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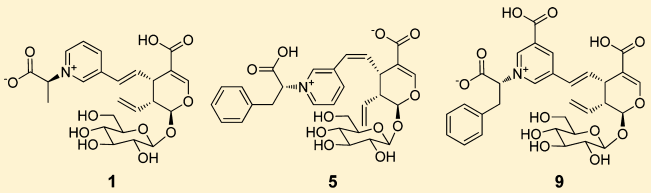
Homosecoiridoid Alkaloids with Amino Acid Units from the Flower Buds of *Lonicera japonica*

Yang Yu,[†] Chenggen Zhu,[†] Sujuan Wang, Weixia Song, Yongchun Yang, and Jiangong Shi*

State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China

Supporting Information

ABSTRACT: Nine new homosecoiridoid alkaloids, named lonijaposides O–W (**1**–**9**), along with 19 known compounds, were isolated from an aqueous extract of the flower buds of *Lonicera japonica*. Their structures and absolute configurations were determined by spectroscopic data analysis and chemical methods. Lonijaposides O–W have structural features that involve amino acid units sharing the N atom with a pyridinium (**1**–**5**) or nicotinic acid (**6**–**9**) moiety. The absolute configurations of the amino acid units were determined by oxidation of each pyridinium ring moiety with potassium ferricyanide, hydrolysis of the oxidation product, and Marfey's analysis of the hydrolysate. This procedure was validated by oxidizing and hydrolyzing synthetic model compounds. The phenylalanine units in compounds **4**, **5**, and **9** have the D-configuration, and the other amino acid units in **1**–**3** and **6**–**8** possess the L-configuration. Compounds **1**, **4**, **6**, and **9** and the known compounds 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 5′-*O*-methyladenosine exhibited antiviral activity against the influenza virus A/Hanfang/359/95 (H3N2) with IC_{50} values of 3.4–11.6 μM, and **4** inhibited Coxsackie virus B3 replication with an IC_{50} value of 12.3 μM.



The flower buds of *Lonicera japonica* Thunb., known as "Jin Yin Hua" in Chinese, provide some of the most common ingredients for formulations used in traditional Chinese medicine to treat influenza, cold, fever, and infections.[1−5] The constituents of the extracts of *L. japonica* flower buds, which exhibit differing structural features and biological activities, have been characterized in chemical and pharmacological studies; some of the identified compound classes have been caffeoyl quinic acids, secoiridoids, flavonoids, saponins, cerebrosides, polyphenols, and nitrogen-containing iridoids.[6−14] As part of a program to assess the chemical and biological diversity of traditional Chinese medicines, a detailed chemical study was conducted on an aqueous extract of *L. japonica* flower buds, since their decoctions are used in a variety of formulations. Our previous studies on the aqueous extract led to the isolation of 18 homosecoiridoids with the following structural characteristics: secoiridoid nuclei coupled with N-substituted nicotinic acid or pyridine units (lonijaposides A–N) and secoiridoid nuclei coupled with phenylpyruvic acid-derived moieties (loniphenyruviridosides A–D).[15,16] Additionally, aqueous extractions were performed on the flower buds; then the residue was further extracted with EtOH (95%), which resulted in the characterization of six new aromatic glycosides and 48 known compounds.[17] The present report describes a continuation of this investigation on the aqueous extract, and nine new homosecoiridoid alkaloids containing amino acid units (**1**–**9**) have been isolated in addition to 19 known compounds. Reported herein are the isolation, structure determination, and biological activity of these isolates.

RESULTS AND DISCUSSION

The IR spectrum of compound **1** indicated the presence of hydroxy group, carboxylic acid, conjugated double bond, and aromatic ring absorptions. Its molecular formula of $C_{24}H_{29}NO_{11}$ was determined by HRESIMS combined with the NMR data (Table 1 and Experimental Section). The NMR spectra of **1** showed resonances attributable to a terminal monosubstituted double bond, a *trans*-disubstituted double bond, and a trisubstituted double bond. They also displayed resonances due to four methines (dioxygen-bearing and nitrogen-bearing), a methyl group vicinal to a methine, and two quaternary carboxyl groups; there were also diagnostic resonances for pyridinium-3″-yl and β-glucopyranosyl moieties. These data indicated that **1** is an analogue of a homosecoiridoid possessing a N-substituted pyridinium moiety.[15,16] The presence of a β-glucopyranosyl unit was confirmed by enzymatic hydrolysis of **1** with snailase, which produced glucose as identified by TLC comparison using an authentic sugar sample. The glucose isolated from the hydrolysate gave a positive optical rotation, $[\alpha]^{20}_{D}$ +42.7 (*c* 0.09, H_2O), indicating it to be D-glucose.[15,16] The structure of the aglycone moiety of **1** was established by 2D-NMR data analysis. The proton resonances and protonated carbon resonances in the NMR spectra were assigned on the basis of the gHSQC data. In the $^1H-^1H$ gCOSY spectrum of **1**, homonuclear vicinal coupling correlations between H-1/H-9/H-5/H-6/H-7 and H-9/H-8/H_2-10, combined with the chemical shifts and coupling

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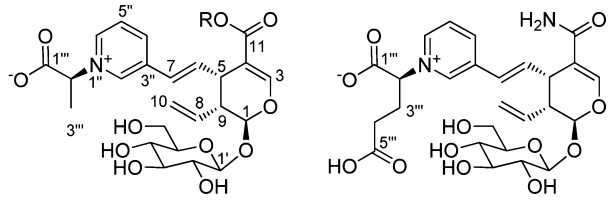

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2 R = Me

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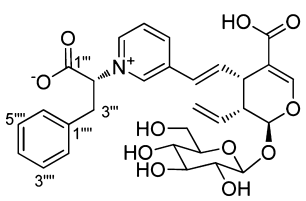

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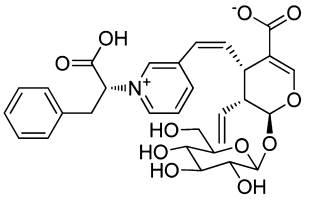

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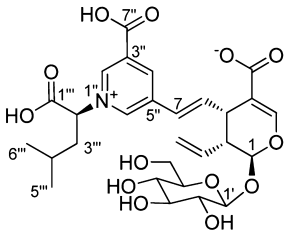

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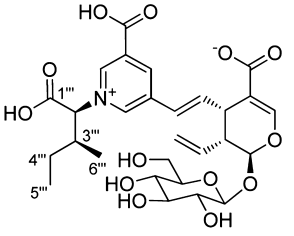

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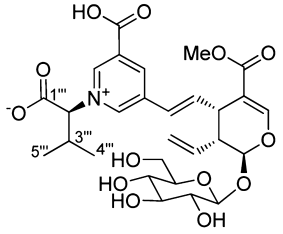

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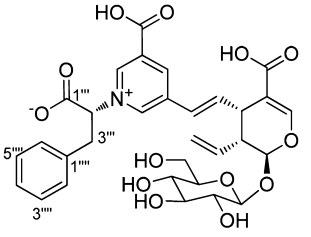

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constants of these protons, demonstrated the presence of a secoiridoid moiety with a C-6−C-7 *trans*-double bond and a vinyl group at C-9. This was confirmed in the gHMBC spectrum by the two- and three-bond heteronuclear correlations of H-1/C-3, C-5, and C-8; H-3/C-1, C-4, C-5, and C-11; H-5/C-1, C-3, C-4, C-6, C-7, C-8, C-9, and C-11; H-6/C-4, C-5, C-7, and C-9; H-7/C-5 and C-6; H-8/C-1, C-5, and C-9; H-9/C-1, C-4, C-5, C-6, C-8, and C-10; and H_2-10/C-8 and C-9. Also, gCOSY correlations of H-1′/H-2′/H-3′/H-4′/H-5′/H_2-6′, combined with gHMBC correlations of H-1/C-1′ and H-1′/C-1, confirmed that the *β*-D-glucopyranosyl unit is located at C-1 in **1**. The gCOSY correlations of H-5″ with H-4″ and H-6″, together with gHMBC correlations of H-2″/C-3″, C-4″, C-6″, and C-7; H-4″/C-2″, C-6″, and C-7; H-5″/C-3″, C-4″, and C-6″; H-6″/C-2″, C-4″, and C-5″; H-6/C-3″; and H-7/C-2″, C-3″, and C-4″, provided evidence for the occurrence of the pyridinium-3″-yl moiety at C-7. In addition, a vicinal coupling gCOSY correlation between H-2‴ and H_3-3‴, gHMBC correlations of H-2‴/C-1‴, C-3‴, C-2″, and C-6″ and H_3-3‴/C-1‴ and C-2‴, and their chemical shifts revealed an alanine unit sharing the N atom with the pyridinium moiety. Accordingly, the planar structure of **1** was elucidated as shown, which is different from lonijaposides I and K−M[16] with respect to the substituent at the N atom of the pyridinium moiety. Hence, the deshielded shift of C-3 and the shielded shifts of C-4 and C-11 suggested that the inner salt is formed via an alanine carboxylate in **1**. Similarities between the NMR and circular dichroism (CD) data of compound **1** and lonijaposides H and K−M demonstrated that these compounds have the same configuration in the secoiridoid moiety, which was determined by a single-crystal X-ray crystallographic analysis of the co-occurring secologanic acid using anomalous scattering of Cu K*α* radiation.[16] To determine the absolute configuration of the alanine unit, the pyridinium moiety in **1** was oxidized with potassium ferricyanide $[K_3Fe(CN)_6]$,[18] then hydrolyzed using 6 N HCl to liberate the amino acid, and finally analyzed by Marfey's method.[19] This resulted in the assignment of the L-configuration to the alanine unit in **1** (Figure S12, Supporting Information). Therefore, the structure of compound **1** was determined as shown, with this isolate assigned as lonijaposide O.

Compound **2** gave the molecular formula $C_{25}H_{31}NO_{11}$, with one more CH_2 unit than **1**, as indicated by HRESIMS. Comparison of the NMR data between these compounds (Tables 1 and 2) indicated the presence of an additional methoxy group [δ_H 3.73 (s) and δ_C 54.8] in **2**. Additionally, the H-3 and C-3 resonances in **2** were deshielded by $\Delta\delta_H$ +0.16 and $\Delta\delta_C$ +2.3 ppm, respectively, whereas the C-4 and C-11 resonances were shielded, in turn, by $\Delta\delta_C$ −2.7 and −3.1 ppm. This revealed that **2** is the methyl 11-carboxylate of **1**, which was confirmed by 2D-NMR data analysis. In particular, a HMBC correlation of OCH_3/C-11 supported the location of the methoxy group. The chemical shifts of the alanine unit in **2** were in good agreement with those of **1**, which confirmed the inner salt to be formed via the alanine carboxylate in both compounds. The L-configuration of the alanine unit in **2** was verified by the same protocol as described for **1**. Thus, the structure of compound **2** (lonijaposide P) was determined as shown.

The spectroscopic data of compound **3**, lonijaposide Q, indicated that it is an analogue of **1**, with the molecular formula $C_{26}H_{32}N_2O_{12}$. Comparison of the NMR data between **3** and **1** (Tables 1 and 2) revealed that instead of containing the alanine unit and OH-11 group of **1**, **3** contains a glutamic acid unit and an amino group at these respective positions. The 11-carbamide was indicated by the chemical shift of H-3 (δ_H 7.52) in the ^1H NMR spectrum and the undetected C-4 and C-11 resonances in the ^{13}C NMR spectrum of **3**, which distinguished it from those of **1**, **2**, and other derivatives with an 11-carboxylate and 11-carboxylic acid unit.[15,16] The presence of the glutamic acid unit was confirmed by two- and three-bond correlations between H-2″ and H-6″/C-2‴; H-2‴/C-1‴, C-4‴, C-2″, and C-6″; H_2-3‴/C-1‴, C-2‴, C-4‴, and C-5‴; and H_2-4‴/C-2‴, C-3‴, and C-5‴ in the gHMBC spectrum of **3**. The chemical shifts of C-1‴ (δ_C 174.5) and C-5‴ (δ_C 179.9) indicated that the inner salt is formed via the 1‴-carboxylate. Oxidation of **3** using $K_3Fe(CN)_6$ followed by acid hydrolysis and Marfey's analysis of the hydrolysate supported the L-configuration for the glutamic acid unit in **3**. Therefore, the structure of compound **3** was determined as shown.

Compound **4** exhibited spectroscopic data similar to those of **1**. The molecular formula $C_{30}H_{33}NO_{11}$ of **4** was determined from its HRESIMS and NMR data. On comparison of their NMR data, this indicated that instead of containing an alanine unit as in **1**, **4** contains a phenylalanine unit, as verified by 2D-NMR analysis (Figures S40−S42, Supporting Information). A gCOSY correlation of H-2‴/H_2-3‴ and HMBC correlations of H-2‴/C-1‴; H_2-3‴/C-1‴, C-2‴, and C-6‴; and H-2″ and H-6″/C-2‴, in addition to their shifts, confirmed the presence of the phenylalanine unit that shared the N atom with the

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Table 1. ^{1}H NMR Spectroscopic Data (δ) for Lonijaposides O–W (1–9)[a]

no.	1	2	3	4[b]	5[b]	6	7	8	9[b]
1	5.53 d (7.2)	5.57 d (7.8)	5.50 d (7.5)	5.50 d (7.5)	5.48 d (8.4)	5.57 d (7.2)	5.48 d (7.2)	5.60 d (7.5)	5.51 d (7.2)
3	7.60 s	7.76 s	7.52 s	7.75 s	7.26 s	7.69 s	7.41 s	7.78 s	7.71 s
5	3.70 dd (7.8, 7.2)	3.71 dd (7.8, 7.2)	3.72 dd (7.5, 7.0)	3.61 dd (8.0, 6.5)	3.11 dd (12.0,5.4)	3.70 dd (7.2, 6.6)	3.70 dd (7.2, 6.6)	3.75 dd (7.5, 7.5)	3.64 dd (7.8, 7.8)
6	6.68 dd (15.6, 7.8)	6.67 dd (16.2, 7.8)	6.68 dd (15.5, 7.0)	6.23 dd (16.0, 8.0)	5.83 t (12.0)	6.73 dd (15.6, 7.2)	6.71 dd (16.2, 7.2)	6.73 dd (16.0, 7.5)	6.28 dd (16.2, 7.8)
7	6.59 d (15.6)	6.59 d (16.2)	6.58 dd (15.5)	6.43 d (16.0)	6.53 d (12.0)	6.63 d (15.6)	6.59 d (16.2)	6.67 d (16.0)	6.49 d (16.2)
8	5.81 ddd (17.4, 10.2, 7.8)	5.79 ddd (18.0, 10.8, 7.8)	6.81 ddd (17.5, 10.5, 8.5)	5.70 ddd (16.5, 10.0, 7.5)	5.68 ddd (16.8, 10.2, 7.2)	5.82 ddd (16.8, 10.2, 7.8)	5.82 ddd (17.4, 10.8, 7.8)	5.82 ddd (18.0, 10.0, 7.5)	5.73 ddd (17.4, 10.2, 7.8)
9	2.86 ddd (7.8, 7.2, 7.2)	2.85 ddd (7.8, 7.8, 7.2)	2.86 m	2.80 ddd (7.5, 7.5, 6.5)	2.36 ddd (8.4, 7.2, 5.4)	2.87 ddd (7.8, 7.2, 6.6)	2.85 ddd (7.8, 7.2, 6.6)	2.87 ddd (7.5, 7.5, 7.5)	2.82 ddd (7.8, 7.8, 7.2)
10a	5.38 d (17.4)	5.37 d (18.0)	5.37 d (17.5)	5.33 d (17.0)	5.13 d (10.2)	5.38 d (16.8)	5.37 d (17.4)	5.39 d (18.0)	5.34 d (17.4)
10b	5.33 d (10.2)	5.33 d (10.8)	5.32 d (10.5)	5.31 d (10.5)	4.96 d (16.8)	5.34 d (10.2)	5.32 d (10.8)	5.35 d (10.0)	5.33 d (10.2)
1′	4.90 d (8.0)	4.90 d (8.0)	4.90 d (8.0)	4.90 d (8.0)	4.85 d (7.8)	4.90 d (8.0)	4.88 d (7.8)	4.93 d (8.0)	4.91 d (7.8)
2′	3.35 dd (8.0, 9.0)	3.34 dd (9.0, 8.0)	3.34 dd (9.0, 8.0)	3.34 dd (9.0, 8.0)	3.30 dd (9.0, 7.8)	3.35 dd (9.0, 8.0)	3.34 dd (9.0, 7.8)	3.35 dd (9.0, 8.0)	3.35 dd (9.0, 7.8)
3′	3.54 dd (9.0, 9.0)	3.53 dd (9.0, 9.0)	3.54 dd (9.0, 9.0)	3.54 dd (9.0, 9.0)	3.52 dd (9.0, 9.0)	3.54 dd (9.0, 9.0)	3.53 dd (9.0, 9.0)	3.55 dd (9.0, 9.0)	3.55 dd (9.0, 9.0)
4′	3.41 dd (9.0, 9.0)	3.40 dd (9.0, 9.0)	3.41 dd (9.0, 9.0)	3.42 dd (9.0, 9.0)	3.38 dd (9.0, 9.0)	3.41 dd (9.0, 9.0)	3.41 dd (9.0, 9.0)	3.42 dd (9.0, 9.0)	3.43 dd (9.0, 9.0)
5′	3.50 m	3.49 m	3.49 m	3.50 m	3.48 m	3.49 m	3.48 m	3.50 m	3.51 m
6′a	3.92 d (12.0)	3.91 d (12.0)	3.91 d (12.5)	3.91 d (12.0)	3.92 d (12.0)	3.91 d (12.0)	3.91 d (12.0)	3.91 d (12.0)	3.92 d (12.0)
6′b	3.72 dd (12.0, 6.0)	3.70 dd (12.0, 6.0)	3.71 dd (12.5, 6.0)	3.72 dd (12.0, 6.0)	3.71 dd (12.0, 6.0)	3.72 dd (12.0, 6.0)	3.73 dd (12.0, 6.0)	3.71 dd (12.0, 6.0)	3.75 dd (12.0, 6.0)
2″	8.77 s	8.76 s	8.80 s	8.34 s	8.72 s	9.03 s	9.08 s	9.10 s	8.88 s
4″	8.55 d (7.2)	8.53 d (7.8)	8.58 d (8.0)	8.35 d (8.0)	8.40 d (8.4)	8.89 s	8.89 s	8.90 s	8.70 s
5″	7.99 dd (7.2, 6.6)	7.99 dd (7.8, 6.0)	8.01 dd (8.0, 6.5)	7.87 dd (8.0, 6.0)	7.98 dd (8.4, 6.0)				
6″	8.68 d (6.6)	8.69 d (6.0)	8.70 d (6.5)	8.59 d (6.0)	8.74 d (6.0)	8.85 s	8.88 s	8.90 s	8.38 s
2‴	5.35 q (7.2)	5.35 q (7.2)	5.22 m	5.49 m	5.43 dd (12.0, 4.2)	5.32 m	4.89 d (8.4)	4.82 d (7.0)	5.58 dd (9.0, 4.2)
3‴	1.93 d (7.2)	1.92 d (7.2)	2.33 m; 2.38 m	3.75 m; 3.50 m	3.90 m; 3.51 m	2.29 m; 2.22 m	2.45 m	2.65 m	3.72 m; 3.57 m
4‴			2.56 m; 2.76 m			1.35 m	1.24 m; 1.08 m	1.15 d (7.0)	
5‴						0.92 d (6.6)	0.87 t (7.2)	0.85 d (7.0)	
6‴						0.97 d (6.6)	1.11 d (7.2)		
OMe		3.73 s						3.75 s	

[a]NMR data (δ) were measured in D_2O at 600 MHz for **1**, **2**, **5**–**7**, and **9** and at 500 MHz for **3**, **4**, and **8**. Coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, ^{1}H–^{1}H COSY, gHSQC, and HMBC experiments, and the data were presented as calculated using the solvent peak (δ 4.80 ppm) as the reference. Data of the phenyl unit: δ 7.08 (2H, d, J = 7.0 Hz, H-2‴″ and H-6‴″), 7.25 (3H, m, H-3‴″, H-4‴″, and H-5‴″) for **4**; 7.11 (2H, d, J = 7.2 Hz, H-2‴″ and H-6‴″), 7.29 (2H, t, J = 7.2 Hz, H-3‴″ and H-5‴″), and 7.25 (H, t, J = 7.2 Hz, H-4‴″) for **5**; 7.09 (2H, d, J = 7.2 Hz, H-2‴″ and H-6‴″) and 7.27 (3H, m, H-3‴″, H-4‴″, and H-5‴″) for **9**.

pyridinium moiety in **4**. The chemical shifts of C-3, C-4, C-11, and C-1‴, as compared with those of lonijaposides I and K–M,[16] suggested that **4** possesses 11-carboxylic acid and 1‴-carboxylate units. The phenylalanine unit was determined to have the D-configuration using the same protocol as described for **1**. Therefore, the structure of compound **4** (lonijaposide R) was determined as shown.

The spectroscopic data of compound **5** indicated it to be an isomer of **4**. Comparison of the NMR data of these two compounds revealed a variation in the coupling constant between H-6 and H-7 ($J_{6,7}$), which was 16.0 Hz in **4** and 12.0 Hz in **5**. This suggests that **5** is a geometrical isomer of **4** at the C-6–C-7-*cis* double bond, which was corroborated by 2D-NMR and NOE difference spectroscopic data analysis (Figures S52–S55, Supporting Information). As observed in the NOE difference spectrum of **5**, irradiation of H-6 enhanced H-1 and H-7, which verified that H-6 and H-7 are *cis*-oriented and that the secoiridoid moiety has the same configuration as in **4**. The chemical shifts of the C-3, C-4, and C-11 resonances in **5** were consistent with those of lonijaposides I and K–M.[16] This demonstrated that the inner salt is formed via the 11-carboxylate. By applying Marfey's method, the D-configuration was assigned to the phenylalanine unit in **5** (lonijaposide S), for which the structure was determined as shown.

Compound **6** gave the molecular formula $C_{28}H_{35}NO_{13}$, as indicated by its (+)-HRESIMS and NMR data. The NMR data of **6** (Tables 1 and 2) revealed the presence of leucine and 3″,5″-disubstituted nicotinic acid units in place of the respective alanine and pyridinium-3″-yl moieties in **1**. This was verified by the gCOSY cross-peaks of H-2‴/H_2-3‴, H-4‴/H_3-5‴, and H_3-6‴ and gHMBC correlations of H-2‴and H_2-3‴/C-1‴; H_3-5‴, H_3-6‴/C-3‴, and C-4‴; H-2″/ C-3″, C-4″, C-6″, and C-7″; H-4″/C-2″, C-5″, C-6″, and C-7″; and H-6″/C-2″, C-4″, and C-5″. gHMBC correlations of H-2″ and H-6″/C-2‴, in addition to their chemical shifts, also confirmed that the leucine and nicotinic acid units share the same N atom. The chemical shifts of the C-3, C-4, and C-11 resonances indicated the formation of the inner salt via the 11-carboxylate. Oxidation of **6** followed by acid hydrolysis and Marfey's analysis of the hydrolysate revealed an L-configuration for the leucine unit. Thus, the structure of compound **6** (lonijaposide T) was determined as shown.

The spectroscopic data of compound **7** (Tables 1 and 2 and Experimental Section) demonstrated that it is an isomer of **6**

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Table 2. ^{13}C NMR Spectroscopic Data (δ) for Lonijaposides O–W (1–9)[a]

no.	1	2	3	4[b]	5[b]	6	7	8	9[b]
1	99.9	100.1	100.0	100.2	98.9	99.8	99.7	100.2	100.0
3	154.9	157.2	154.4	157.5	151.5	151.8	151.7	157.3	156.6
4	112.6	109.9	N.D.	110.1	116.1	116.0	115.9	109.9	111.0
5	40.9	40.8	41.3	40.6	37.2	41.3	41.1	40.9	40.6
6	140.4	139.9	140.9	140.1	139.9	141.7	141.6	140.2	140.5
7	128.3	128.6	128.2	128.4	126.0	127.9	127.9	128.5	128.1
8	136.3	136.0	1366	136.1	137.5	136.8	136.7	136.1	136.1
9	47.5	47.4	47.8	47.5	47.2	47.8	47.7	47.4	47.5
10	122.5	122.7	122.6	122.9	122.5	122.4	122.4	122.7	122.8
11	175.3	172.2	N.D.	173.3	177.5[c]	177.9	177.7	172.2	174.2
1′	101.9	102.0	102.0	102.1	101.9	101.9	101.8	102.1	102.0
2′	75.4	75.4	75.5	75.5	75.4	75.5	75.4	75.4	75.4
3′	78.4	78.5	78.6	78.6	78.5	78.5	78.4	78.5	78.5
4′	72.3	72.3	72.5	72.4	72.4	72.5	72.4	72.3	72.3
5′	79.1	79.2	79.3	79.3	79.2	79.2	79.1	79.2	79.2
6′	63.5	63.5	63.5	63.5	63.5	63.5	63.5	63.5	63.5
2″	144.4	144.5	144.9	145.0	145.8	145.3	145.4	145.5	145.6
3″	140.5	140.4	141.1	140.1	140.6	139.4	139.3	139.3	139.2
4″	144.5	144.6	145.2	145.1	148.7	144.3	145.2	144.3	144.6
5″	130.2	130.2	130.5	130.4	130.0	140.7	140.5	140.2	139.8
6″	144.4	144.3	144.9	144.7	145.0	145.5	144.3	145.2	145.4
7″						170.9	170.8	170.5	170.9[c]
1‴	176.5	176.5	174.5	174.5	174.6	176.1	175.0	175.3[c]	172.3[c]
2‴	74.0	74.0	78.5	79.7	79.9	77.5	84.3	88.9	79.9
3‴	20.7	20.7	34.4	42.0	41.6	43.9	40.4	34.5	41.9
4‴			31.1			27.9	27.3	21.5	
5‴			179.9			24.9	12.8	20.5	
6‴						23.2	17.8		
OMe		54.8						54.8	

[a]Data were measured in D_2O at 125 MHz for **1**–**5**, **7**, and **9** and at 150 MHz for **6** and **8**. The assignments were based on DEPT, ^{1}H–^{1}H COSY, HSQC, HMQC, and HMBC experiments, and the data were presented as calculated using C-6′ (δ 63.5 ppm) as the reference. "N.D." means that the carbon resonance was not displayed in the spectrum. [b]Data of the phenyl unit: δ 138.4 (C-1⁗), 131.8 (C-2⁗′ and H-6⁗), 132.1 (C-3⁗ and C-5⁗), and 130.6 (C-4⁗) for **4**; 138.3 (C-1⁗), 131.4 (C-2⁗ and H-6⁗), 132.1 (C-3⁗ and C-5⁗), and 130.4 (C-4⁗) for **5**; 138.2 (C-1⁗), 131.8 (C-2⁗ and H-6⁗), 132.0 (C-3⁗ and C-5⁗), and 130.6 (C-4⁗) for **9**. [c]The resonance data were obtained from the HMBC spectrum.

with an isoleucine unit in place of the leucine unit. This was corroborated by gCOSY correlations of H-2‴/H-3‴/H_2-4‴/H_3-5‴ and H-3‴/H_3-6‴ and gHMBC correlations of H_2-4‴/C-2‴ and C-6‴; H_3-5‴/C-3‴ and C-4‴; H_3-6‴/C-2‴, C-3‴, and C-4‴; and H-2″ and H-6″/C-2‴ (Figures S77–S79, Supporting Information). The L-configuration of the isoleucine unit was determined by applying Marfey's analysis in combination with the chemical shifts of the C-5‴ and C-6‴ resonances.[20−22] Therefore, the structure of compound **7** (lonijaposide U) was determined as shown.

Compound **8** was found to be a further isomer of **6**, as indicated from its spectroscopic data (Tables 1 and 2 and Experimental Section). The NMR data of **8** suggested the presence of valine and methoxy units in place of the leucine moiety in **6**. Although resonances for the carboxylic carbons (C-7″ and C-1‴) were not observed in the ^{13}C NMR spectrum of **8** due to the limited amount of sample obtained, their chemical shifts were assigned on the basis of the correlations from H-2″ and H-4″ to the carbon resonance at δ_C 170.5 (C-7″) and from H-2‴ to the carbon resonance at δ_C 175.3 (C-1‴), respectively, in the HMBC spectrum (Figure S90, Supporting Information). Additionally, the gCOSY spectrum of **8** displayed vicinal coupling correlations between H-3‴ and H-2‴, H_3-4‴, and H_3-5‴, and the gHMBC spectrum exhibited long-range correlations from H-3 and OCH_3 to C-11 and from H-2″, H-6″, H_3-4‴, and H_3-5‴ to C-2‴. These correlations provided evidence that the OCH_3 group is located at C-11 and that the valine unit shares the N atom with the nicotinic acid moiety in **8**. Similarities between the chemical shifts of the nicotinic acid moieties of **8** with **7** suggested that the inner salt is formed via the 1‴-carboxylate. Therefore, the L-configuration was assigned to the valine unit based on Marfey's analysis. Accordingly, the structure of compound **8** (lonijaposide V) was determined as shown.

The spectroscopic data of compound **9** (Tables 1 and 2 and Experimental Section) indicated that it is an analogue of **6** with a phenylalanine unit in place of the leucine unit. This was corroborated by 2D-NMR data analysis (Figures S100–S102, Supporting Information). As compared to those of **6**, the shifts of the C-3, C-4, C-11, and C-1‴ resonances suggested that the inner salt is formed via the 1‴-carboxylate in **9**. By application of the aforementioned Marfey's method, the phenylalanine unit in **9** was determined to have the D-configuration. Therefore, the structure of compound **9** (lonijaposide W) was determined as shown.

Since the sample amounts of the isolated compounds **1**–**9** were limited, the protocol used to determine the absolute configurations of the amino acid units was first established by the oxidation and hydrolysis of synthetic model compounds. Alkylation of nicotinic acid (**10**) and pyridine (**10′**) with 1-

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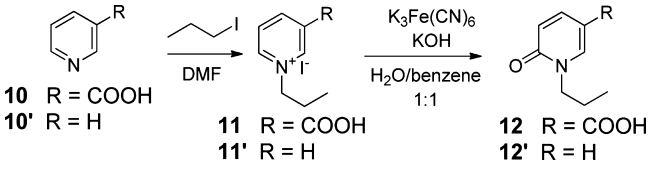
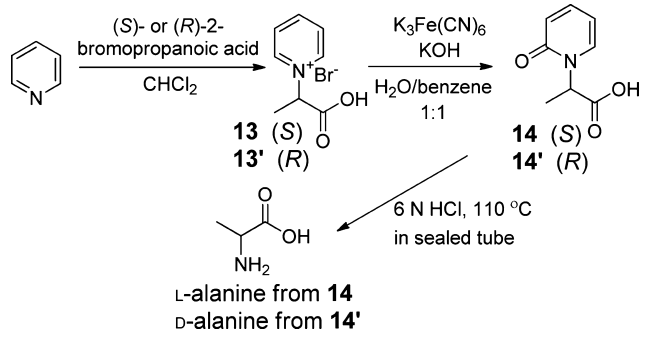
iodopropane yielded 3-carboxy-1-propylpyridin-1-ium iodide (**11**) and 1-propylpyridin-1-ium iodide (**11′**), respectively, which were oxidized by $K_3Fe(CN)_6$ to produce 6-oxo-1-propyl-1,6-dihydropyridine-3-carboxylic acid (**12**) and 1-propylpyridin-2(1*H*)-one (**12′**) (Scheme 1).[18] This demonstrated that

Scheme 1. Synthesis and Oxidation of 3-Carboxy-1-propylpyridin-1-ium Iodide (11) and 1-Propylpyridin-1-ium Iodide (11′)



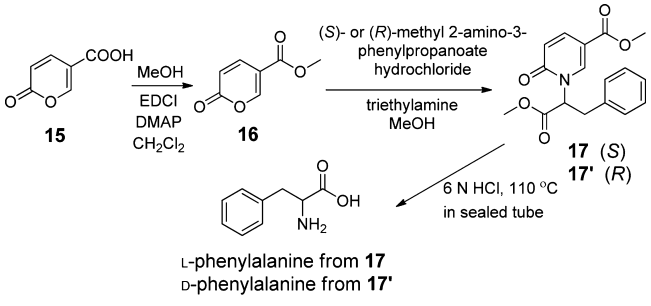
the pyridinium moiety in compounds **1**–**9** could be oxidized readily by $K_3Fe(CN)_6$ to produce the hydrolyzable amide derivatives. In addition, (*S*)- and (*R*)-2-[2-oxopyridin-1(2*H*)-yl]propanoic acid (**14** and **14′**) were prepared by alkylation of pyridine with (*S*)- and (*R*)-2-bromopropanoic acid, respectively, and oxidation of the alkylated products (**13** and **13′**) with $K_3Fe(CN)_6$ (Scheme 2). (*S*)- and (*R*)-Methyl 1-(1-

Scheme 2. Synthesis and Hydrolysis of (*S*)- and (*R*)-2-[2-Oxopyridin-1(2*H*)-yl]propanoic Acid (14 and 14′)



methoxy-1-oxo-3-phenylpropan-2-yl)-6-oxo-1,6-dihydropyridine-3-carboxylate (**17** and **17′**) were synthesized from commercially available coumalic acid (2-oxo-2*H*-pyran-5-carboxylic acid, **15**) and L- or D-methyl 2-amino-3-phenylpropanoate hydrochloride,[23] respectively (Scheme 3). Hydrolysis of the two pairs of stereoisomers **14** and **14′** and **17** and **17′** with 6 N HCl at 110 °C for 16 h produced L- and D-alanine and L- and D-phenylalanine, respectively, as identified by

Scheme 3. Synthesis and Hydrolysis of (*S*)- and (*R*)-Methyl 1-(1-Methoxy-1-oxo-3-phenylpropan-2-yl)-6-oxo-1,6-dihydropyridine-3-carboxylate (17 and 17′)



comparing the NMR and $[\alpha]^{20}_D$ data with those of the authentic samples. This confirmed that the amide derivatives could be hydrolyzed to release the corresponding amino acids, and their configurations could then be determined by Marfey's method.[19]

The known compounds were identified by comparing their spectroscopic data with reported data as adinoside A,[24] stryspinoside,[25] dimethyl secoxyloganoside,[26] ketologanin,[27] chlorogenic acid, methyl chlorogenate, 3,5-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid, caffeic acid, protocatechuic acid,[28] 5-caffeoylquinic acid,[29] 4-caffeoylquinic acid,[30] syringin,[31] caffeic acid 4-*O*-*β*-D-glucopyranoside,[30] vanillic acid 4-*O*-*β*-D-(6-*O*-benzoyl)glucopyranoside,[28] gentisic acid 5-*O*-*β*-D-glucopyranoside,[32] benzyl 2-*O*-*β*-D-glucopyranosyl-2,6-dihydroxybenzoate,[33] adenosine, and 5′-*O*-methyladenosine.[34]

In in vitro assays, compounds **1**, **4**, **6**, and **9** and the known compounds 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 5′-*O*-methyladenosine showed antiviral activity against the influenza virus A/Hanfang/359/95 (H3N2),[35] with respective IC_{50} values of 11.6, 6.8, 10.3, 8.2, 10.2, 4.9, and 3.4 μM and respective SI values of 23.0, 41.5, 16.2, 32.3, 120.3, 88.7, and 48.3. (The positive control, oseltamivir, gave an IC_{50} value of 1.3 μM and a SI value of 1164.2.) Compound **4** inhibited Coxsackie virus B3 replication with an IC_{50} of 12.3 μM (the positive control, pleconaril, gave an IC_{50} value of 0.3 μM).[35] Compounds **1**, **2**, and **4** and caffeic acid, at 10 μM, showed inhibitory activity against the release of glucuronidase in rat polymorphonuclear leukocytes (PMN) induced by PAF with 63.2 ± 3.6%, 72.3 ± 2.8%, 53.8 ± 5.6%, and 40.3 ± 3.3% inhibition rates, respectively, while the other isolates exhibited inhibition rates lower than 30%. The positive control (ginkgolide B) gave an inhibition rate of 78.0 ± 4.3% at the same concentration.[15,36] These results, combined with our previous studies,[15,16] show that diverse compounds contribute toward pharmacological efficacy, which supports the traditional uses of *L. japonica* flower buds. In addition, these compounds were also assessed for their inhibitory activity against HIV-1 replication[37] and several human cancer cell lines,[38] but all were inactive at a concentration of 10 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a model 343 polarimeter (Perkin-Elmer). 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 300, 400, 500, or 600 MHz for ^{1}H and 75, 100, 125, or 150 MHz for ^{13}C, respectively, on INOVA 300 MHz, 400 MHz, 500 MHz, or SYS 600 MHz spectrometers (Varian), with solvent peaks serving as references (unless otherwise noted). ESIMS data were measured with a Q-Trap LC/MS/MS (Turbo Ionspray source) spectrometer. HRESIMS data were, in turn, measured on an AccuTOF-CS JMS-T100CS spectrometer (JEOL). 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 with a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual λ absorbance detector (detecting wavelength: 250 nm) on a Prevail (250 × 10 mm i.d.) semipreparative column packed with C_{18} (5 μm) (flow rate: 2 mL/min). Glass, precoated silica gel GF254 plates were used for TLC. Spots were visualized under UV light or by spraying with 7% H_2SO_4 in 95% EtOH followed by heating.

Plant Material. See refs 15 and 16.

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Extraction and Isolation. For the extraction and preliminary fractionation of the extract, see ref 15. Fraction B_3-10 (22.1 g) was separated by MPLC over RP silica gel and eluted with a gradient of EtOH (0−50%) in H_2O to give subfractions B_3-10-1−B_3-10-13. Subfraction B_3-10-7 (1.6 g) was separated further by flash chromatography over RP silica gel, eluted with a gradient of CH_3CN (0−30%) in H_2O, to obtain subfractions B_3-10-7-1−B_3-10-7-5. Of these, subfraction B_3-10-7-2 (102 mg) was subjected to RP-HPLC using CH_3CN−H_2O (7:93) containing 0.1% HOAc as the mobile phase to afford **1** (33 mg, 0.00027%, t_R = 16.3) and **2** (2 mg, 0.000016%, t_R = 20.1). Subfraction B_3-10-10 (2.1 g) was separated by flash chromatography over RP silica gel and eluted with a gradient of EtOH (0−50%) in H_2O to give subfractions B_3-10-10-1−B_3-10-10-4. Subfraction B_3-10-10-2 (58 mg) was subjected to RP-HPLC using CH_3CN−H_2O (7:93) containing 0.1% HOAc as mobile phase to yield **6** (6 mg, 0.00005%, t_R = 17.4), **7** (5 mg, 0.000042%, t_R = 17.9), and **8** (1.5 mg, 0.000012%, t_R = 21.1).

Fraction B_3-14 (4.3 g) was separated by flash chromatography over RP silica gel and eluted with a gradient of EtOH (0−50%) in H_2O to give subfractions B_3-14-1−B_3-14-5. Subfraction B_3-14-2 (36 mg) was subjected to RP-HPLC using CH_3CN−H_2O (7:93), containing 0.1% HOAc as the mobile phase, to afford **3** (3 mg, 0.000024%, t_R = 19.6). Subfraction B_4 (86 g) was separated over a RP silica gel column and eluted with a gradient of EtOH (0−100%) in H_2O to yield subfractions B_4-1−B_4-7. Of these, subfraction B_4-6 (9.8 g) was separated further by flash chromatography over RP silica gel and eluted with a gradient of MeOH (0−50%) in H_2O to give subfractions B_4-6-1−B_4-6-4. Subfractions B_4-6-1 (176 mg) and B_4-6-2 (90 mg) were separately subjected to RP-HPLC, using for B_4-6-1 CH_3CN−H_2O (13:87) containing 0.1% HOAc as the mobile phase, to afford **4** (120 mg, 0.001%, t_R = 23.1) and **9** (1 mg, 0.000 008 3%, t_R = 19.6), and using for B_4-6-2 CH_3CN−H_2O (15:85) containing 0.1% HOAc as the mobile phase, to afford **5** (2 mg, 0.000 016%, t_R = 23.7).

*Lonijaposide O (**1**):* beige, amorphous solid; $[\alpha]^{20}_D$ −160.0 (*c* 0.18, H_2O); UV (H_2O) λ_{max} (log ε) 230 (4.39), 256 (4.21, sh), 304 (3.71) nm; CD (H_2O) 195 ($\Delta\varepsilon$ −3.45), 214 ($\Delta\varepsilon$ +0.19), 237 ($\Delta\varepsilon$ −2.88), 243 ($\Delta\varepsilon$ −2.72), 261 ($\Delta\varepsilon$ −5.63); IR (Nujol) ν_{max} 3352, 2926, 1630, 1504, 1453, 1394, 1360, 1283, 1195, 1161, 1074, 1042, 755, 689, 629 cm^{-1}; ^1H NMR (D_2O, 600 MHz) data, see Table 1; ^{13}C NMR (D_2O, 125 MHz) data, see Table 2; (+)-ESIMS *m*/*z* 508 $[M + H]^+$, 530 $[M + Na]^+$, 546 $[M + K]^+$; HRESIMS *m*/*z* 508.1827 $[M + H]^+$ (calcd for $C_{24}H_{30}NO_{11}$, 508.1813), 530.1641 $[M + Na]^+$ (calcd for $C_{24}H_{29}NO_{11}Na$, 530.1633).

*Lonijaposide P (**2**):* beige, amorphous solid; $[\alpha]^{20}_D$ −129.5 (*c* 0.10, H_2O); UV (H_2O) λ_{max} (log ε) 232 (4.07), 253 (3.95, sh), 300 (3.43) nm; CD (H_2O) 198 ($\Delta\varepsilon$ −1.42), 207 ($\Delta\varepsilon$ −0.39), 221 ($\Delta\varepsilon$ −0.29), 240 ($\Delta\varepsilon$ −1.62), 262 ($\Delta\varepsilon$ −4.31); IR (Nujol) ν_{max} 3330, 2906, 1695, 1627, 1505, 1436, 1394, 1360, 1302, 1252, 1196, 1162, 1072, 948, 794, 770, 687 cm^{-1}; ^1H NMR (D_2O, 600 MHz) data, see Table 1; ^{13}C NMR (D_2O, 125 MHz) data, see Table 2; (+)-ESIMS *m*/*z* 522 $[M + H]^+$, 544 $[M + Na]^+$, 560 $[M + K]^+$; HRESIMS *m*/*z* 522.1973 $[M + H]^+$ (calcd for $C_{25}H_{32}NO_{11}$, 522.1970), 544.1788 $[M + Na]^+$ (calcd for $C_{25}H_{31}NO_{11}Na$, 544.1789).

*Lonijaposide Q (**3**):* beige, amorphous solid; $[\alpha]^{20}_D$ −129.0 (*c* 0.08, H_2O); UV (H_2O) λ_{max} (log ε) 229 (4.14), 258 (3.98), 300 (3.47) nm; IR (Nujol) ν_{max} 3317, 2920, 1727, 1665, 1636, 1548, 1394, 1276, 1239, 1188, 1153, 1072, 1042, 796, 683, 614, 582 cm^{-1}; ^1H NMR (D_2O, 500 MHz) data, see Table 1; ^{13}C NMR (D_2O, 125 MHz) data, see Table 2; (+)-ESIMS *m*/*z* 565 $[M + H]^+$, 587 $[M + Na]^+$; HRESIMS *m*/*z* 565.2034 $[M + H]^+$ (calcd 565.2035 for $C_{26}H_{33}N_2O_{12}$).

*Lonijaposide R (**4**):* beige, amorphous solid; $[\alpha]^{20}_D$ −195.0 (*c* 0.30, H_2O); UV (H_2O) λ_{max} (log ε) 204 (4.44, sh), 230 (4.37), 257 (4.23, sh), 305 (3.75) nm; CD (H_2O) 194 ($\Delta\varepsilon$ −1.92), 207 ($\Delta\varepsilon$ −0.85), 202 ($\Delta\varepsilon$ −0.90), 223 ($\Delta\varepsilon$ + 0.95), 242 ($\Delta\varepsilon$ −3.17), 263 ($\Delta\varepsilon$ −8.70), 299 ($\Delta\varepsilon$ −1.08); IR (Nujol) ν_{max} 3348, 2921, 2851, 1681, 1630, 1501, 1366, 1277, 1199, 1164, 1075, 1045, 950, 928, 754, 703 cm^{-1}; ^1H NMR (D_2O, 500 MHz) data, see Table 1; ^{13}C NMR (D_2O, 125 MHz) data, see Table 2; (+)-ESIMS *m*/*z* 584 $[M + H]^+$, 606 $[M + Na]^+$; HRESIMS *m*/*z* 584.2141 $[M + H]^+$ (calcd for $C_{30}H_{34}NO_{11}$, 584.2126), 606.1951 $[M + Na]^+$ (calcd for $C_{30}H_{33}NO_{11}Na$, 606.1946).

*Lonijaposide S (**5**):* beige, amorphous solid; $[\alpha]^{20}_D$ −120.0 (*c* 0.05, H_2O); UV (H_2O) λ_{max} (log ε) 202 (4.35, sh), 231 (4.02, sh), 285 (3.51, sh) nm; CD (H_2O) 204 ($\Delta\varepsilon$ −7.04), 210 ($\Delta\varepsilon$ −6.69), 222 ($\Delta\varepsilon$ −9.07), 247 ($\Delta\varepsilon$ +3.62), 278 ($\Delta\varepsilon$ −0.05), 291 ($\Delta\varepsilon$ +0.09); IR (Nujol) ν_{max} 3375, 2954, 2918, 2851, 1735, 1641, 1575, 1543, 1500, 1466, 1396, 1185, 1164, 1078, 961, 927, 831, 797, 722, 702, 662 cm^{-1}; ^1H NMR (D_2O, 600 MHz) data, see Table 1; ^{13}C NMR (D_2O, 125 MHz) data, see Table 2; (+)-ESIMS *m*/*z* 584 $[M + H]^+$, 606 $[M + Na]^+$, 622 $[M + K]^+$; (−)-ESIMS *m*/*z* 582 $[M - H]^-$; HRESIMS *m*/*z* 584.2129 $[M + H]^+$ (calcd for $C_{30}H_{34}NO_{11}$, 584.2126).

*Lonijaposide T (**6**):* beige, amorphous solid; $[\alpha]^{20}_D$ −185.8 (*c* 0.12, H_2O); UV (H_2O) λ_{max} (log ε) 209 (4.35, sh), 233 (4.26, sh), 262 (4.09, sh), 309 (3.60) nm; CD (H_2O) 197 ($\Delta\varepsilon$ −6.29), 216 ($\Delta\varepsilon$ +0.65), 232 ($\Delta\varepsilon$ −1.55), 244 ($\Delta\varepsilon$ −2.75), 268 ($\Delta\varepsilon$ −6.78); IR (Nujol) ν_{max} 3347, 2959, 2925, 1633, 1605, 1390, 1278, 1199, 1165, 1074, 938, 786, 755 cm^{-1}; ^1H NMR (D_2O, 600 MHz) data, see Table 1; ^{13}C NMR (D_2O, 150 MHz) data, see Table 2; (+)-ESIMS *m*/*z* 594 $[M + H]^+$, 632 $[M + K]^+$; (−)-ESIMS *m*/*z* 592 $[M - H]^-$; HRESIMS *m*/*z* 594.2194 $[M + H]^+$ (calcd for $C_{28}H_{36}NO_{13}$, 594.2181), 616.2006 $[M + Na]^+$ (calcd for $C_{28}H_{35}NO_{13}Na$, 616.2001).

*Lonijaposide U (**7**):* beige, amorphous solid; $[\alpha]^{20}_D$ −169.3 (*c* 0.15, H_2O); UV (H_2O) λ_{max} (log ε) 232 (4.28, sh), 264 (4.07, sh), 310 (3.61) nm; CD (H_2O) 198 ($\Delta\varepsilon$ −4.95), 218 ($\Delta\varepsilon$ +0.19), 227 ($\Delta\varepsilon$ −0.55), 235 ($\Delta\varepsilon$ −0.34), 246 ($\Delta\varepsilon$ −0.84), 270 ($\Delta\varepsilon$ −2.17); IR (Nujol) ν_{max} 3399, 2958, 2919, 2851, 1640, 1597, 1543, 1466, 1392, 1288, 1163, 1075, 947, 791, 720, 680 cm^{-1}; ^1H NMR (D_2O, 600 MHz) data, see Table 1; ^{13}C NMR (D_2O, 125 MHz) data, see Table 2; (−)-ESIMS *m*/*z* 592 $[M - H]^-$; HRESIMS at *m*/*z* 594.2194 $[M + H]^+$ (calcd for $C_{28}H_{36}NO_{13}$, 594.2181), 616.2006 $[M + Na]^+$ (calcd for $C_{28}H_{35}NO_{13}Na$, 616.2001).

*Lonijaposide V (**8**):* beige, amorphous solid; $[\alpha]^{20}_D$ −117.5 (*c* 0.04, H_2O); UV (H_2O) λ_{max} (log ε) 212 (4.32, sh), 233 (4.25, sh), 264 (4.07, sh), 308 (3.64) nm; CD (H_2O) 199 ($\Delta\varepsilon$ −6.13), 216 ($\Delta\varepsilon$ +2.19), 238 ($\Delta\varepsilon$ −0.23), 266 ($\Delta\varepsilon$ −4.29); IR (Nujol) ν_{max} 3375, 2961, 2920, 2851, 1728, 1697, 1627, 1599, 1390, 1306, 1261, 1165, 1076, 1047, 940, 867, 789, 770, 660 cm^{-1}; ^1H NMR (D_2O, 500 MHz) data, see Table 1; ^{13}C NMR (D_2O, 150 MHz) data, see Table 2; (−)-ESIMS *m*/*z* 592 $[M - H]^-$; HRESIMS *m*/*z* 594.2175 $[M + H]^+$ (calcd for $C_{28}H_{36}NO_{13}$, 594.2181).

*Lonijaposide W (**9**):* beige, amorphous solid; $[\alpha]^{20}_D$ −112.0 (*c* 0.05, H_2O); UV (H_2O) λ_{max} (log ε) 203 (4.15, sh), 232 (3.83, sh), 266 (3.64, sh), 312 (3.34) nm; CD (H_2O) 197 ($\Delta\varepsilon$ −4.20), 203 ($\Delta\varepsilon$ −3.48), 217 ($\Delta\varepsilon$ −1.23), 227 ($\Delta\varepsilon$ −1.05), 244 ($\Delta\varepsilon$ −2.49), 268 ($\Delta\varepsilon$ −4.94); IR (Nujol) ν_{max} 3375, 2923, 2853, 1728, 1640, 1402, 1243, 1164, 1074, 932, 706, 596 cm^{-1}; ^1H NMR (D_2O, 600 MHz) data, see Table 1; ^{13}C NMR (D_2O, 125 MHz) data, see Table 2; (−)-ESIMS *m*/*z* 627 $[M]^-$; HRESIMS *m*/*z* 628.2034 $[M + H]^+$ (calcd for $C_{31}H_{34}NO_{13}$, 628.2025), 650.1846 $[M + Na]^+$ (calcd for $C_{31}H_{33}NO_{13}Na$, 650.1844).

Enzymatic Hydrolysis of Compounds 1 and 4. A solution of each compound (5 mg) in H_2O (1 mL) was treated with snailase (20 mg, LJ0427B2011Z, Shanghai Sangon Biotech Co., Ltd., Shanghai, People's Republic of China) at 37 °C for 12 h. The reaction mixture was extracted with EtOAc (2 × 3 mL). The H_2O phases of the hydrolysates of **1** and **4** were separately concentrated to dryness and then eluted with CH_3CN−H_2O (6:1) on a silica gel column to yield glucose with an $[\alpha]^{20}_D$ value of +42.7 (*c* 0.09, H_2O) and +43.6 (*c* 0.16, H_2O), respectively. The solvent system CH_3CN−H_2O (4:1) was used for the TLC identification of glucose (R_f = 0.38).

Synthesis and Oxidation of 3-Carboxy-1-propylpyridin-1-ium Iodide (11) and 1-Propylpyridin-1-ium Iodide (11′). A solution of either nicotinic acid (50 mg) or pyridine (0.1 mL) and 1-iodopropane (70 mg) in *N*,*N*-dimethylformamide (DMF, 2 mL) was stirred at room temperature for 12 h, then evaporated under reduced pressure to give a residue. The residue was separated over silica gel and eluted with $CHCl_3$−MeOH (8:1) to yield 3-carboxy-1-propylpyridin-1-ium iodide (**11**, 63 mg) from nicotinic acid, and 1-propylpyridin-1-ium iodide (**11′**, 86 mg) from pyridine, which were identified by ESIMS and NMR analysis (Figures S106−S110, Supporting Information). $K_3Fe(CN)_6$ (100 mg) and KOH (40 mg) were

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successively added into a solution of **11** (30 mg) or **11′** (40 mg) in H_2O–benzene (1:1, 2 mL). The mixture was stirred at room temperature for 2 h, then partitioned with EtOAc (2 × 2 mL). The EtOAc phase was separated over silica gel and eluted with petroleum ether–EtOAc (4:1) to give 6-oxo-1-propyl-1,6-dihydropyridine-3-carboxylic acid (**12**) from **11**, and 1-propylpyridin- 2(1*H*)-one (**12′**) from **11′**, as identified by ESIMS and NMR spectroscopic analysis (Figures S111–S116, Supporting Information).

Synthesis and Hydrolysis of (*S*)- and (*R*)-2-[2-Oxopyridin-1(2*H*)-yl]propanoic Acid (14 and 14′). A solution of pyridine (1.24 g) and either (*S*)- or (*R*)-2-bromopropanoic acid (2 g) in CH_2Cl_2 (10 mL) was refluxed for 4 h. The solvent was removed under reduced pressure to give a residue. The residue was separated over silica gel and eluted with $CHCl_3$–MeOH (5:1) to yield (*S*)-1-(1-carboxyethyl)-pyridin-1-ium bromide {**13**, 2.6 g, $[\alpha]^{20}_D$ −5.1 (*c* 2.3, MeOH)} from (*S*)-2-bromopropanoic acid, and **13′** [the (*R*)-isomer, 2.4 g, $[\alpha]^{20}_D$ +5.2 (*c* 1.8, MeOH)] from (*R*)-2-bromopropanoic acid. $K_3Fe(CN)_6$ (700 mg) and KOH (40 mg) were successively added into a solution of **13** or **13′** (200 mg) in H_2O–benzene (1:1, 5 mL). The mixture was stirred at room temperature for 8 h, then acidified with 6 N HCl to pH 5 and filtered. The filtrate was evaporated under reduced pressure to yield a residue, which was chromatographed over silica gel, eluting with $CHCl_3$–MeOH (6:1), to afford (*S*)-2-[2-oxopyridin-1(2*H*)-yl]propanoic acid {**14**, 123 mg, $[\alpha]^{20}_D$ +4.4 (*c* 1.0, MeOH)} from **13**, and the (*R*)-isomer {**14′**, 144 mg, $[\alpha]^{20}_D$ −4.2 (*c* 1.3, MeOH)} from **13′**. The synthesized compounds **13**, **13′**, **14**, and **14′** were characterized by ESIMS, NMR, and CD spectroscopic techniques (Figures S117–S129, Supporting Information). The enantiomers **14** and **14′** (100 mg each) were separately hydrolyzed with 6 N HCl (4 mL) to liberate the corresponding L-alanine {24.3 mg, $[\alpha]^{20}_D$ +14.3 (*c* 1.3, H_2O)} from **14** and D-alanine {21.2 mg, $[\alpha]^{20}_D$ −14.5 (*c* 1.1, H_2O)} from **14′**, as identified by ESIMS and NMR spectroscopic analyses (Figures S130–S135, Supporting Information) and their $[\alpha]^{20}_D$ values.

Synthesis and Hydrolysis of (*S*)- and (*R*)-Methyl 1-(1-methoxy-1-oxo-3-phenylpropan-2-yl)-6-oxo-1,6-dihydropyridine-3-carboxylate (17 and 17′). A solution of coumalic acid (**15**, 500 mg), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 1 g), and 4-dimethylaminopyridine (DMAP, 800 g) in anhydrous MeOH (20 mL) was stirred at room temperature for 4 h. The solvent was removed under reduced pressure to give a residue, which was then partitioned between H_2O (20 mL) and CH_2Cl_2 (2 × 20 mL). The CH_2Cl_2 phase was concentrated and purified by column chromatography over silica gel to afford methyl coumalate (**16**, 412 mg), which was identified by ESIMS and NMR spectroscopic analysis (Figures S136–S138, Supporting Information). Methyl coumalate (50 mg) and either L-phenylalanine or D-phenylalanine (80 mg) in anhydrous MeOH (5 mL) were stirred at room temperature for 2 h, then evaporated under reduced pressure. The residue was separated over silica gel and eluted with petroleum ether–EtOAc (4:1) to yield **17** (59 mg) from L-phenylalanine and **17′** [the (*R*)-isomer, 63 mg] from D-phenylalanine, which were characterized using the ESIMS, HRESIMS, NMR, and CD spectroscopic techniques (Figures S139–S147, Supporting Information). These enantiomers (**17** and **17′**; 30 mg each) were separately hydrolyzed with 6 N HCl (2 mL) to liberate the corresponding L-phenylalanine {12.4 mg, $[\alpha]^{20}_D$ −34.8 (*c* 0.62, H_2O)} and D-phenylalanine {11.6 mg, $[\alpha]^{20}_D$ +35.2 (*c* 0.58, H_2O)}, as identified by ESIMS and NMR spectroscopic analyses (Figures S148–S153, Supporting Information) and their $[\alpha]^{20}_D$ values.

Determination of the Absolute Configurations of Amino Acid Units in Compounds 1–9. Compounds **1**–**9** (0.5–5 mg) were individually oxidized with $K_3Fe(CN)_6$ (10–20 mg) in 2 N KOH solution (2 mL) at room temperature for 4 h. Each of the reaction solutions was passed through a small column packed with MCI resin (5 g), then successively eluted with H_2O and EtOH (15 mL each). The EtOH eluent was concentrated to dryness under reduced pressure. The residue was hydrolyzed with 6 N HCl (1 mL) into a sealed glass bomb and kept at 110 °C for 16 h, then evaporated under reduced pressure. The residue was suspended in 250 μL of H_2O, and solutions of (1-fluoro-2,4-dinitrophenyl)-5-L-alanine amide (FDAA) in acetone (1%, 115 μL) and 6% aqueous triethylamine (60 μL) were added. The mixture was kept at 40 °C for 2 h, then diluted with H_2O (150 μL) and filtered. The standard FDAA-amino acids were prepared in the same manner using the corresponding L- and D-amino acids (0.8–1.3 mg). The FDAA-amino acid derivatives from the hydrolysate were compared with standard FDAA-amino acids using HPLC analysis: Previl C_{18} (250 × 4.6, 5 μm); flow rate, 1 mL/min; UV detection at 340 nm; linear gradient elution increasing CH_3CN from 25% to 45% in 0.1% aqueous TFA buffer over a period of 45 min. The absolute configuration of each amino acid was determined by comparing the retention time of the hydrolysate FDAA derivatives with those of the standard D- and L-amino acid FDAA derivatives.

Anti-influenza Virus, Coxsackie Virus, and Herpes Simplex Virus Assay. See ref 35.

Anti-inflammatory Activity Assay. See refs 15 and 36.

HIV-1 Replication Inhibition Assay. See ref 37.

Cells, Culture Conditions, and Cell Proliferation Assay. See ref 38.

■ ASSOCIATED CONTENT

Supporting Information

Isolation of the known compounds, IR, MS, 1D/2D NMR, UV, and CD spectra of compounds **1**–**9**, HPLC chromatograms of FDAA derivatives of the standard amino acids, and hydrolysates of the oxidation products of compounds **1**–**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 86-10-83154789. Fax: 86-10-63017757. E-mail: shijg@imm.ac.cn (J.S).

Author Contributions

†Y. Yu and C. Zhu contributed equally to this study.

Notes

The authors declare no competing financial interest.

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