\Delta\epsilon$) 212 (−0.77), 228 (−2.15), 242 (−1.82), 261 (3.72); IR ν_{max} 3352, 2926, 1695, 1635, 1612, 1436, 1387, 1304, 1165, 1075, 938, 893, 786, 770 cm^{-1}; ^1H NMR (D_2O, 500 MHz) and ^{13}C NMR (D_2O, 125 MHz) data, see Table 1; positive-mode ESIMS *m/z* 538 $[M + H]^+$ and 560 $[M + Na]^+$; negative-mode ESIMS *m/z* 536 $[M - H]^-$; HRESIMS *m/z* 538.1908 $[M + H]^+$ (calcd for $C_{25}H_{32}N_1O_{12}$, 538.1925).

Lonijaposide C (3). Beige amorphous solid; $[\alpha]^{20}_D$ –81.0 (*c* 0.32, H_2O); UV (H_2O) λ_{max} (log ϵ) 231 (5.03), 264 (4.82), 311 (4.32) nm; CD (H_2O) λ_{max} (*c* 0.000701 mol/L, $\Delta\epsilon$) 212 (−1.70), 223 (−3.35), 245 (−1.54), 266 (−3.97); IR ν_{max} 3339, 2926, 1635, 1607, 1544, 1385, 1155, 1065, 1041, 941, 788, 679 cm^{-1}; ^1H NMR (D_2O, 500 MHz) and ^{13}C NMR (D_2O, 125 MHz) data, see Table 1; positive-mode ESIMS *m/z* 524 $[M + H]^+$, 546 $[M + Na]^+$ and 562 $[M + K]^+$; negative-mode ESIMS *m/z* 522 $[M - H]^-$; HRESIMS *m/z* 524.1729 $[M + H]^+$ (calcd for $C_{24}H_{30}NO_{12}$, 524.1768).

Enzymatic Hydrolysis of 1. A solution of compound **1** (10 mg) in H_2O (3 mL) was treated with β-glucosidase from almonds (Fluka) (10 mg) at 37 °C for 30 h. The reaction mixtures were extracted with EtOAc (3 × 3 mL). The aqueous phase of the hydrolyzate was dried using a stream of N_2 and then subjected to column chromatography over silica gel, eluting with CH_3CN−H_2O (8:1) to yield glucose (1.8 mg). It gave a positive optical rotation, $[\alpha]^{20}_D$ + 45.1 (*c* 0.37, H_2O). The solvent system CH_3CN−H_2O (6:1) was used for TLC identification of glucose (R_f = 0.33).

Anti-Inflammatory Activity Assay.[15] Test compounds were dissolved in DMSO with a concentration of 0.1 mol/L and diluted with RPMI-1640 to 10^{-3} mol/L. A suspension of rat polymorphonuclear leukocytes (PMNs) (245 μL) at a density of 2.5 × 10^6 cells/mL and the test samples (2.5 μL) were incubated at 37 °C for 15 min. Cytochalasin B (2.5 μL, 1 mM) (Sigma) was added and incubated for 5 min, followed by the addition of 0.2 μM of platelet-activating factor (PAF) (Sigma) (2.5 μL). After 10 min, the reaction was terminated in an ice bath. The supernatant was obtained by centrifugation at 4000 rpm for 5 min. Then, 25 μL of the supernatant and 2.5 mM phenolphthalein glucuronic acid (25 μL) were incubated with 100 μL of 0.1 M acetic acid buffer (pH 4.6) at 37 °C, 5% CO_2, for 18 h. The reaction was stopped by addition of 0.3 M NaOH (150 μL). The absorbance was read at 550 nm. The inhibitory ratio (IR) was calculated as follows

$$\text{IR}\ (\%) = (A_{PAF} - A_t)/(A_{PAF} - A_C) \times 100\%$$

A_{PAF}, A_t, and A_c refer to the average absorbance of three wells of PAF, the test compound, and control groups, respectively.

Ginkgolide B was used as the positive control and gave an inhibition rate of 86.6% at 10 μm.

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Supporting Information Available: IR, MS, 1D, and 2D NMR, CD, and UV spectra of compounds **1**, **2**, and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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