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Article

Renoprotective Mono- and Triterpenoids from the Fruit of *Gardenia jasminoides*

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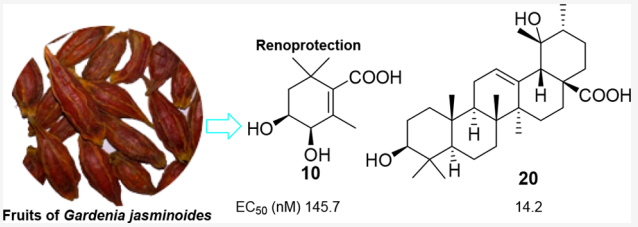
ABSTRACT: This paper describes the isolation and characterization of 17 new and 12 known terpenoids from the fruit of *Gardenia jasminoides*. The structures of eight new triterpenoids and nine new monoterpenoids, including their absolute configurations, were defined by spectroscopic analysis in combination of quantum chemical electronic circular dichroism (ECD), vibrational circular dichroism (VCD), and gauge-independent atomic orbital (GIAO) NMR calculations. The cytoprotective effects of the isolated compounds against lipopolysaccharide (LPS)-induced apoptosis in normal rat kidney tubule epithelioid (NRK 52e) cells were investigated in vitro. Compounds **10**, **18**, **20**, **21**, **24**, and **26** exhibited significant protective effects with EC_{50} values from 14.2 nM to 1.6 μM.



The genus *Gardenia* (Rubiaceae) comprises more than 200 species spread among the tropical and subtropical climate zones.[1] In folk medicine, many species of the *Gardenia* genus are used as sedative, diuretic, hypotensive, and hepatoprotective agents.[2,3] Many mono- and triterpenoids and iridoid glycosides have been isolated from this genus,[1−7] and a few of these compounds possess cytoprotective effects.[2,8,9]

Gardenia jasminoides is distributed extensively over some southern provinces of China, and its fruit is commonly used as a folk medicine.[10] However, limited research has been done regarding its phytochemical and biological properties.[6,10−12] In our ongoing efforts to discover natural products with cytoprotective effects,[13,14] eight new triterpenoids (**1**−**8**) and nine new monoterpenoids (**9**−**17**), including a pair of enantiomers, (+)-**16** and (−)-**16**, accompanied by 12 known terpenoids (**18**−**29**), were isolated from the fruit of *G. jasminoides*. Some compounds showed cytoprotective effects against lipopolysaccharide (LPS)-induced apoptosis in normal rat kidney tubule epithelioid (NRK 52e) cells.

RESULTS AND DISCUSSION

Multiple column chromatography separations of the EtOAc layer from the 50% aqueous acetone extract of *G. jasminoides* fruit yielded 17 new compounds (**1**−**17**), including a pair of enantiomers, (+)-**16** and (−)-**16**, and 12 known compounds (**18**−**29**).

Compound **1** was isolated as a colorless, amorphous solid (Figure 1). The HRESIMS and ^{13}C NMR data corresponded to the molecular formula $C_{30}H_{48}O_5$. The 1D NMR and HSQC data (Tables 1 and 2) revealed the presence of a carboxylic carbon at δ_C 177.8; an olefinic bond, including a methylene at δ_H 4.82 (br s), 4.74 (br s)/δ_C 112.2 and an olefinic quaternary carbon at δ_C 150.8; two oxymethylenes at δ_H 4.08, 3.47 (each d, J = 11.5 Hz)/δ_C 71.6 and δ_H 3.50, 3.42 (each d, J = 11.2 Hz)/δ_C 68.0; an oxymethine at δ_H 3.35 (dd, J = 10.8, 2.4 Hz)/δ_C 82.5; and an oxygenated quaternary carbon at δ_C 75.3. The above NMR data resembled those of lithocarpic acid B, except that the 26-methyl group of lithocarpic acid B was oxidized into a hydroxymethyl group in **1**.[15]

The NOESY cross-peaks (Figure 2) between H-8 at δ_H 1.61 and H-19β at δ_H 0.76 and H_3-18 at δ_H 1.00 (Figure S10, Supporting Information) suggested the β orientations of H-8 and H_3-18. Conversely, H-5 at δ_H 2.51, H-17 at δ_H 2.47, and H_3-30 at δ_H 1.05 were α-oriented. The orientations of H-20 and H-24 were determined by the coupling constants of H-21α at δ_H 3.47 (dd, J = 11.5, 2.2 Hz), H-21β at δ_H 4.08 (d, J = 11.5 Hz), and H-24 at δ_H 3.35 (dd, J = 10.8, 2.4 Hz), which were confirmed by the NOESY correlations between H-21β and H-20 at δ_H 1.48 and H-24.[15]

The (5*S*,10*R*) absolute configuration was defined by comparing the experimental and simulated electronic circular dichroism (ECD) spectra (Figure 3), which were calculated at the B3LYP/6-311G(d,p) level in MeOH. The predicted ECD spectra of four possible structures **1a**−**1d** were identical to the experimental ECD spectrum of **1** (Figure S198, Supporting

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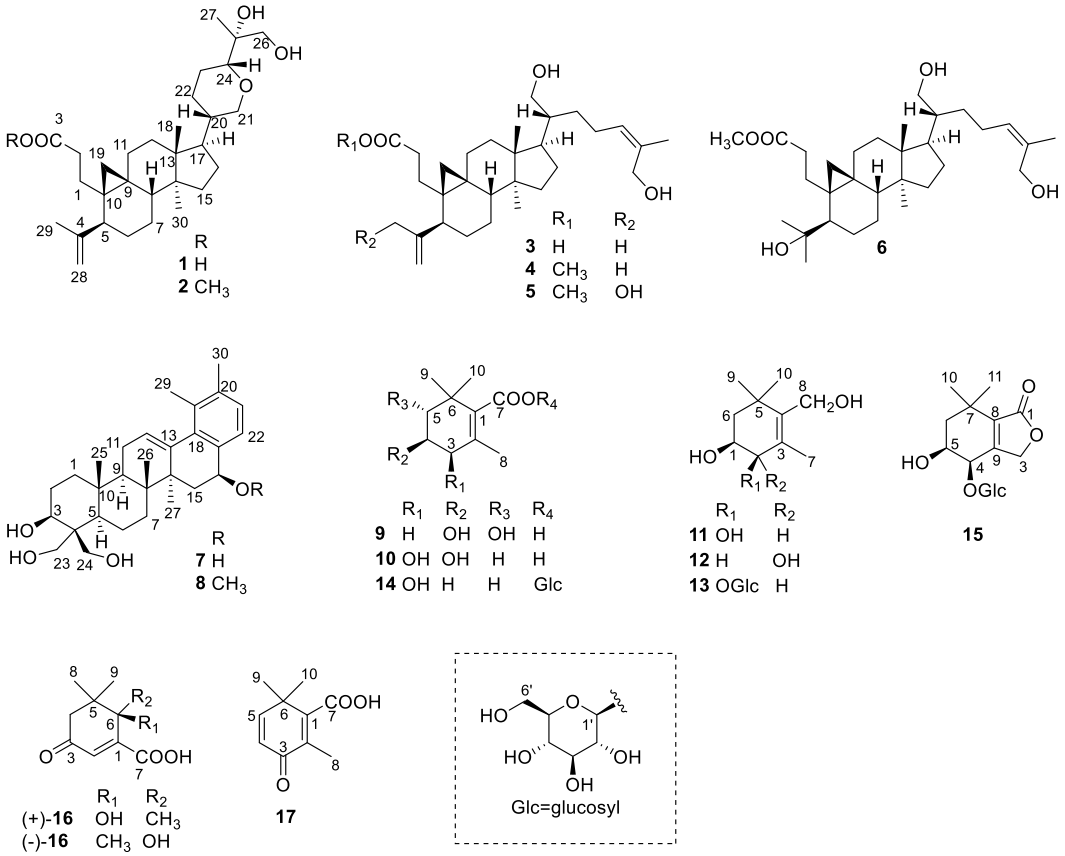

Figure 1. Compounds isolated from the fruits of *G. jasminoides*.

Information). In order to determine the absolute configurations of C-20, C-24, and C-25, calculations of the gauge-independent atomic orbital (GIAO) 1D NMR data for **1a**−**1d** were performed at the mPW1PW91/6-31G(d,p) level using MeOH as the solvent, and the data were compared with the experimental values. As a result, the isomer **1c** was predicted as the correct structure with the higher correlation coefficient (R^2) of 0.9992 (Figure 4) and DP4+ probability of 95.62% (Figure S200, Supporting Information).[16] The IR and vibrational circular dichroism (VCD) frequencies of **1a**−**1d** were calculated at the B3LYP/6-31G(d,p) level in $CHCl_3$ and the predicted VCD spectrum of **1c** matched well with the experimental spectrum of **1** (Figure 5). Therefore, the structure of 26-hydroxylithocarpic acid B (**1**) was defined as (5*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*,20*S*,24*R*,25*R*)-21,24-epoxy-25,26-dihydroxy-3,4-*seco*-cycloart-4(28)-en-3-oic acid.

Compound **2** was obtained as a colorless, amorphous solid, and its HRESIMS and ^{13}C NMR data corresponded to the molecular formula $C_{31}H_{50}O_5$. The 1D NMR data of **2** (Tables 1 and 2) resembled those of **1** and differed only in the -OCH_3 group (δ_H 3.62/δ_C 52.0) connected to C-3 (δ_C 176.2). The position of this substituent was elucidated from the HMBC correlation between the methoxy group protons and C-3 (Figure S19, Supporting Information). Interpretation of its NOESY spectrum (Figure S20, Supporting Information) suggested that its relative configuration was consistent with that of **1**. The ECD spectrum (Figure S199, Supporting Information), 1D NMR data, and specific rotation of **2** were similar to those of **1**, which suggested that its absolute configuration was the same as that of **1**. Thus, the structure of **2** (methyl 26-hydroxylithocarpic acid B) was elucidated as methyl (5*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*,20*S*,24*R*,25*R*)-21,24-epoxy-25,26-dihydroxy-3,4-*seco*-cycloart-4(28)-en-3-oate.

Compound **3** was obtained as a colorless, amorphous solid, with the molecular formula ($C_{30}H_{48}O_4$) determined by its HRESIMS and ^{13}C NMR data. The 1D NMR data of **3** (Tables 1 and 2) revealed similarities with those of lithocarpic acid N, except for the presence of a hydroxy group at C-21.[15] Its NOESY correlations (Figure S30, Supporting Information) showed that the relative configuration of **3** was similar to that of lithocarpic acid N. The chemical shift of C-26 was closer to those of *Z* isomers (*E* configuration: δ_C approximately 69.5;[15,17,18] *Z* configuration: δ_C approximately 61.0),[19,20] indicating the *Z* geometry of the $\Delta^{24(25)}$ double bond. Therefore, the structure of compound **3** [(24*Z*)-21-hydroxylithocarpic acid N] was defined as (24*Z*)-21,26-dihydroxy-3,4-*seco*-cycloart-4(28),24-dien-3-oic acid.

Compound **4** [methyl (24*Z*)-21-hydroxylithocarpic acid N] was isolated as a colorless, amorphous solid with the molecular formula of $C_{31}H_{50}O_4$ as indicated by the HRESIMS and ^{13}C NMR data. The 1D NMR data of **4** (Tables 1 and 2) revealed that it was structurally similar to compound **3** with the exception of a methoxy group (δ_H 3.62/δ_C 52.0). The HMBC correlation (Figure S39, Supporting Information) from the methoxy group protons to C-3 (δ_C 176.1) revealed the location of the methoxy moiety. Its NOESY correlations (Figure S40, Supporting Information) and the chemical shift of C-26 were consistent with those of **3**. Thus, the structure of methyl (24*Z*)-21-hydroxylithocarpic acid N was established as methyl (24*Z*)-21,26-dihydroxy-3,4-*seco*-cycloart-4(28),24-dien-3-oate.

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Table 1. ^{1}H NMR Data of Compounds 1−8[a]

NO.	1[b]	2[b]	3[b]	4[b]	5[b]	6[b]	7[b]	8[c]
1	2.04, m; 1.37, m	2.03, m; 1.28, m	2.03, m; 1.36, m	2.03, m; 1.36, m	2.10, m; 1.37, m	2.19, m; 1.35, m	1.75, m; 1.08, m	1.62, m; 0.96, m
2	2.46, m; 2.26, m	2.51, m; 2.28, m	2.48, m; 2.24, m	2.51, m; 2.26, m	2.52, m; 2.27, m	2.70, m	1.82, m; 1.73, m	1.67, m; 1.57, m
3							3.77, dd (11.8, 4.6)	3.60, dd (11.5, 5.2)
5	2.51[d]	2.49[d]	2.50[d]	2.49[d]	2.52[d]	1.88, m	1.34[d]	1.25[d]
6	1.53, m; 1.26, m	1.53, m; 1.13, m	1.40, m; 1.13, m	1.30, m; 1.14, m	1.68, m; 1.38, m	1.04, m; 0.71, m	1.68, m; 1.53, m	1.58, m; 1.47, m
7	α: 1.34, m; β: 1.17, m	α: 1.34, m; β: 1.16, m	α: 1.33, m; β: 1.17, m	α: 1.33, m; β: 1.16, m	α: 1.31, m; β: 1.14, m	α: 1.30, m; β: 1.17, m	α: 1.65, m; β: 1.62, m	α: 1.57, m; β: 1.47, m
8	1.61[d]	1.62[d]	1.62[d]	1.62[d]	1.60[d]	1.37[d]		
9							1.70[d]	1.59[d]
11	2.20, m; 1.30, m	2.17, m; 1.36, m	2.17, m; 1.31, m	2.15, m; 1.29, m	2.17, m; 1.28, m	2.18, m; 1.30, m	2.18, m; 2.05, m	2.13, m; 1.98, m
12	1.94, m; 1.66, m	1.94, m; 1.66, m	1.78, m; 1.64, m	1.77, m; 1.64, m	1.77, m; 1.64, m	1.78, m; 1.63, m	5.46, br s	5.46, br s
15	1.38, m	1.37, m	1.37, m	1.37, m	1.36, m	1.37, m	2.33, dd (13.0, 4.7); 0.86, t (13.0)	2.47, t (12.6); 0.58, t (12.6)
16	1.83, m; 1.29, m	1.83, m; 1.29, m	1.92, m; 1.30, m	1.91, m; 1.31, m	1.90, m; 1.34, m	1.92, m; 1.31, m	4.36, dd (12.0, 4.4)	3.87, dd (11.8, 3.1)
17	2.47, m	2.48, m	1.98, m	1.98, m	1.98, m	1.96, m		
18	1.00, s	0.99, s	1.02, s	1.02, s	1.02, s	1.01, s		
19	α: 0.44, d (4.4); β: 0.76, d (4.4)	α: 0.43, d (4.4); β: 0.76, d (4.4)	α: 0.42, d (4.3); β: 0.76, d (4.3)	α: 0.42, d (4.3); β: 0.76, d (4.3)	α: 0.47, d (4.2); β: 0.75, d (4.2)	α: 0.52, d (4.6);β: 0.71 d , (4.6)		
20	1.48, m	1.50, m	1.52, m	1.53, m	1.52, m	1.53, m		
21	α: 3.47, dd (11.5, 2.2); β: 4.08, br d (11.5)	α: 3.48, dd (11.5, 2.3); β: 4.08, br d (11.5)	3.69, dd (11.0, 3.3); 3.48, dd (11.0, 6.2)	3.69, dd (11.0, 3.3); 3.48, dd (11.0, 6.2)	3.69, dd (11.0, 3.3); 3.48, dd (11.0, 6.2)	3.69, dd (11.0, 3.3); 3.48, dd (11.0, 6.3)	6.99 d, (7.6)	6.97, d (7.6)
22	1.90, m; 1.14, m	1.92, m; 1.30, m	1.51, m; 1.38, m	1.52, m; 1.38, m	1.52, m; 1.38, m	1.52, m; 1.38, m	7.11 d, (7.6)	7.01, d (7.6)
23	1.61, m; 1.41, m	1.66, m; 1.41, m	2.14, m; 2.05, m	2.14, m; 2.05, m	2.14, m; 2.05, m	2.10, m; 2.02, m	4.07, d (11.4); 3.67, d (11.4)	3.79, d (11.0); 3.48, d (11.0)
24	3.35, dd (10.8, 2.4)	3.35, dd (10.8, 2.4)	5.28, t (7.2)	5.28, t (7.2)	5.28, t (7.1)	5.28, t (7.0)	4.13, d (11.6); 3.58, d (11.6)	3.82, d (11.9); 3.45, d (11.9)
25							1.06, s	0.94, s
26	3.50, d (11.2); 3.42, d (11.2)	3.50, d (11.1); 3.41, d (11.1)	4.07, dd (14.4, 11.8)	4.07, dd (14.8, 12.2)	4.07, s	4.07, dd (15.4, 12.6)	1.02, s	0.98, s
27	1.11, s	1.10, s	1.75, s	1.75, s	1.75, s	1.75, s	0.93, s	0.83, s
28	4.82, br s; 4.74, br s	4.81, brs; 4.73, br s	4.83, br s; 4.74, br s	4.82, br s; 4.73, br s	5.10, br s; 5.05, br s	1.20, s		
29	1.70, s	1.69, s	1.70, s	1.69, s	4.06, s	1.19, s	2.24, s	2.19, s
30	1.05, s	1.04, s	1.00, s	1.00, s	1.00, s	0.98, s	2.25, s	2.21, s
-OCH_3		3.62, s		3.62, s	3.62, s	3.62, s		3.43, s

[a]Recorded at 500 MHz, δH in ppm, J in Hz. [b]Recorded in methanol-d_4. [c]Recorded in DMSO-d_6. [d]Overlapped signals.

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Table 2. ^{13}C NMR Data of Compounds 1−8[a]

NO.	1[b]	2[b]	3[b]	4[b]	5[b]	6[b]	7[b]	8[c]
1	30.4	30.3	30.3	30.3	30.2	31.3	39.7	36.6
2	32.5	32.4	32.4	32.4	32.5	32.4	28.1	27.0
3	177.8	176.2	177.8	176.1	176.1	176.7	74.7	71.8
4	150.8	150.9	150.8	150.8	154.1	76.8	47.2	45.6
5	47.2	47.2	47.1	47.1	43.5	46.6	49.1	47.0
6	29.0	29.0	28.9	28.9	30.1	27.0	19.6	18.3
7	26.2	26.2	26.2	26.2	26.5	26.2	34.9	33.4
8	49.5	49.5	49.3	49.3	49.6	50.2	40.8	39.1
9	22.6	22.7	22.6	22.6	23.1	23.9	49.4	47.2
10	28.4	28.4	28.5	28.4	28.8	27.9	37.6	36.0
11	28.1	28.1	28.1	28.1	28.1	27.7	24.5	23.0
12	33.7	33.7	33.4	33.4	33.4	33.5	126.6	125.3
13	46.0	46.0	46.2	46.2	46.2	46.0	138.7	136.6
14	50.4	50.4	50.2	50.2	50.1	50.1	43.5	41.5
15	36.7	36.7	36.7	36.7	36.8	37.0	42.4	38.3
16	28.3	28.3	28.4	28.4	28.4	28.5	69.6	77.1
17	43.7	43.8	47.4	47.4	47.4	47.5	141.8	138.5
18	19.3	19.2	19.0	19.0	19.1	19.4	137.5	135.8
19	31.1	31.1	31.0	31.0	31.3	32.8	136.4	134.8
20	37.2	37.3	43.5	43.6	43.5	43.5	134.1	132.9
21	71.6	71.6	62.7	62.7	62.7	62.7	128.5	127.2
22	28.3	28.3	30.9	30.9	30.9	30.9	119.1	118.0
23	21.8	21.8	25.1	25.1	25.1	25.1	63.2	60.9
24	82.5	82.6	129.5	129.5	129.5	129.5	63.7	61.7
25	75.3	75.3	135.4	135.4	135.4	135.4	16.8	15.9
26	68.0	68.0	61.4	61.4	61.4	61.4	17.4	16.5
27	20.2	20.2	21.6	21.6	21.6	21.6	28.0	27.3
28	112.2	112.1	112.2	112.2	110.3	31.5		
29	20.1	20.1	20.1	20.1	64.6	26.4	17.1	16.5
30	20.1	20.1	20.0	20.0	20.1	20.2	21.0	20.5
-OCH$_3$		52.0		52.0	52.0	51.9		56.7

[a]Recorded at 125 MHz. [b]Recorded in methanol-d_4. [c]Recorded in DMSO-d_6.

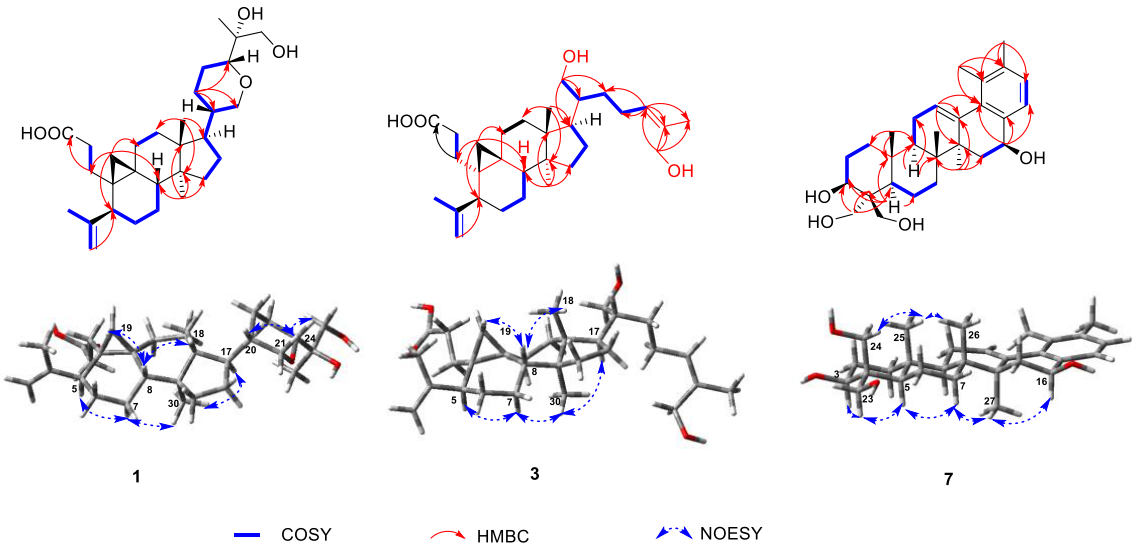

Figure 2. Key 2D NMR correlations for 1, 3, and 7.

Comparing the NMR data of 5 and 6 with those of 4 showed that they shared identical methyl (24Z)-21,26-dihydroxy-3,4-*seco*-cycloart-24-en-3-oate skeletons but had different C-5 side chains. Compound 5 was obtained as a colorless, amorphous solid. The molecular formula of 5 ($C_{31}H_{50}O_5$) was obtained from its HRESIMS and ^{13}C NMR data. Its 1D NMR data (Tables 1 and 2) revealed similar resonances to those of 4 but varied in the C-5 substituent. In compound 5, this was a 3-hydroxyprop-1-en-2-yl group [δ_C 154.1, δ_H 5.10 (br s), 5.05 (br s)/δ_C 110.3, δ_H 4.06 (s)/δ_C 64.6]. Thus, the structure of compound 5 [methyl (24Z)-21,29-dihydroxylithocarpic acid N] was assigned as methyl

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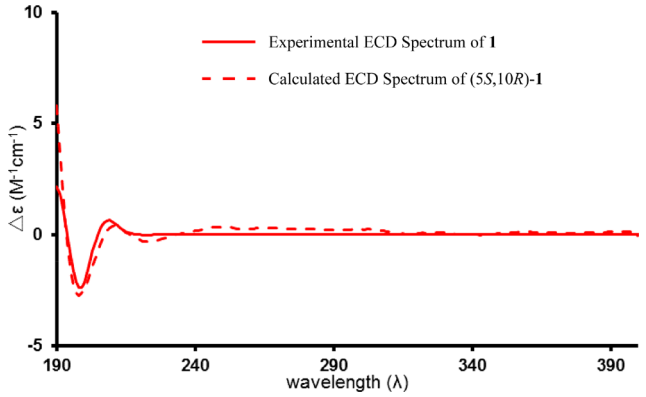

Figure 3. Experimental and calculated ECD spectra of **1**.

(24*Z*)-21,26,29-trihydroxy-3,4-*seco*-cycloart-4(28),24-dien-3-oate.

Compound **6** was obtained as a colorless, amorphous solid with the molecular formula of $C_{31}H_{52}O_5$ deduced from the HRESIMS and ^{13}C NMR data. Its 1D NMR data (Tables 1 and 2) resembled those of **4**, with the exception of the 2-hydroxypropan-2-yl moiety at C-5, which was deduced from the HMBC cross-peaks between H-5 (δ_H 1.88) and C-4 (δ_C 76.8), C-28 (δ_C 31.5), and C-29 (δ_C 26.4) as well as those between H_3-28 (δ_H 1.20), H_3-29 (δ_H 1.19), and C-4 and C-5 (δ_C 46.6) (Figure S59, Supporting Information). Therefore, the structure of compound **6** [methyl (24*Z*)-4,21-dihydroxy-4*H*,28*H*-lithocarpic acid N] was defined as methyl (24*Z*)-4,21,26-trihydroxy-3,4-*seco*-cycloart-24-en-3-oate. Compounds **2**, **4**, **5**, and **6** were detected in the CH_2Cl_2 fraction of 50% aqueous acetone extract by UPLC-Q/TOF-MS (Figures S194−S197, Supporting Information).

Compound **7** was isolated as a white, amorphous powder. The molecular formula ($C_{29}H_{42}O_4$) was determined from the HRESIMS and ^{13}C NMR data. Its 1D NMR (Tables 1 and 2) and HSQC data revealed the presence of an 1,2,3,4-tetrasubstituted benzene ring [two methines at δ_H 6.99 (d, J = 7.6 Hz)/δ_C 128.5 and δ_H 7.11 (d, J = 7.6 Hz)/δ_C 119.1 and four quaternary carbons at δ_C 141.8, 137.5, 136.4, and 134.1]; an olefinic bond [a methine at δ_H 5.46 (brs)/δ_C 126.6 and a quaternary carbon at δ_C 138.7]; two oxymethines [δ_H 3.77 (dd, J = 11.8, 4.6 Hz)/δ_C 74.7 and δ_H 4.36 (dd, J = 12.0, 4.4 Hz)/δ_C 69.6]; and two diastereotopic hydroxymethyls [δ_H 4.13, 3.58 (each d, J = 11.6 Hz)/δ_C 63.7 and δ_H 4.07, 3.67 (each d, J = 11.4 Hz)/δ_C 63.2]. The above data revealed similarities to those of kakidiol with the exception of two hydroxyl groups connected to C-16 and C-23, respectively.[21] The α orientations of H-3, H-5, H-9, H-16, and Me-14 were determined based on the coupling constants of H-3 (J = 11.8 Hz) and H-16 (J = 12.0 Hz) as well as the NOESY correlations from H-5 to H-3 and H-9 and from H-9 to H_3-27 (Figure 2). Thus, the structure of compound **7** was defined as 3β,16β,23,24-tetrahydroxy-28-nor-ursane-12,17,19,21-tetraene.

Compound **8** was obtained as a white, amorphous powder with the molecular formula ($C_{30}H_{44}O_4$) determined by the HRESIMS and ^{13}C NMR data. Its 1D NMR data resembled those of **7** (Tables 1 and 2) except for the methoxy group (δ_H 3.43/δ_C 56.7) linked to C-16 (δ_C 77.1), which was supported by the HMBC correlation of the methoxy protons with C-16 (Figure S79, Supporting Information). Key NOESY correlations of compound **8** (Figure S80, Supporting Information) were similar to those of **7**. Thus, the structure of compound **8** was defined as 3β,23,24-trihydroxy-16β-methoxy-28-nor-ursane-12,17,19,21-tetraene.

Compound **9** (crocusatin N) was isolated as a colorless, amorphous solid. The molecular formula ($C_{10}H_{16}O_4$) was

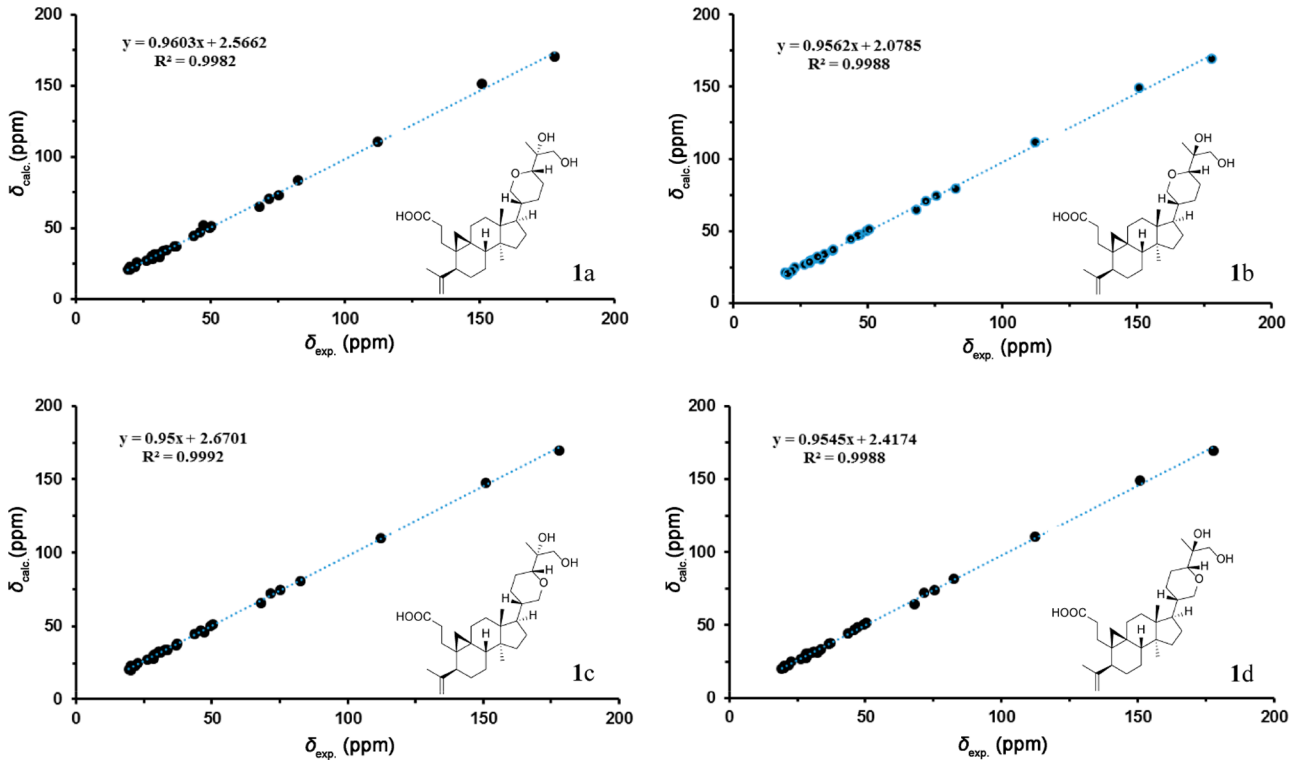

Figure 4. Correlations between calculated ^{13}C NMR chemical shifts of **1a**−**1d** and experimental ^{13}C NMR chemical shifts of **1**.

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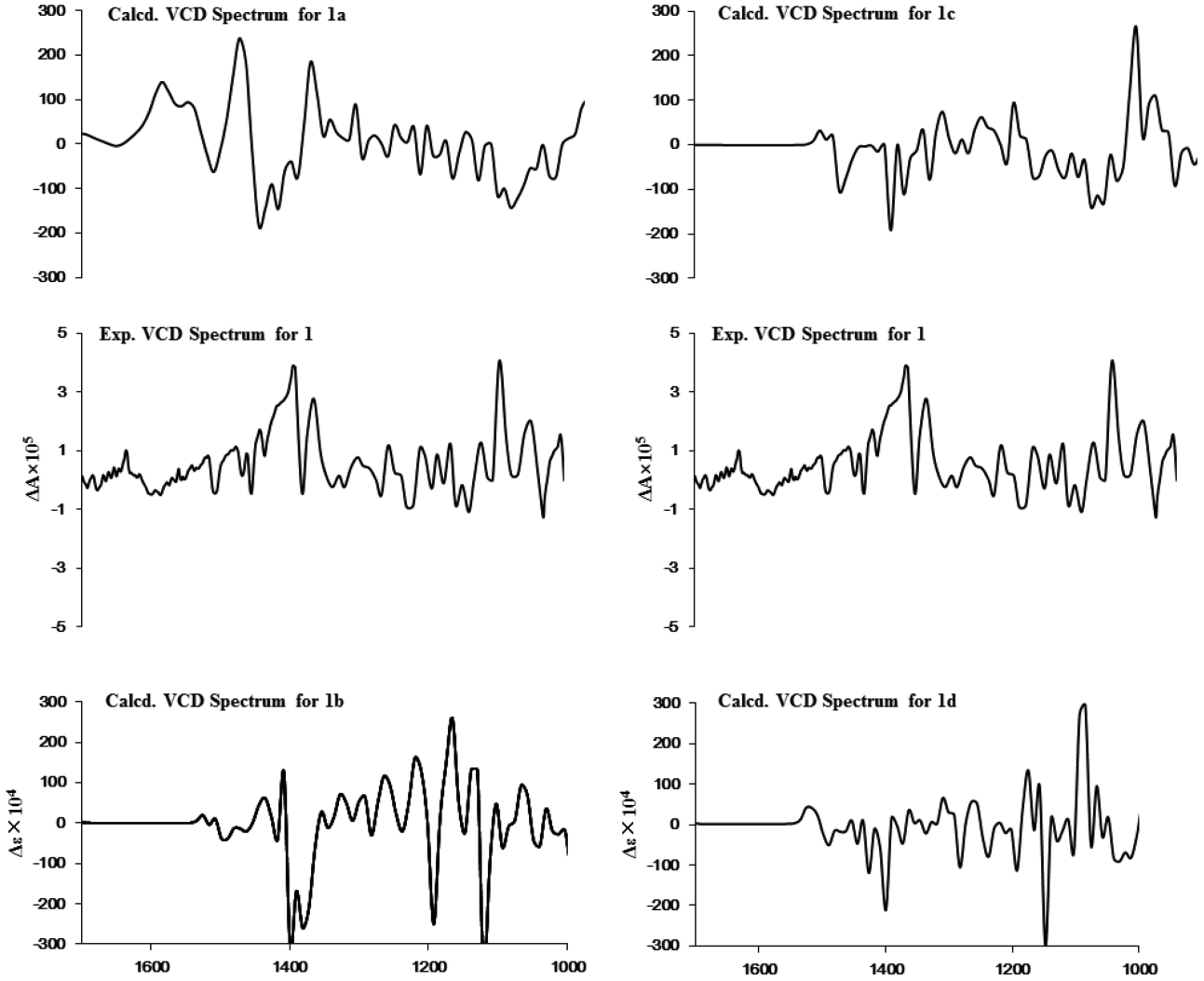

Figure 5. Comparison of the calculated VCD spectra of **1a**−**1d** and the experimental VCD spectrum of **1**.

obtained from the HRESIMS and ^{13}C NMR data. Its 1D NMR (Tables 3 and 4) and HSQC data showed signals for a carboxylic carbon at δ_C 174.8; two quaternary olefinic carbons at δ_C 137.7 and 131.8; two oxymethines at δ_H 3.82 (d, J = 8.0 Hz)/δ_C 69.8 and δ_H 3.76 (ddd, J = 12.0, 8.0, 3.8 Hz)/δ_C 75.0; a methylene at δ_H 1.66 (dd, J = 12.8, 3.8 Hz), 1.51 (d, J = 12.8 Hz)/δ_C 43.7; and three methyl groups at δ_H 1.63/δ_C 15.5, δ_H 1.11/δ_C 27.4, and δ_H 0.97/δ_C 28.4. The 2D structure of **9** was assigned based on its $^1H-^1H$ COSY and HMBC spectra (Figure S87 and S89, Supporting Information). The equatorial orientations of HO-4 and HO-5 were determined based on the coupling constants of H-4 (ddd, J = 12.0, 8.0, 3.7 Hz) and H-5 (d, J = 8.0 Hz). The absolute configuration of compound **9** was elucidated by comparing its experimental and calculated ECD spectra (Figure 6). Subsequently, the structure of crocusatin N was defined as (4*R*,5*R*)-4,5-dihydroxy-2,6,6-trimethylcyclohex-1-ene-1-carboxylic acid.

Crosantin O (**10**) was isolated as a colorless, amorphous solid. The molecular formula ($C_{10}H_{16}O_4$) was obtained from its HRESIMS and ^{13}C NMR data. The 1D NMR data of **10** (Tables 3 and 4) revealed similarities to those of **9**. The only differences were in the substituents at C-3 (a hydroxy group replaced a hydrogen) and C-5 (a hydrogen replaced a hydroxy group). The coupling constants of H-3 (d, J = 3.9 Hz) and H-4 (dt, J = 12.6, 3.9 Hz) suggested a *cis* orientation, which was supported by the NOESY correlation between H-3 (δ_H 3.83) and H-4 (δ_H 3.78) (Figure S100, Supporting Information). The calculated ECD curve for the (3*R*,4*S*) diastereoisomer of **10** matched well with the experimental ECD curve (Figure 6). Therefore, the structure of crosantin O (**10**) was elucidated as (3*R*,4*S*)-3,4-dihydroxy-2,6,6-trimethylcyclohex-1-ene-1-carboxylic acid.

The molecular formula of **11** ($C_{10}H_{18}O_3$) was determined from its HRESIMS and ^{13}C NMR data. Its 1D NMR data (Tables 3 and 4) exhibited similarities to those of **9**, but for replacement of the 7-hydroxycarbonyl group with a hydroxymethyl group. The spatial orientation of each −OH group was resolved based on the coupling constants between H-1 [δ_H 3.73 (dt, J = 12.8, 3.8 Hz)] and H-2 [δ_H 3.78 (d, J = 3.8 Hz)]. The (1*S*,2*R*) absolute configuration was determined based on the agreement between the experimental and calculated ECD curves (Figure 6). Thus, the structure of crocusatin P (**11**) was defined as (1*S*,2*R*)-4-(hydroxymethyl)-3,5,5-trimethylcyclohex-3-ene-1,2-diol.

The molecular formula of crocusatin Q (**12**) was the same as that of **11**. Their 1D NMR data (Tables 3 and 4) were similar, but the coupling constants between H-1 [δ_H 3.69 (ddd, J = 11.2, 7.8, 3.8 Hz)] and H-2 [δ_H 3.74 (d, J = 7.8 Hz)] in **12**

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Table 3. ^{1}H NMR Data of Compounds 9−17[a]

NO.	9[b]	10[c]	11[c]	12[c]	13[c]	14[c]	15[c]	16[c]	17[c]
1			3.73, dt (12.8, 3.8)	3.69, ddd (11.2, 7.8, 3.8)	3.77, dt (12.4, 3.1)				
2			3.78, t (3.8)	3.74, d (7.8)	3.93, d (3.1)			6.36, s	
3	1.66, dd (12.8, 3.8); 1.51, t (12.8)	3.83, t (3.9)				3.91, t (5.2)	5.02, d (17.8); 4.89, d (17.8)		
4	3.76, ddd (12.0, 8.0, 3.8)	3.78, dt (12.6 3.9)				1.92, m; 1.72, m	4.59, d (3.8)	2.54, d (16.9); 2.30, d (16.9)	6.18, d (10.0)
5	3.82, d (8.0)	1.75, t (12.5); 1.44, dd (12.5, 2.8)				1.65, m; 1.42, m	4.13, dt (9.3, 3.8)		6.96, d (10.0)
6			1.76, t (12.6); 1.40, dd (12.6, 2.5)	1.64, dd (12.7, 3.4); 1.50, t (12.7)	1.79, t (12.6); 1.45, dd (12.6, 2.2)		1.93, dd (13.6, 9.3); 1.64, dd (13.6, 2.8)		
7			1.87, s	1.81, s	1.91, s				
8	1.63, s	1.82, s	4.12, d (11.5); 4.07, d (11.5)	4.12, d (11.4); 4.10, d (11.4)	4.13, d (11.2); 4.04, d (11.2)	1.79, s		1.08, s	1.86, s
9	1.11, s	1.20, s	1.09, s	1.09, s	1.09, s	1.14, s		1.05, s	1.36, s
10	0.97, s	1.09, s	1.06, s	1.07, s	1.04, s	1.13, s	1.31, s	1.60, s	1.36, s
11							1.21, s		
1’					4.42, d (7.8)	5.55, d (8.2)	4.43, d (7.8)		
2’					3.25[d]	3.32[d]	3.25[d]		
3′					3.38[d]	3.39[d]	3.38[d]		
4’					3.33[d]	3.37[d]	3.33[d]		
5′					3.34, m	3.43, m	3.34, m		
6’					3.86, d (11.8); 3.66, dd (11.8, 5.0)	3.84, dd (11.9, 1.6); 3.70, dd (11.9, 4.4)	3.86, dd (11.8, 1.7); 3.68, dd (11.8, 5.0)		

[a]Recorded at 500 MHz, δ_H in ppm, J in Hz. [b]Recorded in D_2O. [c]Recorded in methanol-d_4. [d]Overlapped signals

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Table 4. ^{13}C NMR Data of Compounds 9−17[a]

NO.	9[b]	10[c]	11[c]	12[c]	13[c]	14[c]	15[c]	16[c]	17[c]
1	137.7	140.2	67.9	71.6	67.4	138.5	174.4	156.0	156.0
2	131.8	132.1	72.8	78.2	84.2	136.5		130.6	131.6
3	43.7	71.3	133.6	134.9	132.2	69.3	71.5	201.2	187.8
4	75.0	67.6	142.0	140.1	142.4	29.4	74.4	51.0	126.2
5	69.8	41.2	37.7	37.3	37.9	35.8	68.5	42.3	159.2
6	34.7	36.4	41.8	46.2	42.9	34.7	43.1	75.4	39.5
7	174.8	173.6	18.1	15.0	18.2	170.4	32.5	169.9	170.6
8	15.5	19.4	58.3	58.6	58.1	18.2	135.1	23.3	13.2
9	27.4	27.9	29.5	29.8	29.4	28.6	158.9	23.8	26.6
10	28.4	29.7	27.7	28.3	27.4	28.0	27.2	23.3	26.6
11							27.0		
1′					106.0	95.9	104.8		
2′					75.2	74.0	74.4		
3′					77.8	78.9	77.7		
4′					71.4	71.1	71.5		
5′					78.2	78.4	78.3		
6′					62.5	62.4	62.5		

[a]Recorded at 125 MHz. [b]Recorded in D_2O. [c]Recorded in methanol-d_4.

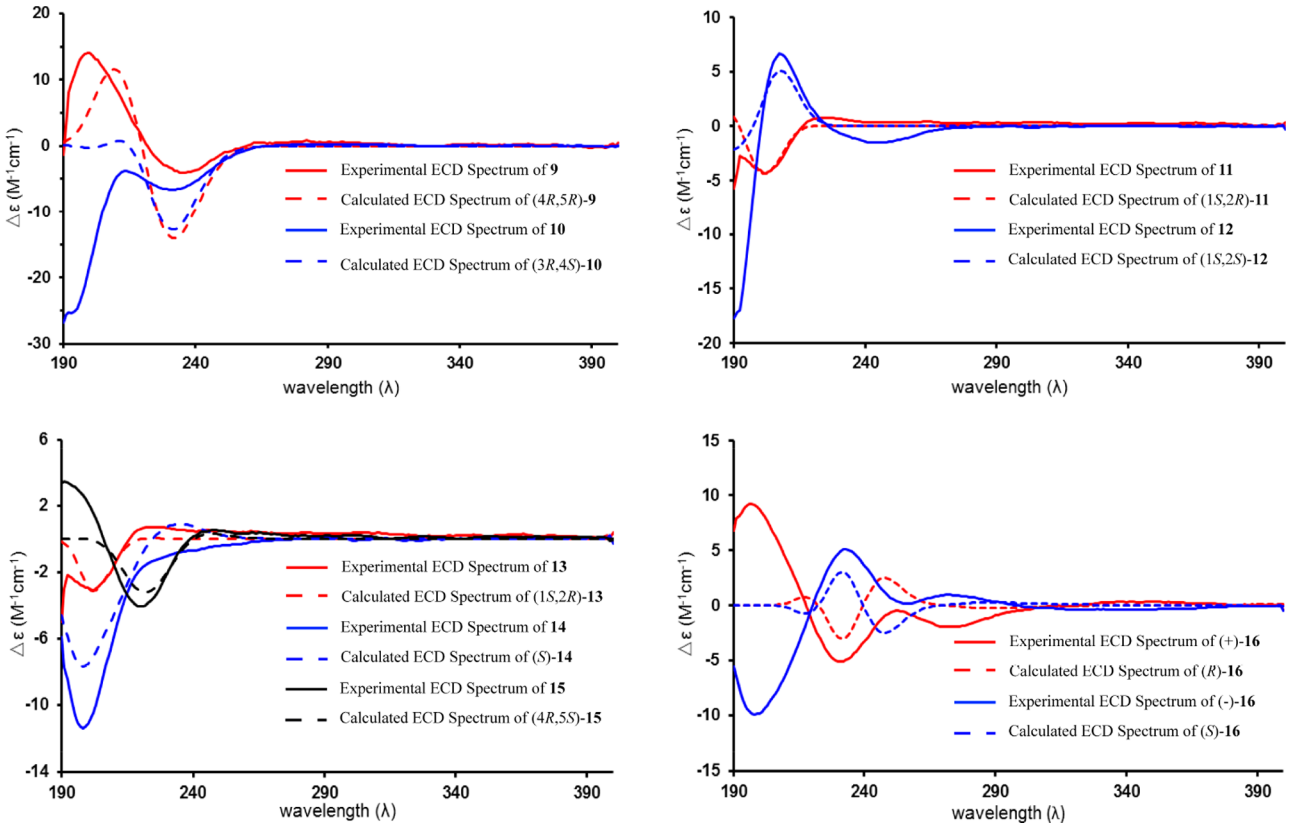

Figure 6. Experimental and calculated ECD spectra of **9**−**15**, (+)-**16**, and (−)-**16**.

were different from those of **11** and suggested that the 1-OH and 2-OH of **12** were equatorially oriented. Its absolute configuration was confirmed by the matching of the experimental and calculated ECD curves (Figure 6). Therefore, the structure of compound **12** was determined as (1*S*,2*S*)-4-(hydroxymethyl)-3,5,5-trimethylcyclohex-3-ene-1,2-diol.

Compound **13** was isolated as a colorless, amorphous solid, with a molecular formula of $C_{16}H_{28}O_8$ as defined by its HRESIMS and ^{13}C NMR data. The 1D NMR data of **13** (Tables 3 and 4) resembled those of **11**, with the main difference being the presence of a hexose moiety. The hexose moiety was identified as D-glucose by chiral-phase HPLC analysis of the acid hydrolysate of **13** (Figure S131, Supporting Information). The anomeric proton (δ_H 4.42) had a large coupling constant (J = 7.8 Hz), indicating a β-glucosidic bond. The D-glucose moiety was attached to C-2 (δ_C 84.2) as supported by the HMBC correlation between H-1′ (δ_H 4.42) of the D-glucose moiety and C-2 of the aglycone moiety (Figure S129, Supporting Information). The equatorial orientation of HO-1 and the axial orientation of the 2-*O*-β-D-

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glucose moiety were deduced from the coupling constants between H-1 [δ_H 3.77 (dt, J = 12.4, 3.1 Hz)] and H-2 [δ_H 3.93 (d, J = 3.1 Hz)]. The calculated ECD spectrum of the (1*S*,2*R*) isomer of compound **13** matched the experimental ECD curve (Figure 6). Thus, the structure of crocusatin R (**13**) was identified as (1*S*,2*R*)-4-(hydroxymethyl)-3,5,5-trimethylcyclohex-3-ene-1,2-diol-2-*O*-*β*-D-glucopyranoside.

The molecular formula of crocusatin S (**14**), $C_{16}H_{26}O_8$, was deduced from its HRESIMS and ^{13}C NMR data. The 1D NMR data of **14** (Tables 3 and 4) closely resembled those of rehmapicrogenin[22] but for a hexosyl moiety, which was identified as a D-glucose in the same manner as described for **13** (Figure S131, Supporting Information). The anomeric proton (δ_H 5.55) had a large coupling constant (J = 8.2 Hz), indicating a *β*-glucosidic linkage. The HMBC correlation between the anomeric proton of the D-glucose unit and C-7 of the aglycone unit (Figure S140, Supporting Information) implied a bond between the anomeric carbon of the D-glucose moiety and C-7 of the aglycone moiety. Subsequently, the absolute configuration of **14** was elucidated by comparing the experimental and simulated ECD curves (Figure 6). Hence, the structure of compound **14** was defined as (*S*)-3-hydroxy-2,6,6-trimethylcyclohex-1-ene-1-carboxylic acid-7-*O*-*β*-D-glucopyranoside.

Compound **15** was purified as a colorless, amorphous solid. The molecular formula ($C_{16}H_{24}O_9$) was obtained from the HRESIMS and ^{13}C NMR data. The 1D NMR data of **15** (Tables 3 and 4) resembled those of **10** and differed only in the presence of a hexose moiety and an oxymethylene and the absence of a methyl group. The monosaccharide was elucidated as D-glucose in the manner described for compound **13** (Figure S131, Supporting Information). The anomeric proton (δ_H 4.43) had a large coupling constant (J = 7.8 Hz), indicating a *β*-glucosidic bond. The HMBC correlation between H-1′ (δ_H 4.43) and C-4 (δ_C 74.4) (Figure S150, Supporting Information) suggested that the D-glucose unit was attached to C-4. Furthermore, the HMBC correlations of H_2-3 [δ_H 5.02, 4.89 (each d, J = 17.8 Hz)] with C-1 (δ_C 158.9), C-8 (δ_C 135.1), and C-9 (δ_C 174.4) (Figure S150, Supporting Information) indicated the presence of an *α*,*β*-unsaturated lactone moiety. The axial orientation of the 4-*O*-*β*-D-glucose moiety and the equatorial orientation of HO-5 were deduced from the coupling constants between H-4 [δ_H 4.59 (d, J = 3.8 Hz)] and H-5 [δ_H 4.13 (dt, J = 9.3, 3.8 Hz)]. Its absolute configuration was assigned by matching the experimental ECD curve with the calculated curve for the (4*R*,5*S*) diastereoisomer (Figure 6). Therefore, the structure of **15** was defined as (4*R*,5*S*)-4,5-dihydroxy-7,7-dimethyl-4,5,6,7-tetrahydroisobenzofuran-1(3*H*)-one-4-*O*-*β*-D-glucopyranoside.

Compound **16** was isolated as a colorless, amorphous solid with a molecular formula of $C_{10}H_{14}O_4$ as deduced from its HRESIMS and ^{13}C NMR data. The 1D NMR data of **16** (Tables 3 and 4) revealed similar resonances to those of crocusatin M (**23**).[23] Slight structural differences were observed at C-1 (a carboxylic carbon replaced a methyl group) and C-6 (a methyl group replaced a hydroxymethyl group). Compound **16** was racemic and was resolved by chiral-phase HPLC to yield (+)-**16** and (−)-**16**. The absolute configuration of each enantiomer was determined by comparing its experimental and simulated ECD curves (Figure 6). Therefore, the structures of (+)-**16** [(+)-crocusatin U] and (−)-**16** [(−)-crocusatin U] were characterized as (*R*)-6-hydroxy-5,5,6-trimethyl-3-oxocyclohex-1-ene-1-carboxylic acid and (*S*)-6-hydroxy-5,5,6-trimethyl-3-oxocyclohex-1-ene-1-carboxylic acid, respectively.

Compound **17** was obtained as a colorless, amorphous solid, with a molecular formula of $C_{10}H_{12}O_3$ deduced from the HRESIMS and ^{13}C NMR data. Its 1D NMR data (Tables 3 and 4) revealed similarities with those of the known compound, 3-oxo-2,6,6-trimethylcyclohex-1-ene-carboxylic acid,[24] except for the presence of an olefinic bond [δ_H 6.96 (d, J = 10.0 Hz)/δ_C 159.2, 6.18 (d, J = 10.0 Hz)/δ_C 126.2] and the absence of two methylene moieties. Accordingly, the structure of crocusatin V (**17**) was defined as 2,6,6-trimethyl-3-oxocyclohexa-1,4-diene-1-carboxylic acid.

In addition to the eight new triterpenoids and nine new monoterpenoids, 12 known terpenoids, including five triterpenoids and seven monoterpenoids, were isolated from *G. jasminoides*. Their structures were deduced by comparing their experimental spectra with the literature data. These compounds were 3*β*,6*β*,19*α*,23-tetrahydroxyolean-12-en-28-oic acid (**18**),[25,26] 23-hydroxyursolic acid (**19**),[27] pomolic acid (**20**),[28] 3*β*,19*α*,23,24-tetrahydroxyurs-12-en-28-oic acid (**21**),[29] 3*β*,6*β*,19*α*,23-tetrahydroxyurs-12-en-28-oic acid (**22**),[30] crocusatin M (**23**),[23] crocusatin C (**24**),[5] epijasminoside A (**25**),[31] jasminoside B (**26**),[5] 6′-*O*-sinapoyljasminoside C (**27**),[5] 6′-*O*-sinapoyljasminoside A (**28**),[5] and 5-hydroxy-7,7-dimethyl-4,5,6,7-tetrahydro-3*H*-isobenzofuran-1-one (**29**).[32]

Many natural products can alleviate acute kidney injury (AKI) via a variety of routes.[33−36] Some compounds isolated from *Gardenias* species, such as genipin, geniposidic acid, crocetin, and glycoprotein have cytoprotective activity.[9,37−39] In preliminary in vitro bioassays, the cytoprotective activities of the isolated compounds against LPS-induced NRK 52e cell death were evaluated using a real-time cell analysis (RTCA) system. The results indicated that compounds **10**, **18**, **20**, **21**, **24**, and **26** significantly protected NRK 52e cells against LPS-induced apoptosis, and low EC_{50} values, from 14.2 nM to 1.6 *μ*M, were observed (Table 5).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations and ECD spectra were obtained using a Rudolph AP-IV polarimeter (Rudolph, Hackettstown, NJ, USA) and an Applied Photophysics Chirascan qCD spectropolarimeter (AppliedPhotophysics, Leatherhead, Surrey, UK), respectively. UV and IR spectra were recorded on a Thermo EVO 300 spectrometer (Thermo, Waltham, MA, USA) and a Thermo Nicolet IS 10 spectrometer (Thermo, Waltham, MA, USA), respectively. NMR and mass spectra were acquired using a Bruker Avance III 500 spectrometer (Bruker, Germany) and a Bruker maXis HD mass spectrometer (Bruker, Germany), respectively. Semi-preparative HPLC separations were performed on a Saipuruisi LC 50 HPLC system, equipped with an UV/vis 50 detector (Saipuruisi, Beijing, China). Chiral-phase separation of **16** was conducted on a Waters liquid chromatograph with a Waters 2489 tunable absorbance detector (Waters, Milford, MA, USA), using a CHIRALPAK OD-H column (250 × 10 mm) (Daicel Chiral Technologies Co., Ltd., China). Monosaccharide elucidation was conducted on a Waters 2695 separation module with an evaporative light scattering detector (ELSD) (Waters, Milford, MA, USA) using a CHIRALPAK AD-H column (250 × 4.6 mm) (Daicel Chiral Technologies Co., Ltd., China). MCI gel CHP-20, ODS gel (50 *μ*m), Sephadex LH-20 (40−70 *μ*m), and silica gel (160−200 mesh) were acquired from TOSOH Corp., Tokyo, Japan, YMC Group, Kyoto, Japan, Amersham Pharmacia Biotech AB, Uppsala, Sweden, and Marine Chemical Industry, Qingdao, China, respectively.

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Table 5. Renoprotective Effects of Compounds 1−29

Compounds	EC_{50} (μM)
1	38.4
2	>100
3	11.5
4	>100
5	>100
6	26.8
7	>100
8	>100
9	>100
10[a]	145.7
11	>100
12	>100
13	>100
14	>100
15	>100
(+)-**16**	>100
(−)-**16**	>100
17	>100
18[a]	117.5
19	>100
20[a]	14.2
21	1.6
22	>100
23	>100
24	1.4
25	>100
26	1.2
27	>100
28	35.6
29	>100
Trolox[b]	1.9

[a]EC50 in nM. [b]Positive control compound.

Plant Material. The *G. jasminoides* fruit gathered in Tanghe, Henan province, China, in January 2016, was identified by Professor Suiqing Chen, School of Pharmacy, Henan University of Chinese Medicine, Zhengzhou, China. A voucher specimen (20160109) was deposited at the Department of Pharmaceutical Chemistry, Henan University of Chinese Medicine.

Extraction and Isolation. The dried and powdered fruit (9.8 kg) was extracted with 50% aqueous acetone (3 × 80 L, smashed tissue extraction). The extract (2.3 kg) was dispersed in H_2O (8 L) and sequentially extracted with petroleum ether (5 × 8 L), EtOAc (5 × 8 L), and *n*-BuOH (5 × 8 L). After concentration, the petroleum ether fraction (104.8 g), the EtOAc fraction (199.1 g), and the *n*-BuOH fraction (650.2 g) were collected. The EtOAc fraction was separated by silica gel column chromatography (CC, 12 × 130 cm) eluted with a CH_2Cl_2:MeOH (100:0−0:100) gradient and yielded eight fractions (A1−A8).

Fraction A3 (34.2 g) was chromatographed with silica gel CC eluted with a petroleum ether:acetone (50:1−1:1) gradient to obtain nine subfractions (A3-1−A3-9). Subfraction A3-8 (900.2 mg) was further separated by Sephadex LH-20 CC (MeOH) followed by semipreparative HPLC (MeOH:H_2O 80:20) to afford compound **20** (7.5 mg, t_R 18.1 min).

Fraction A4 (21.0 g) was subjected to MCI gel CHP-20 CC eluted with a MeOH:H_2O (10:90−100:0) gradient to give seven subfractions (A4-1−A4-7). Subfraction A4-1 (12.6 g) was further separated by ODS CC eluted with a MeOH:H_2O (10:90−100:0) gradient to yield 18 subfractions (A4-1-1−A4-1-18). Subfraction A4-1-2 (259.4 mg) was passed through a Sephadex LH-20 column (MeOH:H_2O 70:30) and then purified by semipreparative HPLC (CH_3CN:H_2O 20:80) to produce compound **24** (5.8 mg, t_R 17.3 min). Compound **10** (8.5 mg, t_R 24.3 min) was obtained from fraction A4-1-3 (539.3 mg) by semipreparative HPLC (CH_3CN:H_2O 5:95). Compounds **17** (3.8 mg, t_R 32.3 min) and **23** (73.9 mg, t_R 15.1 min) were obtained from fraction A4-1-4 (244.8 mg) by semipreparative HPLC (MeOH:H_2O 30:70). Subfraction A4-1-5 (213.1 mg) was further purified via semipreparative HPLC (MeOH:H_2O 35:65) to yield compound **16** (11.1 mg, t_R 21.0 min). The enantiomers were resolved using a CHIRALPAK OD-H column (cyclohexane:isopropanol:TFA, 850:150:0.15, 3 mL·min^{-1}) to afford (+)-**16** (2.3 mg, t_R 7.1 min) and (−)-**16** (1.5 mg, t_R 7.9 min). Subfraction A4-3 (1.7 g) was passed through a silica gel column eluted with a CH_2Cl_2:MeOH (50:1−10:1) gradient and then separated by semipreparative HPLC (CH_3CN:H_2O 20:80) to yield compound **2** (4.6 mg, t_R 21.9 min). Subfraction A4-4 (1.4 g) was separated by passage through a silica gel column eluted with a CH_2Cl_2:MeOH (16:1−8:1) gradient. Eight subfractions (A4-4-1−A4-4-8) were obtained. Further separation of subfraction A4-4-6 (59.7 mg) via semipreparative HPLC (CH_3CN:H_2O 65:35) yielded compound **19** (29.7 mg, t_R 22.0 min). Compound **1** (199.8 mg, t_R 21.0 min) was obtained from A4-4-7 (262.4 mg) by semipreparative HPLC (CH_3CN:H_2O 80:20). Separation of subfraction A4-4-8 (235.7 mg) using semipreparative HPLC (CH_3CN:H_2O 90:10) resulted in compounds **3** (17.2 mg, t_R 16.7 min) and **4** (16.4 mg, t_R 27.4 min). Subfraction A4-6 (642.9 mg) was chromatographed with Sephadex LH-20 CC (MeOH:H_2O 70:30) and semipreparative HPLC (CH_3CN:H_2O 40:60) to produce compound **29** (3.9 mg, t_R 16.9 min).

The separation of fraction A5 (12.3 g) using ODS as the stationary phase and a gradient of MeOH:H_2O (10:90−100:0) as the mobile phase resulted in 33 subfractions (A5-1−A5-33). Subfraction A5-8 (114.5 mg) was further purified by semipreparative HPLC (CH_3CN:H_2O 8:92) to obtain compound **9** (15.3 mg, t_R 15.6 min). Compound **12** (15.7 mg, t_R 13.1 min) was isolated from subfraction A5-10 (64.4 mg) by semipreparative HPLC (CH_3CN:H_2O 40:60). Compound **11** (8.2 mg, t_R 15.5 min) was isolated from subfraction A5-11 (99.1 mg) by semipreparative HPLC (CH_3CN:H_2O 10:90). Subfraction A5-20 (1.0 g) was separated by Sephadex LH-20 CC (MeOH:H_2O 30:70) to give five subfractions (A5-20-1−A5-20-5). Compounds **27** (47.1 mg, t_R 21.5 min) and **28** (41.4 mg, t_R 24.3 min) were isolated from A5-20-2 (203.8 mg) by semipreparative HPLC (MeOH:H_2O 55:45). Compound **18** (19.0 mg) was isolated from A5-24 (230.0 mg) using silica gel CC (CH_2Cl_2:MeOH 16:1). Subfraction A5-25 (416.2 mg) was separated by silica gel CC with CH_2Cl_2:MeOH (8:1) as the eluent. Further separation using semipreparative HPLC (MeOH:H_2O 65:36) gave compound **21** (99.7 mg, t_R 50.7 min). Compound **22** (13.0 mg, t_R 37.3 min) was isolated from A5-26 (324.7 mg) using Sephadex LH-20 CC (CH_2Cl_2:MeOH 1:1) and semipreparative HPLC (MeOH:H_2O 68:32). Compound **7** (33.7 mg) was obtained from subfraction A5-28 (228.9 mg) using Sephadex LH-20 CC (MeOH). Compound **5** (16.6 mg, t_R 28.5 min) was isolated from subfraction A5-30 using semipreparative HPLC (MeOH:H_2O 80:20). Subfraction A5-32 (247.7 mg) was chromatographed via Sephadex LH-20 CC (CH_2Cl_2:MeOH 1:1) followed by semipreparative HPLC (CH_3CN:H_2O 75:25) to give compound **6** (5.6 mg, t_R 15.1 min). Compound **8** (10.4 mg) was isolated from A5-33 (163.5 mg) using silica gel CC (CH_2Cl_2:MeOH 16:1) and Sephadex LH-20 CC (CH_2Cl_2:MeOH 1:1).

Fraction A6 (69.3 g) was separated by MCI gel CHP-20 CC eluted with a MeOH:H_2O (10:90−100:0) gradient and gave five subfractions (A6-1−A6-5). Subfraction A6-2 (4.9 g) was subjected to ODS CC eluted with a MeOH:H_2O (10:90−100:0) gradient and yielded seven subfractions (A6-2-1−A6-2-7). Fraction A6-2-4 (700.1 mg) was rechromatographed by Sephadex LH-20 CC (MeOH:H_2O 30:70) and semipreparative HPLC (MeOH:H_2O 32:68) to afford compound **25** (11.1 mg, t_R 20.0 min).

Fraction A7 (31.5 g) was subjected to MCI gel CHP-20 CC eluted with a MeOH:H_2O (10:90−100:0) gradient to provide 13 subfractions (A7-1−A7-13). Further separation of A7-4 (1.3 g) using silica gel CC (CH_2Cl_2:MeOH 7:1) resulted in six subfractions

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(A7-4-1−A7-4-6). Compounds **26** (22.9 mg, t_R 13.6 min), **15** (2.0 mg, t_R 17.4 min), **13** (16.2 mg, t_R 18.9 min), and **14** (10.9 mg, t_R 27.9 min) were obtained from subfraction A7-4-3 (161.1 mg) using semipreparative HPLC (MeOH:H_2O 35:65).

*26-Hydroxylithocarpic Acid B (**1**).* Colorless, amorphous solid; $[\alpha]_D^{20}$ +31 (*c* 0.8, MeOH); UV (MeOH) λ_{max} (logε) 201 (3.76) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 198 (−2.3), 211 (0.7), 222 (−0.6) nm; IR (iTR) ν_{max} 3419, 2944, 1708, 1452, 1377, 1205, 1030, 892 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m*/*z* 487.3421 $[M - H]^-$ (calcd for $C_{30}H_{47}O_5$, 487.3418).

*Methyl 26-Hydroxylithocarpic Acid B (**2**).* Colorless, amorphous solid; $[\alpha]_D^{20}$ +26 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 201 (3.66) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 198 (−2.4), 212 (0.3), 221 (−0.7) nm; IR (iTR) ν_{max} 3366, 2947, 1683, 1404, 1125, 1031 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m*/*z* 537.3342 $[M + Cl]^-$ (calcd for $C_{30}H_{50}O_5Cl$, 537.3341).

*(24Z)-21-Hydroxylithocarpic Acid N (**3**).* Colorless, amorphous solid; $[\alpha]_D^{20}$ +57 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.93), 229 (2.43) nm; IR (iTR) ν_{max} 3368, 2942, 1709, 1454, 1378, 1031, 891 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m*/*z* 495.3450 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_4Na$, 495.3448).

*Methyl (24Z)-21-Hydroxylithocarpic Acid N (**4**).* Colorless, amorphous solid; $[\alpha]_D^{20}$ +50 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.85), 226 (2.91) nm; IR (iTR) ν_{max} 3405, 2943, 1736, 1437, 1377, 1169, 1029, 892 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m*/*z* 509.3602 $[M + Na]^+$ (calcd for $C_{31}H_{50}O_4Na$, 509.3601).

*Methyl (24Z)-21,29-Dihydroxylithocarpic Acid N (**5**).* Colorless, amorphous solid; $[\alpha]_D^{20}$ +48 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.86) nm; IR (iTR) ν_{max} 3360, 2942, 1736, 1454, 1379, 1170, 1031, 900 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m*/*z* 525.3557 $[M + Na]^+$ (calcd for $C_{31}H_{50}O_5Na$, 525.3550).

*Methyl (24Z)-4,21-Dihydroxy-4H,28H-lithocarpic Acid N (**6**).* Colorless, amorphous solid; $[\alpha]_D^{20}$ +46 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.72) nm; IR (iTR) ν_{max} 3364, 2948, 1720, 1455, 1378, 1203, 1033, 898 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m*/*z* 527.3712 $[M + Na]^+$ (calcd for $C_{31}H_{52}O_5Na$, 527.3707).

*3β,16β,23,24-Tetrahydroxy-28-nor-ursane-12,17,19,21-tetraene (**7**).* White, amorphous powder; $[\alpha]_D^{20}$ +67 (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.40), 244 (4.02) nm; IR (iTR) ν_{max} 3331, 2944, 1679, 1451, 1385, 1373, 1083, 1030, 818 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m*/*z* 477.2971 $[M + Na]^+$ (calcd for $C_{29}H_{42}O_4Na$, 477.2975).

*3β,23,24-Trihydroxy-16β-methoxy-28-nor-ursane-12,17,19,21-tetraene (**8**).* White, amorphous powder; $[\alpha]_D^{20}$ +59 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.31), 243 (3.92) nm; IR (iTR) ν_{max} 3336, 2943, 1682, 1452, 1385, 1355, 1195, 1128, 1107, 1043, 1004, 823 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; HRESIMS *m*/*z* 491.3137 $[M + Na]^+$ (calcd for $C_{30}H_{44}O_4Na$, 491.3132).

*Crocusatin N (**9**).* Colorless, amorphous solid; $[\alpha]_D^{20}$ −26 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.75) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 199 (18.9), 235 (−5.5) nm; IR (iTR) ν_{max} 3366, 2966, 1698, 1652, 1453, 1367, 1255, 1230, 1066, 1035 cm^{-1}; ^1H and ^{13}C NMR data, Tables 3 and 4; HRESIMS *m*/*z* 223.0941 $[M + Na]^+$ (calcd for $C_{10}H_{16}O_4Na$, 223.0940).

*Crocusatin O (**10**).* Colorless, amorphous solid; $[\alpha]_D^{20}$ −103 (*c* 0.7, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.29) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 195 (−23.5), 214 (5.7), 232 (−6.0) nm; IR (iTR) ν_{max} 3355, 2942, 1701, 1654, 1454, 1407, 1252, 1196, 1076, 1033 cm^{-1}; ^1H and ^{13}C NMR data, Tables 3 and 4; HRESIMS *m*/*z* 223.0942 $[M + Na]^+$ (calcd for $C_{10}H_{16}O_4Na$, 223.0940).

*Crocusatin P (**11**).* Colorless, amorphous solid; $[\alpha]_D^{20}$ −132 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.02), 244 (3.13) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 202 (−5.5), 227 (0.2) nm; IR (iTR) ν_{max} 3365, 2959, 1677, 1458, 1409, 1180, 1072, 1029, 997 cm^{-1}; ^1H and ^{13}C NMR data, Tables 3 and 4; HRESIMS *m*/*z* 209.1149 $[M + Na]^+$ (calcd for $C_{10}H_{18}O_3Na$, 209.1148).

*Crocusatin Q (**12**).* Colorless, amorphous solid; $[\alpha]_D^{20}$ −35 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.91) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 207 (7.1), 244 (−1.6) nm; IR (iTR) ν_{max} 3332, 2935, 1672, 1446, 1413, 1365, 1179, 1124, 1064, 1023, 991 cm^{-1}; ^1H and ^{13}C NMR data, Tables 3 and 4; HRESIMS *m*/*z* 209.1147 $[M + Na]^+$ (calcd for $C_{10}H_{18}O_3Na$, 209.1148).

*Crocusatin R (**13**).* Colorless, amorphous solid; $[\alpha]_D^{20}$ −83 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.85), 235 (2.39) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 200 (−27.2), 225 (0.1) nm; IR (iTR) ν_{max} 3375, 2938, 1678, 1424, 1365, 1102, 1076, 1033, 994 cm^{-1}; ^1H and ^{13}C NMR data, Tables 3 and 4; HRESIMS *m*/*z* 371.1684 $[M + Na]^+$ (calcd for $C_{16}H_{28}O_8Na$, 371.1676).

*Crocusatin S (**14**).* Colorless, amorphous solid; $[\alpha]_D^{20}$ −10 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 201 (3.63) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 198 (−22.6) nm; IR (iTR) ν_{max} 3354, 2929, 1724, 1452, 1365, 1291, 1222, 1203, 1074, 1027 cm^{-1}; ^1H and ^{13}C NMR data, Tables 3 and 4; HRESIMS *m*/*z* 369.1527 $[M + Na]^+$ (calcd for $C_{16}H_{26}O_8Na$, 369.1520).

*Crocusatin T (**15**).* Colorless, amorphous solid; $[\alpha]_D^{20}$ −81 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.07) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 194 (3.4), 219 (−10.9), 249 (0.8) nm; IR (iTR) ν_{max} 3363, 2935, 1738, 1674, 1445, 1349, 1199, 1163, 1076, 1025, 787 cm^{-1}; ^1H and ^{13}C NMR data, Tables 3 and 4; HRESIMS *m*/*z* 383.1312 $[M + Na]^+$ (calcd for $C_{16}H_{24}O_9Na$, 383.1313).

*(±)-Crocusatin U (**16**).* Colorless, amorphous solid; UV (MeOH) λ_{max} (log ε) 228 (3.84) nm; IR (iTR) ν_{max} 3325, 2948, 2833, 1675, 1583, 1410, 1316, 1206, 1144, 1030, 801, 748, 724 cm^{-1}; ^1H and ^{13}C NMR data, Tables 3 and 4; HRESIMS *m*/*z* 221.0785 $[M + Na]^+$ (calcd for $C_{10}H_{14}O_4Na$, 221.0784). (+)-**16**: [α]20D + 21 (*c* 0.1, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 198 (11.1), 233 (−6.1), 257 (−0.6), 273 (−2.4) nm; (−)-**16**: [α]20D − 21 (*c* 0.1, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 197 (−12.4), 231 (6.3), 253 (0.2), 273 (1.2) nm.

*Crocusatin V (**17**).* Colorless, amorphous solid; UV (MeOH) λ_{max} (log ε) 239 (3.75) nm; IR (iTR) ν_{max} 3405, 2975, 1717, 1656, 1626, 1470, 1396, 1376, 1323, 1299, 1249, 1158, 1132, 1057, 1029, 835 cm^{-1}; ^1H and ^{13}C NMR data, Tables 3 and 4; HRESIMS *m*/*z* 179.0702 $[M - H]^-$ (calcd for $C_{10}H_{12}O_3Na$, 179.0703).

Computational Analysis. The conformations of **1**, **9**−**15**, (+)-**16**, and (−)-**16** were determined by GMMX software using the MMFF94 force field. The geometry optimizations and predictions of the ECD spectra of the conformers were carried out using density functional theory (DFT) at the B3LYP/6-311G(d,p) level in the Gaussian 16W.[40,41] SpecDis software, version 1.71 was used to simulate the ECD curves according to Boltzmann distribution theory.[42] The ^1H and ^{13}C NMR chemical shifts of **1a**−**1d** were calculated with the GIAO method at the mPW1PW91/6-31G(d,p) level in MeOH.[43] The calculations of IR and VCD frequencies for **1a**−**1d** were performed at the B3LYP/6-31G(d,p) levels in $CHCl_3$.[44]

Acid-Catalyzed Hydrolysis of Compounds 13−15. Compounds **13** (1.2 mg), **14** (1.4 mg), and **15** (1.0 mg) were treated with 2 N aqueous HCl (2.5 mL) (sealed flask, 80 °C, 3 h). For each compound, the acidic aqueous mixture was dried, H_2O (2 mL) was added, and the mixture was extracted with EtOAc (3 × 2 mL).[45] The dry aqueous layer was subjected to chiral-phase HPLC. The carbohydrate products of the hydrolysis of **13**−**15** were separated by a CHIRALPAK AD-H column (250 × 4.6 mm) using *n*-hexane:EtOH:TFA (750:250:0.25) as the mobile phase (0.5 mL·min^{-1}) and detected by an evaporative light scattering detector (ELSD). For all three compounds, the sugar was found to be D-glucose[46] (Figure S131, Supporting Information).

Cell Culture. Normal rat kidney tubule epithelioid (NRK 52e) cells were grown in DMEM (Gibco) containing 10% FBS (HyClone), penicillin (50 kU·L^{-1}), and streptomycin (50 mg·L^{-1}) in a controlled, humidified atmosphere at 37 °C and 5% CO_2.

Evaluation of the Protective Activities Toward NRK 52e Cells. The RTCA assay was performed over 72 h using an xCELLigence instrument (Acea Biosciences, Inc.). Fifty microliters of cell culture medium from each well was used to measure the background impedance signal. After digestion into a single-cell suspension with 0.25% trypsin, exponentially growing NRK 52e cells were distributed into 16-well E-plates. The final cell density per well was 2 × 10^4 cells in 150 μL of medium. The impedance was

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monitored every 15 min. After 24 h, the cells were treated with lipopolysaccharide (1 $\mu g \cdot mL^{-1}$) and the test compounds or Trolox at five concentrations (0.1, 1, 10, 50, 100 μM). The final concentration of DMSO was 0.1%. After the addition of the test compounds, the signal was monitored at 5 min intervals until the end of the experiment.[47] All tests were conducted in triplicate, and the mean values are reported.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.9b01119.

UV, IR, ESI, and NMR spectra of compounds **1**−**29** (PDF)

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Notes

The authors declare no competing financial interest.

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