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Peroxynitrite-Scavenging Glycosides from the Stem Bark of *Catalpa ovata*

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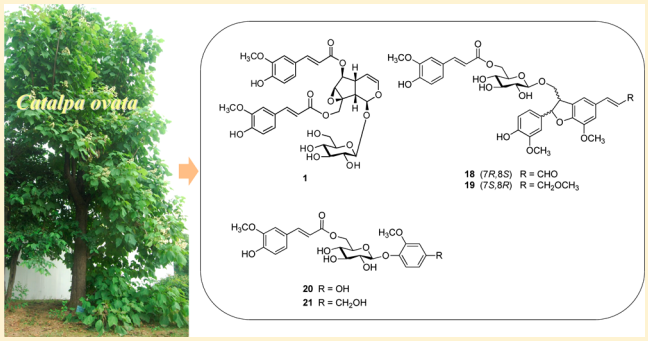
Supporting Information

ABSTRACT: Ten new glycosides, 6,10-*O*-di-*trans*-feruloyl catalpol (**1**), 6,6′-*O*-di-*trans*-feruloyl catalpol (**2**), 3,4-dihydro-6-*O*-di-*trans*-feruloyl catalpol (**10**), (8*R*,7′*S*,8′*R*)-lariciresinol 9′-*O*-*β*-D-(6-*O*-*trans*-feruloyl)glucopyranoside (**17**), and ovatosides A−F (**18**−**22**, **24**), were isolated from the stem bark of *Catalpa ovata* along with 19 known compounds. All isolates, except **6** (catalposide) and **9** (6-*O*-veratroyl catalpol), were found to scavenge peroxynitrite ($ONOO^-$) formed by 3-morpholinosydnonimine. In particular, 12 compounds showed potent activity, with IC_{50} values in the range 0.14−2.2 μM.



Catalpa ovata G. Don. is a deciduous tree belonging to the family Bignoniaceae.[1] Its stem bark has been used traditionally for treating several inflammatory diseases including hepatitis, cholecystitis, and nephritis.[2] Previous phytochemical research on the stem bark of *C. ovata* revealed the occurrence of C_9-type iridoids, some of which were shown to inhibit the production of tumor necrosis factor-α (TNF-α) and nitric oxide (NO), and showed effects on heat shock protein 90 (HSP90), respectively.[3−5]

In the process of inflammation, NO is overproduced by the expression of inducible NO synthase (iNOS), while reactive oxygen species (ROS) are released and accumulated by oxygen uptake of mast cells and leukocytes recruited at the site.[6,7] Although regulation of NO production has been targeted for the treatment of inflammatory diseases, peroxynitrite ($ONOO^-$) is the ultimate cytotoxic agent, made by combining NO and ROS and more potent than both.[7,8] The reactivity of $ONOO^-$ causes a variety of alterations on DNA, protein, and lipids.[8] Accordingly, the scavenging capacity of $ONOO^-$ can prevent further progression of inflammation and the development of chronic disorders. To the best of our knowledge, there has been no research on *C. ovata* for the evaluation of its $ONOO^-$-scavenging effects, even though anti-inflammatory activities have been studied previously in regard to its traditional uses.[2,9]

In the present investigation, an EtOAc extract from a MeOH extract of the stem bark of *C. ovata* was found to be effective in scavenging $ONOO^-$. Subsequent fractionation was conducted using the EtOAc extract, leading to the isolation of 10 new glycosides, 6,10-*O*-di-*trans*-feruloyl catalpol (**1**), 6,6′-*O*-di-*trans*-feruloyl catalpol (**2**), 3,4-dihydro-6-*O*-di-*trans*-feruloyl catalpol (**10**), (8*R*,7′*S*,8′*R*)-lariciresinol 9′-*O*-*β*-D-(6-*O*-*trans*-feruloyl)-glucopyranoside (**17**), and ovatosides A−F (**18**−**22**, **24**), together with 19 known compounds, 6-*O*-*trans*-feruloyl catalpol (**3**),[3,10] specioside (**4**),[11] 6-*O*-(4-methoxy-*trans*-cinnamoyl) catalpol (**5**),[12] catalposide (**6**),[3] 4′-methoxycatalposide (**7**),[13] vanilloyl catalpol (**8**),[14] 6-*O*-veratroyl catalpol (**9**),[15] 3,4-dihydrocatalposide (**11**),[5] 6-*O*-*trans*-feruloyl-5,7-bisdeoxycynanchoside (**12**),[5] 6-*O*-*trans*-isoferuloyl-5,7-bisdeoxycynanchoside (**13**),[5] 6-*O*-(4-hydroxybenzoyl)-5,7-bisdeoxycynanchoside (**14**),[16] 6-*O*-(4-hydroxybenzoyl)ajugol (**15**),[17] 6-*O*-(4-hydroxybenzoyl)phelipaeside (**16**),[5] grayanoside A (**23**),[18] darendoside A (**25**),[19] 2-(4-hydroxyphenyl)ethyl[5-*O*-(4-hydroxybenzoyl)]-*O*-*β*-D-apiofuranosyl-(1→2)-*β*-D-glucopyranoside (**26**),[16] phlomisethanoside (**27**),[20] darendoside B (**28**),[19] and 8,5′-diferulic acid (**29**).[21] All isolates were tested for their $ONOO^-$-scavenging activities.

RESULTS AND DISCUSSION

Compound **1** was obtained as a light yellow, amorphous powder with a molecular formula of $C_{35}H_{38}O_{16}$, based on its ^{13}C NMR data and the $[M + H]^+$ ion at *m*/*z* 715.2242 (calcd 715.2233) in the HRESIMS. The ^1H and ^{13}C NMR spectra of **1** showed typical signals for a C_9-type iridoid at δ_H 5.21 (1H, d, J = 9.6 Hz)/δ_C 95.4 (C-1), 6.39 (1H, dd, J = 6.0, 2.0 Hz)/142.5 (C-3), 5.00 (1H, dd, J = 6.0, 4.4 Hz)/102.7 (C-4), 2.62 (1H, m)/36.8 (C-5), 5.09 (1H, dd, J = 8.4, 1.2 Hz)/81.2 (C-6), 3.77 (1H, d, J = 1.2 Hz)/60.6 (C-7), 2.75 (1H, dd, J = 9.6, 8.0 Hz)/43.4 (C-9), 4.32 and 5.02 (each 1H, d, J = 12.4 Hz)/64.0 (C-10), and δ_C 64.3 (C-8) (Table 1).[5] Besides the iridoid scaffold, proton signals for two ABX spin systems were observed at δ_H 7.22 (1H, d, J = 1.8 Hz, H-2″ exchangeable with H-2‴),

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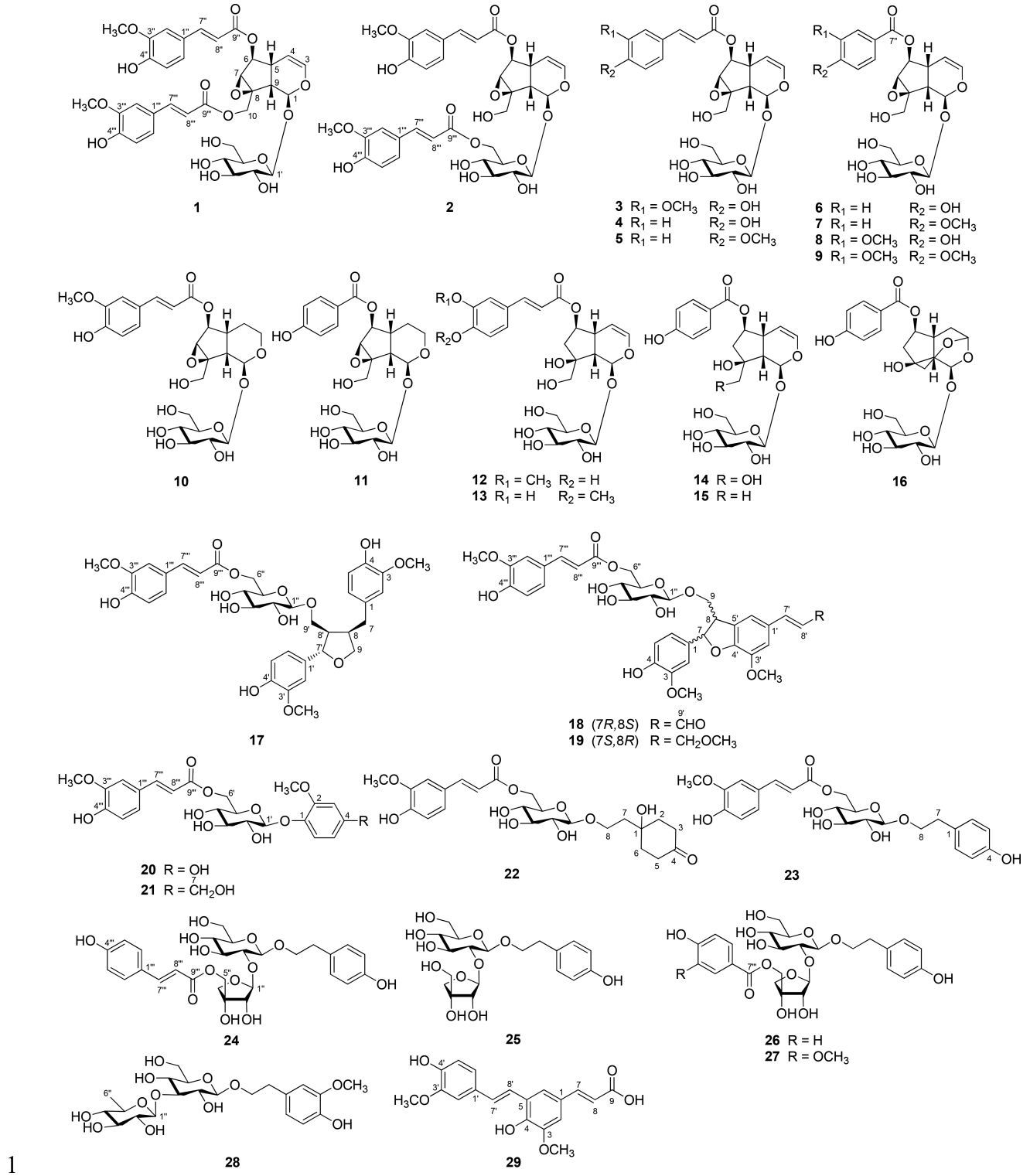
Chart 1



6.815 (1H, d, J = 8.2 Hz, H-5″ exchangeable with H-5‴), 7.10 (1H, dd, J = 8.2, 1.8 Hz, H-6″), 7.21 (1H, d, J = 1.8 Hz, H-2‴ exchangeable with H-2″), 6.805 (1H, d, J = 8.2 Hz, H-5‴ exchangeable with H-5″), and 7.09 (1H, dd, J = 8.2, 1.8 Hz, H-6‴), together with protons of two *trans* double bonds conjugated to carbonyl groups at δ_H 7.68 (1H, d, J = 15.8 Hz, H-7″), 6.43 (1H, d, J = 15.8 Hz, H-8″), 7.64 (1H, d, J = 16.0 Hz, H-7‴), and 6.40 (1H, d, J = 16.0 Hz, H-8‴). The ^{13}C NMR signals of the carbonyl groups resonated at δ_C 168.91 (C-9″) and 168.93 (C-9‴). In the ^{1}H NMR spectrum, two aromatic methoxy group signals appeared at δ_H 3.89 (6H, s, OCH_3-3″), as well as the characteristic signals of a β-glucopyranosyl group at δ_H 4.78 (1H, d, J = 8.0 Hz), with six protons in the range δ_H 3.19−3.96.[22] The two sets of the 1,3,4-trisubstituted benzene, a *trans* double bond conjugated to the carbonyl group, and an aromatic methoxy group were assignable to two *trans*-feruloyl groups on the basis of the HMBC correlations of H-7″/C-2″, C-6″, C-9″, H-8″/C-1″, C-9″, OCH_3-3″/C-3″, H-7‴/C-2‴,

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Table 1. ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR Spectroscopic Data for Compounds 1, 2, and 10[a]

	1		2		10	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	95.4, CH	5.21, d (9.6)	95.3, CH	4.96, d (9.9)	97.5, CH	4.91, d (8.4)
3	142.5, CH	6.39, dd (6.0, 2.0)	142.4, CH	6.32, dd (5.9, 1.4)	62.9, CH_2	3.59, td (12.4, 2.0), 3.90[b]
4	102.7, CH	5.00, dd (6.0, 4.4)	103.1, CH	4.90, dd (5.9, 4.8)	24.0, CH_2	1.54, br d (14.4), 1.80, m
5	36.8, CH	2.62, m	36.9, CH	2.53, m	36.2, CH	2.37[b]
6	81.2, CH	5.09, dd (8.4, 1.2)	81.4, CH	4.88, br d (7.6)	75.8, CH	5.34, d (7.6)
7	60.6, CH	3.77, d (1.2)	60.2, CH	3.67, br s	59.7, CH	3.68, br s
8	64.3, C		66.7, C		66.5, C	
9	43.4, CH	2.75, dd (9.6, 8.0)	43.1, CH	2.61, dd (9.9, 7.8)	43.2, CH	2.34[b]
10	64.0, CH_2	4.32, d (12.4), 5.02, d (12.4)	61.7, CH_2	3.71, d (13.2), 4.21, d (13.2)	61.0, CH_2	3.81, d (13.2), 4.09, d (13.2)
1′	100.3, CH	4.78, d (8.0)	99.9, CH	4.79, d (7.6)	99.3, CH	4.71, d (7.8)
2′	74.9, CH	3.21, dd (9.2, 8.0)	74.9, CH	3.31[b]	74.9, CH	3.23, dd (9.2, 7.8)
3′	77.9, CH	3.37, br t (8.8)	77.6, CH	3.44, br t (8.8)	77.9, CH	3.38, dd (9.2, 8.8)
4′	71.6, CH	3.26[b]	71.9, CH	3.39, br t (8.8)	71.9, CH	3.24, dd (8.8, 8.8)
5′	78.6, CH	3.33[b]	76.2, CH	3.57, m	78.6, CH	3.30[b]
6′	63.1, CH_2	3.67, dd (12.0, 6.6), 3.94, dd (12.0, 1.8)	64.1, CH_2	4.47, dd (11.9, 2.4), 4.54, dd (11.9, 2.6)	63.1, CH_2	3.64, dd (12.1, 6.4), 3.94, dd (12.1, 2.2)
1″	127.7, C		127.6, C		127.5, C	
2″	111.9, CH	7.22, d (1.8)[c]	111.85,[c] CH	7.211, d (1.9)[c]	111.8, CH	7.20, d (1.9)
3″	149.5,[c] C		149.4,[d] C		149.6, C	
4″	150.9,[d] C		150.8,[e] C		151.2, C	
5″	116.54,[e]CH	6.815, d (8.2)[d]	116.48, CH	6.82, d (8.4)	116.6, CH	6.81, d (8.3)
6″	124.4, CH	7.10, dd (8.2, 1.8)	124.3, CH	7.10, dd (8.4, 1.9)	124.4, CH	7.09, dd (8.3, 1.9)
7″	147.6, CH	7.68, d (15.8)	147.5, CH	7.62, d (15.8)	147.5, CH	7.66, d (16.0)
8″	114.9, CH	6.43, d (15.8)	114.9, CH	6.37, d (15.8)	114.8, CH	6.40, d (16.0)
9″	168.91, C		168.7, C		169.3, C	
1‴	127.8, C		127.7, C			
2‴	111.9, CH	7.21, d (1.8)[c]	111.88,[c] CH	7.206, d (1.9)[c]		
3‴	149.4,[c] C		149.5,[d] C			
4‴	150.7,[d] C		150.9,[e] C			
5‴	116.50,[e] CH	6.805, d (8.2)[d]	116.53, CH	6.77, d (8.4)		
6‴	124.3, CH	7.09, dd (8.2, 1.8)	124.5, CH	7.08, dd (8.4, 1.9)		
7‴	147.3, CH	7.64, d (16.0)	147.3, CH	7.66, d (15.8)		
8‴	115.2, CH	6.40, d (16.0)	115.2, CH	6.42, d (15.8)		
9‴	168.93, C		169.0, C			
OCH_3-3″	56.5, CH_3	3.89, s	56.54,[f] CH_3	3.90,[d] s	56.5, CH_3	3.89, s
OCH_3-3‴	56.5, CH_3	3.89, s	56.52,[f] CH_3	3.88,[d] s		

[a]TMS was used as internal standard; data were measured in methanol-d_4. [b]Multiplicity patterns were unclear due to signal overlapping; chemical shifts were estimated by HSQC NMR spectra. [c−f]Data could be exchanged in the same column.

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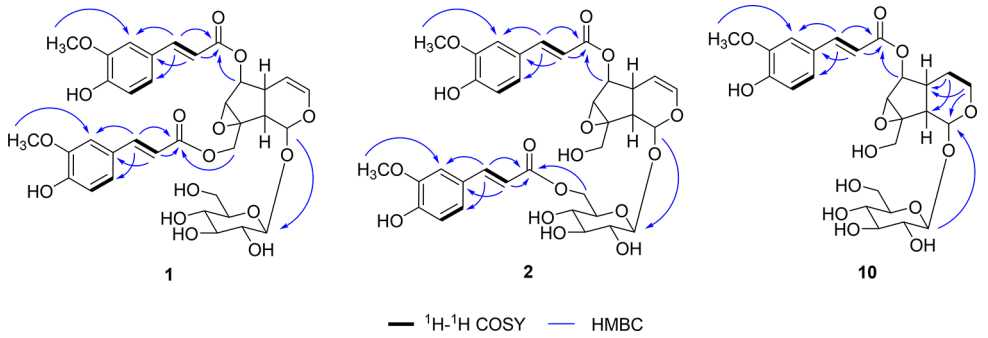

Figure 1. Key ^{1}H−^{1}H COSY and HMBC correlations of **1**, **2**, and **10**.

C-6‴, C-9‴, H-8‴/C-1‴, C-9‴, and OC*H*$_3$-3‴/C-3‴ (Figure 1). The positions of the aromatic methoxy groups were confirmed by the NOE correlations with the *meta*-coupled aromatic proton signals (H-2″ and H-2‴). The three-bond HMBC correlations of H-1/C-1′, H-6/C-9″, and H$_2$-10/C-9‴ provided evidence for the location of the *β*-glucopyranosyl group and the *trans*-feruloyl groups on the iridoid skeleton at C-1, C-6, and C-10, respectively. The connectivity of the *β*-glucopyranosyl group to the iridoid skeleton at C-1 was supported by the NOE correlation between H-1 and H-1′. Moreover, the NOE correlations of H-1/H$_2$-10, H-6, H-4/H-6, and H-7/H-10b together with the coupling constant between H-5 and H-9 (J = 8.0 Hz) supported the stereochemistry of the iridoid skeleton with a *cis* ring fusion.[23−27] Acid hydrolysis of **1** yielded a sugar moiety, which was determined as D-glucose by the HPLC analysis of its thiocarbamoyl-thiazolidine derivative.[28−31] The characteristic NMR data for **1** were similar to those of 6-*O*-*trans*-feruloyl catalpol (**3**), which was also identified in the present study,[10] except for the presence of a *trans*-feruloyl group at C-10. Thus, the structure of **1** was defined as shown for the new compound, 6,10-*O*-di-*trans*-feruloyl catalpol.

Compound **2** was isolated as a light yellow, amorphous powder with a molecular formula of $C_{35}H_{38}O_{16}$, based on ^{13}C NMR data and an $[M + H]^+$ ion at m/z 715.2233 (calcd 715.2233) in the HRESIMS. The ^{1}H and ^{13}C NMR spectra of **2** also showed resonances for a di-*trans*-feruloyl catalpol unit, as found for **1**. However, in the ^{1}H NMR spectrum of **2**, the methylene group protons at C-10 (δ_H 3.71 and 4.21) of the iridoid skeleton were found upfield by 0.61 and 0.81 ppm, respectively, when compared with those of **1**. Downfield shifts of 0.80 and 0.60 ppm were observed for the CH$_2$-6′ resonances of the *β*-glucopyranosyl group (δ_H 4.47 and 4.54), respectively (Table 1). The observation suggested that one of the *trans*-feruloyl groups was positioned at C-6′ in **2**, instead of the C-10 location in **1**. The C-6′ location of the *trans*-feruloyl group was supported by the important HMBC correlations of H-6′a/C-9‴ and H-6′b/C-9‴ (Figure 1). Acid hydrolysis of **2** afforded D-glucose. Therefore, the structure of **2** was defined as shown for the new substance 6,6′-*O*-di-*trans*-feruloyl catalpol.

Compound **10** was isolated as a light yellow, amorphous powder with a molecular formula of $C_{25}H_{32}O_{13}$, based on its ^{13}C NMR data and the $[M + Na]^+$ ion at m/z 563.1729 (calcd 563.1735) in the HRESIMS. Compound **10** exhibited closely comparable ^{1}H and ^{13}C NMR data with **3**, except for two methylene groups replacing the $\Delta^{3,4}$ olefinic group. The methylene groups were observed at δ_H 3.59 (1H, td, J = 12.4, 2.0 Hz) and 3.90 (1H, obscured due to signal overlapping)/δ_C 62.9 (C-3), and 1.54 (1H, br d, J = 14.4 Hz) and 1.80 (1H, m)/24.0 (C-4). The observation suggested that **10** is a 3,4-dihydro derivative of **3**, which was confirmed by the HMBC correlations of H-3a/C-1, C-5, H-4a/C-5, and H-4b/C-9 and the COSY correlations of H-3a with H-4a and H-4b (Figure 1). The sugar moiety in **10** was also identified as D-glucose. Therefore, the structure of **10** was defined as shown for the new substance 3,4-dihydro-6-*O*-di-*trans*-feruloyl catalpol.

Compound **17** was isolated as a light yellow, amorphous gum with a molecular formula of $C_{36}H_{42}O_{14}$, based on the ^{13}C NMR data and the $[M + Na]^+$ ion at m/z 721.2473 (calcd 721.2467) in the HRESIMS. Compound **17** displayed typical ^{1}H and ^{13}C NMR signals for lariciresinol,[32] a tetrahydrofuran-type lignan, at δ_H 6.73 (1H, d, J = 2.0 Hz)/δ_C 113.4 (C-2), 6.66 (1H, d, J = 8.2 Hz)/116.2 (C-5), 6.56 (1H, d, J = 8.2, 2.0 Hz)/122.3 (C-6), 2.90 (1H, dd, J = 13.8, 4.8 Hz) and 2.46 (1H, dd, J = 13.8, 11.2 Hz)/33.9 (C-7), 2.73 (1H, m)/44.2 (C-8), 3.67 (1H, dd, J = 8.4, 6.4 Hz) and 3.94 (1H, dd, J = 8.4, 6.8 Hz)/73.8 (C-9), 6.92 (1H, d, J = 1.9 Hz)/110.9 (C-2′), 6.71 (1H, d, J = 8.2 Hz)/116.0 (C-5′), 6.77 (1H, dd, J = 8.2, 1.9 Hz)/119.8 (C-6′), 4.82 (1H, d, J = 6.8 Hz)/84.6 (C-7′), 2.48 (1H, m)/51.5 (C-8′), 3.83 (1H, obscured due to signal overlapping) and 3.97 (1H, dd, J = 9.6, 6.4)/68.8 (C-9′), 3.78 (3H, s)/56.43 (OCH$_3$-3), and 3.82 (3H, s)/56.47 (OCH$_3$-3′), along with those for a *trans*-feruloyl group and a *β*-glucopyranosyl group. The preliminary assignments were supported by the HMBC correlations of H$_2$-7/C-1, C-2, C-6, C-9, H-7′/C-1′, C-2′, C-6′, H-8′/C-1′, OC*H*$_3$-3/C-3, and OC*H*$_3$-3′/C-3′ and the COSY correlations of H-8/H$_2$-7, H-8′ and H-8′/H-7′, H$_2$-9′ (Figure 2). The connectivity of the *β*-glucopyranosyl group to the lariciresinol nucleus at C-9′ was established by the HMBC correlation from H-1″ to C-9′. Moreover, the HMBC correlation of H$_2$-6″/C-9‴ indicated the C-6″ location of the *trans*-feruloyl group. The aromatic methoxy group proton signals at δ_H 3.78 (OC*H*$_3$-3), 3.82 (OC*H*$_3$-3′), and 3.83 (OC*H*$_3$-3‴) showed NOE correlations with H-2, H-2′, and H-2‴, respectively, which confirmed their position at the aromatic rings. Compound **17** exhibited a specific rotation of $[\alpha]^{22}_D$ +12 (c 0.1, MeOH), and a negative Cotton effect was observed at 239 nm in the electronic circular dichroism (ECD) curve. The absolute configuration of **17** was determined as (8*R*,7′*S*,8′*R*) by comparison of the ECD data with those of alangilignosides C and D.[32−34] The configuration was supported by the NOE correlations of H-7′/H$_2$-9′ and H-9′b/H-7. Acid hydrolysis of **17** gave D-glucose. Accordingly, the structure of **17** was defined as shown for the new substance (8*R*,7′*S*,8′*R*)-lariciresinol 9′-*O*-*β*-D-(6-*O*-*trans*-feruloyl)-glucopyranoside.

Compound **18** was isolated as a colorless, amorphous gum with a molecular formula of $C_{36}H_{38}O_{14}$, based on the ^{13}C NMR data and an $[M + Na]^+$ ion at m/z 717.2159 (calcd 717.2154)

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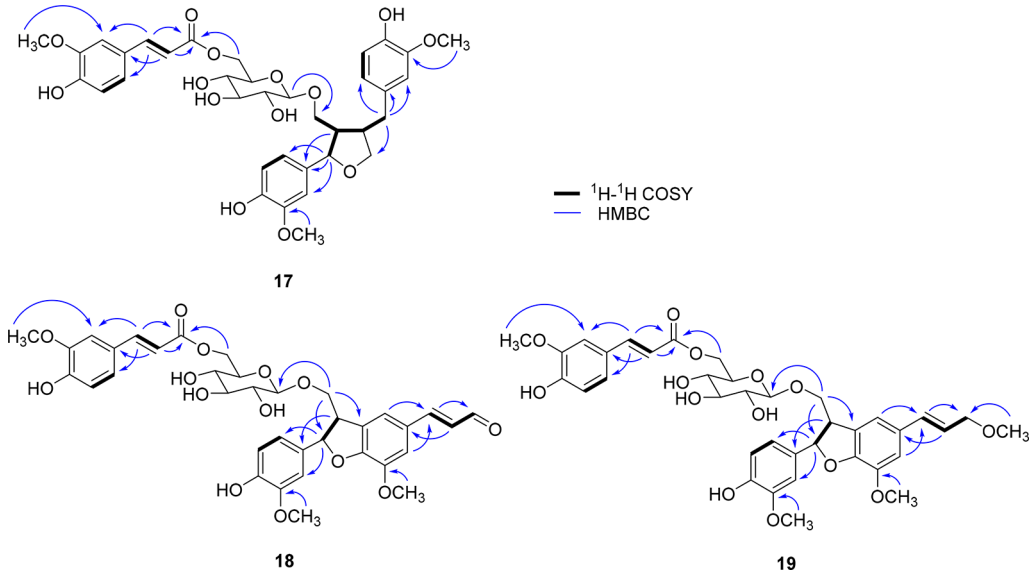

Figure 2. Key ^{1}H–^{1}H COSY and HMBC correlations of **17**–**19**.

in the HRESIMS. The ^{1}H and ^{13}C NMR spectra of **18** also showed resonances for a *β*-(6-*O*-*trans*-feruloyl)glucopyranosyl unit, as found for **17**, and the HPLC sugar analysis after acid hydrolysis indicated the presence of a D-glucose moiety. Upon comparison of the ^{1}H and ^{13}C NMR data of these two compounds, differences were observed in the signals of the aglycones at C-1″. Four methine groups appeared at δ_H 5.69 (d, J = 6.0 Hz)/δ_C 90.2 (C-7), 3.81 (obscured due to signal overlapping)/52.7 (C-8), 7.15 (br s)/114.2 (C-2′), and 7.20 (br s)/120.4 (C-6′) together with four quaternary carbon signals at δ_C 129.7 (C-1′), 146.0 (C-3′), 152.8 (C-4′), and 130.9 (C-5′) (Table 2), indicating the presence of a 2,3,5,7-tetrasubstituted 2,3-dihydrobenzofuran skeleton.[35] In the ^{1}H NMR spectrum, signals for a 1,3,4-trisubstituted phenyl group were observed at δ_H 6.96 (1H, d, J = 2.0 Hz, H-2), 6.75 (1H, d, J = 8.0 Hz, H-5), and 6.85 (1H, dd, J = 8.0, 2.0 Hz, H-6), and an extra aromatic methoxy group resonance appeared at δ_H 3.88 (3H, s, OCH_3-3′). The ^{1}H and ^{13}C NMR data exhibited signals for an olefinic double bond at δ_H 7.42 (1H, d, J = 15.9 Hz)/δ_C 155.9 (C-7′) and 6.58 (1H, dd, J = 15.9, 7.9 Hz)/127.1 (C-8′), which was conjugated to a formyl group at δ_H 9.49 (1H, d, J = 7.9 Hz)/δ_C 196.1 (C-9′). These ^{1}H and ^{13}C NMR assignments were confirmed by further analysis of 2D NMR experiments. Oxygenated methylene protons at δ_H 4.08 (1H, dd, J = 10.2, 5.2 Hz, H-9a) and 3.91 (1H, dd, J = 10.2, 8.0 Hz, H-9b) showed HMBC correlations with C-7 and C-5′ of the dihydrobenzofuran and C-1″ of the *β*-D-glucopyranoside (Figure 2), suggesting that the *β*-D-glucopyranosyl group is connected to the dihydrobenzofuran nucleus through the methylene group. The positions of the 3,4-disubstituted phenyl group and the ethylene formyl group were determined as C-7 and C-1′, respectively, due to the HMBC correlations of H-7/C-2, C-6, H-8/C-1, H-2′/C-7′, H-6′/C-7′, and H-8′/C-1′. One of the three aromatic methoxy groups at δ_H 3.88 was assigned at C-3 based on the HMBC correlation between the aromatic methoxy protons and C-3′. The other two aromatic methoxy groups also showed HMBC correlations of OCH_3-3/C-3 and OCH_3-3‴/C-3‴, indicating their C-3 and C-3′ location, respectively. Compound **18** gave a specific rotation of $[\alpha]^{22}_D$ −102 (*c* 0.1, MeOH). ECD Cotton effects in the region 220–240 nm provide evidence for determining the absolute configurations at C-7 and C-8 of the dihydrobenzofuran.[36−39] A positive Cotton effect at 231 nm observed in the ECD curve of **18** was indicative of a (7*R*,8*S*)-configuration. The NOE correlation between H-6 and H-8 was consistent with the *trans* configuration in the dihydrobenzofuran skeleton.[35] Thus, the structure of **18** was defined as shown for the new compound (2*E*)-3-[(2*R*,3*S*)-2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxy-1-benzofuran-5-yl]prop-2-enal 9-[6-[(2*E*)-3-(4-hydroxy-3-methoxyphenyl)-1-oxoprop-2-enyl]oxy]-*β*-D-glucopyranoside, which was given the trivial name ovatoside A.

Compound **19** was isolated as a colorless, amorphous gum with a molecular formula of $C_{37}H_{42}O_{14}$, based on its ^{13}C NMR data and the $[M + Na]^+$ ion at m/z 733.2471 (calcd 733.2467) in the HRESIMS. The ^{1}H and ^{13}C NMR spectra of **19** were closely comparable to those of **18**, except for signals for a methylene and a methoxy group replacing those of a formyl group. The methylene group appeared at δ_H 3.98 (2H, br d, J = 6.5 Hz)/δ_C 74.4 (C-9′) with an additional methoxy group at δ_H 3.32 (3H, s)/δ_C 58.0 (OCH_3-9′) (Table 2). This observation suggested the presence of a 3-methoxypropenyl group instead of the ethylene formyl group in **18**, which was supported by the HMBC correlations of H-2′/C-7′, H-6′/C-7′, H-7′/C-9′, H-8′/C-1′, and OCH_3-9′/C-9′ and the COSY correlations of H-8′ with H-7′ and H_2-9′ (Figure 2). The specific rotation was measured as $[\alpha]^{22}_D$ −33 (*c* 0.1, MeOH). Compound **19** was found to have a (2*S*,3*R*)-configuration according to its negative Cotton effect at 230 nm in the ECD spectrum, opposite that of **18**.[36,38,39] Like **18**, acid hydrolysis of **19** afforded D-glucose. Therefore, the structure of **19** was defined as shown for the new compound (2*S*,3*R*)-2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxy-5-[(*E*)-3-methoxyprop-1-en-1-yl]-1-benzofuran 9-[6-[(2*E*)-3-(4-hydroxy-3-methoxyphenyl)-1-oxoprop-2-enyl]oxy]-*β*-D-glucopyranoside (ovatoside B).

Compound **20** was isolated as a light yellow, amorphous powder with a molecular formula of $C_{23}H_{26}O_{11}$, based on the ^{13}C NMR data and the $[M + Na]^+$ ion at m/z 501.1367 (calcd 501.1367) in the HRESIMS. Compound **20** exhibited resonances for a *trans*-feruloyl group at δ_H 7.18 (1H, d, J = 1.8 Hz)/δ_C 111.7 (C-2″), 6.82 (1H, d, J = 8.2 Hz)/116.6 (C-5″), 7.07 (1H, dd, J = 8.2, 1.8 Hz)/124.2 (C-6″), 7.61

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Table 2. ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR Spectroscopic Data for Compounds 17−19 and 24[a]

	17		18		19		24	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	133.7, C		133.8, C		134.4, C		130.6, C	
2	113.4, CH	6.73, d (2.0)	110.9, CH	6.96, d (2.0)	110.7, CH	6.95, d (1.9)	131.0, CH	7.02, d (8.6)
3	148.99, C		149.1, C		149.0, C		116.2, CH	6.64, d (8.6)
4	145.8, C		147.8, C		147.5, C		156.8, C	
5	116.2, CH	6.66, d (8.2)	116.2, CH	6.75, d (8.0)	116.1, CH	6.72, d (8.3)	116.2, CH	6.64, d (8.6)
6	122.3, CH	6.56, dd (8.2, 2.0)	120.0, CH	6.85, dd (8.0, 2.0)	119.9, CH	6.85, dd (8.3, 1.9)	131.0, CH	7.02, d (8.6)
7	33.9, CH_2	2.90, dd (13.8, 4.8), 2.46, dd (13.8, 11.2)	90.2, CH	5.69, d (6.0)	89.4, CH	5.63, d (6.0)	36.5, CH_2	2.79, br t (7.6)
8	44.2, CH	2.73, m	52.7, CH	3.81[b]	53.3, CH	3.74, m	71.9, CH_2	3.65, 4.00, m
9	73.8, CH_2	3.67, dd (8.4, 6.4), 3.94, dd (8.4, 6.8)	72.7, CH_2	3.91, dd (10.2, 8.0), 4.08, dd (10.2, 5.2)	72.9, CH_2	3.86, dd (10.3, 8.0), 4.07, dd (10.3, 5.0)		
1′	135.8, C		129.7, C		132.3, C		103.1, C	4.328, d (7.6)
2′	110.9, CH	6.92, d (1.9)	114.2, CH	7.15, br s	112.1, CH	6.91, br s	78.0, CH	3.39, dd (9.2, 7.6)
3′	148.95, C		146.0, C		145.6, C		79.0, CH	3.48, br t (8.8)
4′	147.0, C		152.8, C		149.4, C		71.8, CH	3.27, dd (9.9, 8.4)
5′	116.0, CH	6.71, d (8.2)	130.9, C		130.0, C		77.9, CH	3.21, ddd (9.9, 5.8, 2.1)
6′	119.8, CH	6.77, dd (8.2, 1.9)	120.4, CH	7.20, br s	117.0, CH	6.92, br s	62.8, CH_2	3.64, dd (11.8, 5.8), 3.84, dd (11.8, 2.1)
7′	84.6, CH	4.82, d (6.8)	155.9, CH	7.42, d (15.9)	134.3, CH	6.43, br d (16.0)		
8′	51.5, CH	2.48, m	127.1, CH	6.58, dd (15.9, 7.9)	124.3, CH	6.08, dt (16.0, 6.5)		
9′	68.8, CH_2	3.83,[b] 3.97, dd (9.6, 6.4)	196.1, C	9.49, d (7.9)	74.4, CH_2	3.98, br d (6.5)		
1″	104.6, CH	4.30, d (7.8)	105.1, CH	4.42, d (7.9)	105.1, CH	4.41, d (7.8)	110.1, CH	5.43, d (1.2)
2″	75.2, CH	3.24, dd (9.2, 7.8)	75.1, CH	3.26, dd (9.0, 7.9)	75.1, CH	3.26, dd (8.8, 7.8)	78.4, CH	3.94, d (1.2)
3″	78.0, CH	3.39[b]	78.1, CH	3.39, dd (9.0, 9.0)	78.1, CH	3.39, dd (8.8, 8.8)	79.2, C	
4″	72.0, CH	3.38[b]	72.2, CH	3.32[b]	72.1, CH	3.32[b]	75.5, CH_2	4.10, d (9.6), 3.76, d (9.6)
5″	75.5, CH	3.54, m	75.5, CH	3.57, ddd (10.0, 7.2, 2.4)	75.5, CH	3.56, ddd (9.6, 7.1, 2.2)	68.4, CH_2	4.26, d (11.4), 4.326, d (11.4)
6″	64.5, CH_2	4.40, dd (11.9, 6.2), 4.52, dd (11.9, 2.4)	64.8, CH_2	4.28, dd (11.6, 7.2), 4.51, dd (11.6, 2.4)	64.9, CH_2	4.27, dd (11.8, 7.1), 4.52, dd (11.8, 2.2)		
1‴	127.6, C		127.5, C		127.6, CH		127.2, C	
2‴	111.7, CH	7.11, d (1.9)	111.7, CH	7.00, d (1.9)	111.7, CH	7.03, d (2.0)	131.3, CH	7.41, d (8.6)
3‴	149.5, C		149.4, C		149.4, C		116.9, CH	6.79, d (8.6)
4‴	150.9, C		150.8, C		150.8, C		161.4, C	
5‴	116.7, CH	6.78, d (8.4)	116.5, CH	6.72, d (8.1)	116.5, CH	6.74, d (8.4)	131.3, CH	6.79, d (8.6)
6‴	124.3, CH	7.00, d (8.3, 1.9)	124.2, CH	6.89, d (8.1, 1.9)	124.2, CH	6.90[b]	116.9, CH	7.41, d (8.6)
7‴	147.1, CH	7.60, d (15.8)	147.1, CH	7.48, d (16.0)	147.1, CH	7.50, d (15.8)	147.1, CH	7.64, d (16.0)
8‴	115.2, CH	6.35, d (15.8)	115.2, CH	6.26, d (16.0)	115.2, CH	6.27, d (15.8)	114.9, CH	6.34, d (16.0)
9‴	169.0, C		168.9, C		169.0, C		169.1, C	
OCH_3-3	56.43, CH_3	3.78, s	56.4, CH_3	3.80, s	56.5, CH_3	3.77, s		

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Table 2. continued

	17		18		19		24	
position	δ_C type	δ_H (J in Hz)	δ_C type	δ_H (J in Hz)	δ_C type	δ_H (J in Hz)	δ_C type	δ_H (J in Hz)
OCH_3-3′	56.47, CH_3 3.82, s		56.8, CH_3 3.88, s		56.8, CH_3 3.85, s			
OCH_3-9′					58.0, CH_3 3.32, s			
OCH_3-3″	56.50, CH_3 3.83, s		56.5, CH_3 3.81, s		56.5, CH_3 3.81, s			

[a]TMS was used as internal standard; data were measured in methanol-d_4. [b]Multiplicity patterns were unclear due to signal overlapping; chemical shifts were estimated by HSQC NMR spectra.

(1H, d, J = 16.0 Hz)/147.1 (C-7″), 6.36 (1H, d, J = 16.0 Hz)/115.4 (C-8″), 3.90 (3H, s)/56.5 (OCH_3-3″), and δ_C 169.0 (C-9″), along with a β-glucopyranosyl anomeric proton signal at δ_H 4.69 (1H, d, J = 8.0 Hz, H-1′) (Table 3), as found for **17**−**19**. In the ^{1}H NMR spectrum, an additional ABX spin system was observed at δ_H 6.45 (1H, d, J = 2.7 Hz, H-3), 6.22 (1H, dd, J = 8.7, 2.7 Hz, H-5), and 6.96 (1H, d, J = 8.7 Hz, H-6), and an extra aromatic methoxy group signal appeared at δ_H 3.79 (3H, s, OCH_3-2). The β-glucopyranosyl group and aromatic methoxy group were assigned at C-1 and C-2, respectively, on the basis of the HMBC correlations of H-1′/C-1 and OCH_3-2/C-2 (Figure 3). The C-2 position of the aromatic methoxy group was supported by the NOE correlation between OCH_3-2 and H-3. The location of the *trans*-feruloyl group was determined as C-6′ of the β-glucopyranosyl group according to the HMBC connectivity between H_2-6′ and C-9″. HPLC determination after acid hydrolysis showed the presence of a D-glucopyranosyl moiety. The ^{1}H and ^{13}C NMR data of **20** were also compared with those reported for dunalianoside B,[35] which has a hydroxy group at C-2 instead of the methoxy group in **20**. Therefore, the structure for **20** was defined as shown for the new substance 4-hydroxy-2-methoxybenzene 1-[6-[(2*E*)-3-(4-hydroxy-3-methoxyphenyl)-1-oxoprop-2-enyl]oxy]-β-D-glucopyranoside (ovatoside C).

Compound **21** was isolated as a white, amorphous powder with a molecular formula of $C_{24}H_{28}O_{11}$, based on both the ^{13}C NMR data and the $[M + Na]^+$ ion at m/z 515.1526 (calcd 515.1524) in the HRESIMS. The ^{1}H NMR spectrum of **21** was similar to that of **20**, except for the presence of a singlet signal at δ_H 4.43 (2H, H_2-7) (Table 3). The oxygenated methylene protons showed HMBC correlations with C-3, C-4, and C-5 (Figure 3), as well as the NOE correlations with H-3 and H-5. The observation indicated the location of the hydroxymethyl group at C-4. The sugar moiety was determined as D-glucose. Thus, the structure of **21** was defined as shown for the new compound 4-hydroxymethyl-2-methoxybenzene 1-[6-[(2*E*)-3-(4-hydroxy-3-methoxyphenyl)-1-oxoprop-2-enyl]oxy]-β-D-glucopyranoside (ovatoside D).

Compound **22** was isolated as a white, amorphous powder with a molecular formula of $C_{24}H_{32}O_{11}$, using the ^{13}C NMR data and a $[M + Na]^+$ ion at m/z 519.1830 (calcd 519.1837) in the HRESIMS. The ^{1}H and ^{13}C NMR spectra of **22** also showed resonances for a *trans*-feruloyl group and a β-glucopyranosyl group, similar to those of **20**. When comparing the ^{1}H NMR data of these two compounds, the signals for the 4-hydroxy-2-methoxyphenyl group at C-1′ of the β-glucopyranosyl group in **20** were absent. Instead, the ^{1}H NMR spectrum of **22** exhibited resonances for six methylenes at δ_H 1.98 and 1.83 (each 2H, m, H_2-2 and H_2-6), 2.63 and 2.15 (each 2H, m, H_2-3 and H_2-4), 1.90 (2H, td, J = 6.6, 1.5 Hz, H_2-7), and 4.05 and 3.80 (each 1H, dt, J = 10.4, 6.6 Hz, H_2-8) (Table 3). In the ^{13}C NMR spectrum, quaternary carbon signals appeared at δ_C 70.4 (C-1) and 214.9 (C-4). The observed ^{1}H and ^{13}C NMR signals were assignable to a 1-ethyl-1-hydroxycyclohexan-4-one unit,[40,41] based on the HMBC correlations of H-2b and H-6b/C-4, H-3a and H-5a/C-1, C-4, H-3b and H-5b/C-4, H_2-7/C-1, C-2, C-6, and H_2-8/C-1 and the COSY correlations of H-3a and H-5a/H_2-2 and H_2-6, and H_2-7/H_2-8 (Figure 3). The HMBC correlation from H_2-8 to C-1′ indicated that the 1-ethyl-1-hydroxycyclohexan-4-one moiety is linked to the β-glucopyranosyl group at C-1′. The D-glucopyranosyl configuration was identified based on HPLC analysis following acid hydrolysis. Therefore, the structure of **22** was defined as shown for the new

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Table 3. ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR Spectroscopic Data for Compounds 20−22[a]

	20		21		22	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	140.9, C		147.08, C		70.4, C	
2	152.3, C		151.0, C		37.9, CH_2	1.83, 1.98, m
3	101.9, CH	6.45, d (2.7)	112.7, CH	7.00, d (2.0)	37.83,[c] CH_2	2.15, 2.63, m
4	155.1, C		137.8, C		214.9, C	
5	107.6, CH	6.22, dd (8.7, 2.7)	120.8, CH	6.76, dd (8.2, 2.0)	37.82,[c] CH_2	2.15, 2.63, m
6	120.9, CH	6.96, d (8.7)	118.2, CH	7.07, d (8.2)	37.9, CH_2	1.83, 1.98, m
7			65.0, CH_2	4.43, s	42.1, CH_2	1.90, td, (6.6, 1.5)
8					67.2, CH_2	3.80, dt (10.4, 6.6), 4.05, dt (10.4, 6.6)
1′	104.4, CH	4.69, d (8.0)	102.9, CH	4.86, d (7.6)	104.6, CH	4.31, d (8.0)
2′	75.1, CH	3.46, m	75.0, CH	3.50, br t (7.6)	75.1, CH	3.19, br t (8.6)
3′	77.8, CH	3.44, m	77.8, CH	3.48, br t (8.4)	78.0, CH	3.38[b]
4′	71.8, CH	3.42, m	71.9, CH	3.43, br t (9.2)	71.8, CH	3.37[b]
5′	75.6, CH	3.59, ddd (9.2, 6.7, 2.4)	75.7, CH	3.67, ddd (9.2, 6.8, 2.3)	75.5, CH	3.54, m
6′	64.7, CH_2	4.34, dd (11.9, 6.7), 4.50, dd (11.9, 2.4)	64.6, CH_2	4.36, dd (11.9, 6.8), 4.50, dd (11.9, 2.3)	64.6, CH_2	4.36, dd (11.7, 6.0), 4.50, dd (11.7, 2.2)
1″	127.7, C		127.7, C		127.6, C	
2″	111.7, CH	7.18, d (1.8)	111.7, CH	7.19, d (2.2)	111.7, CH	7.20, d (2.0)
3″	149.5, C		149.5, C		149.5, C	
4″	150.7, C		150.8, C		150.8, C	
5″	116.6, CH	6.82, d (8.2)	116.6, CH	6.83, d (8.0)	116.6, CH	6.81, d (8.2)
6″	124.2, CH	7.07, dd (8.2, 1.8)	124.3, CH	7.06, dd (8.0, 2.2)	124.3, CH	7.07, dd (8.2, 2.0)
7″	147.1, CH	7.61, d (16.0)	147.06, CH	7.61, d (16.0)	147.1, CH	7.63, d (15.6)
8″	115.4, CH	6.36, d (16.0)	115.3, CH	6.36, d (16.0)	115.3, CH	6.38, d (15.6)
9″	169.0, C		168.9, C		169.1, C	
OCH_3-2	56.6, CH_3	3.79, s	56.7, CH_3	3.86, s		
OCH_3-3″	56.5, CH_3	3.90, s	56.4, CH_3	3.90, s	56.5, CH_3	3.89, s

[a]TMS was used as internal standard; data were measured in methanol-d_4. [b]Multiplicity patterns were unclear due to signal overlapping; chemical shifts were estimated by HSQC NMR spectra. [c]Data could be exchanged in the same column.

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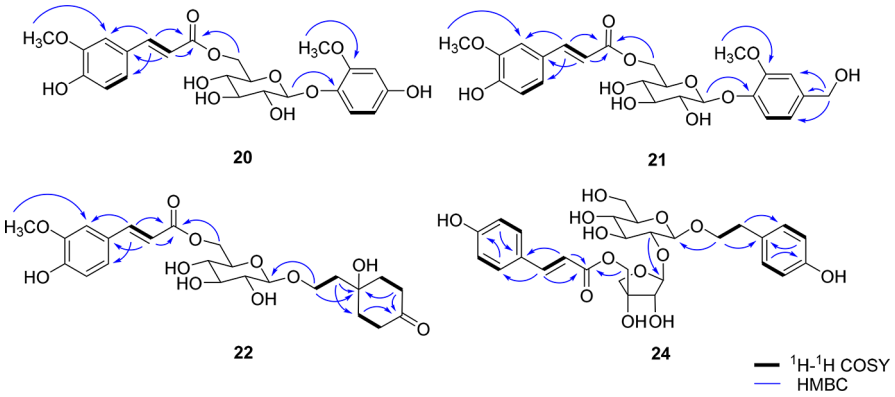

Figure 3. Key ^{1}H−^{1}H COSY and HMBC correlations of **20**−**22** and **24**.

Table 4. Peroxynitrite ($ONOO^-$)-Scavenging Activities of Compounds 1−29[a]

compound	IC_{50} (μM)	compound	IC_{50} (μM)	compound	IC_{50} (μM)
1	0.88 ± 0.05	**11**	58.4 ± 1.95	**21**	0.17 ± 0.03
2	1.1 ± 0.15	**12**	2.0 ± 0.78	**22**	1.02 ± 0.06
3	1.6 ± 0.02	**13**	3.9 ± 0.84	**23**	0.14 ± 0.02
4	13.6 ± 0.47	**14**	39.5 ± 0.67	**24**	4.5 ± 0.36
5	78.5 ± 2.44	**15**	8.3 ± 1.93	**25**	15.7 ± 0.39
6	>100	**16**	76.7 ± 1.32	**26**	36.2 ± 1.07
7	36.4 ± 1.54	**17**	0.81 ± 0.09	**27**	8.6 ± 2.46
8	6.0 ± 0.01	**18**	0.79 ± 0.11	**28**	7.7 ± 0.91
9	>100	**19**	0.34 ± 0.02	**29**	3.5 ± 1.03
10	2.2 ± 0.10	**20**	0.48 ± 0.07	DL-penicillamine	3.4 ± 0.34

[a]Inhibitory activities of $ONOO^-$ from decomposition of SIN-1 are expressed as IC_{50} values. Data were described as mean ± SD (obtained from three different experiments). DL-Penicillamine was used as a positive control.

compound 1-ethyl-1-hydroxycyclohexan-4-one 8-[6-[(2*E*)-3-(4-hydroxy-3-methoxyphenyl)-1-oxoprop-2-enyl]oxy]-*β*-D-glucopyranoside (ovatoside E).

Compound **24** was isolated as a white, amorphous gum with a molecular formula of $C_{28}H_{34}O_{13}$, based on its ^{13}C NMR data and a $[M + Na]^+$ ion at *m*/*z* 601.1897 (calcd 601.1892) in the HRESIMS. In the ^{1}H NMR spectrum of **24**, two AA′BB′ spin systems appeared at δ_H 7.02 (2H, d, *J* = 8.6 Hz, H-2 and H-6), 6.64 (2H, d, *J* = 8.6 Hz, H-3 and H-5), 7.41 (2H, d, *J* = 8.6 Hz, H-2‴ and H-6‴), and 6.79 (2H, d, *J* = 8.6 Hz, H-3‴ and H-5‴) (Table 2). Two coupled doublet signals at δ_H 7.64 (1H, d, *J* = 16.0 Hz, H-7‴) and 6.34 (1H, d, *J* = 16.0 Hz, H-8‴) were attributed to an olefinic double bond conjugated to a carbonyl group (δ_C 169.1). The presence of a *β*-glucopyranosyl group and a *β*-apiofuranosyl group was deduced from their characteristic anomeric proton signals at δ_H 4.328 (1H, d, *J* = 7.6 Hz, H-1′) and 5.43 (1H, d, *J* = 1.2 Hz, H-1″), respectively.[16,19] Additionally, two methylene proton signals resonated at δ_H 2.79 (2H, br t, *J* = 7.6 Hz, H_2-7) and 4.00 and 3.65 (each 1H, m, H-8a and H-8b) and were coupled to each other in the COSY spectrum of **24** (Figure 3). The HMBC correlations of H_2-7/C-2 and C-6 and H_2-8/C-1, C-1′ suggested the presence of a 4-hydroxyphenethyl group attached to C-1′ of the *β*-glucopyranosyl group. The anomeric proton signal of the *β*-apiofuranosyl group showed the HMBC correlation with C-2′ of the *β*-glucopyranosyl group, indicating the connectivity of two sugar moieties. Moreover, a *trans*-4-coumaroyl group was assignable at C-5″ of the *β*-apiofuranosyl group according to the HMBC correlations of H_2-5″/C-9‴, H-7‴/C-2‴ and C-6‴, C-9‴, and H-8‴/C-1‴, C-9‴. Acid hydrolysis of **24** gave a mixture of sugars, which were identified as D-glucose and D-apiose based on HPLC analysis of the thiocarbamoyl-thiazolidine derivatives. The ^{1}H and ^{13}C NMR data for **24** were also compared with those for darendoside A (**25**),[19] in which the *trans*-4-coumaroyl group at C-5″ was absent. Thus, the structure of **24** was defined as shown for the new substance 1-ethyl-4-hydroxybenzene 8-[5-[(2*E*)-3-(4-hydroxyphenyl)-1-oxoprop-2-enyl]oxy]-*β*-D-apiofuranosyl-(1→2)-*β*-D-glucopyranoside (ovatoside F).

$ONOO^-$-scavenging activity was evaluated for all isolates produced by 3-morpholinosydnonimine (SIN-1),[8] using DL-penicillamine as a positive control (Table 4).[42] Of the compounds tested, 27 had the effect of scavenging $ONOO^-$. Two compounds were inactive, namely, **6** and **9**. In particular, 12 compounds, **1**−**3**, **10**, **12**, and **17**−**23** (IC_{50} 0.88, 1.1, 1.6, 2.2, 2.0, 0.81, 0.79, 0.34, 0.48, 0.17, 1.0, and 0.14 μM, respectively), showed more potent activity than DL-penicillamine (IC_{50} 3.4 μM). Compounds **13** and **29** also exhibited $ONOO^-$-scavenging effects similar to the positive control with IC_{50} values of 3.9 and 3.5 μM, respectively. In the present study, the feruloyl group was commonly found in the compounds with significant activity (**1**−**3**, **10**, **12**, **17**−**23**, and **29**), suggesting that this functionality is important for $ONOO^-$-scavenging activity.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a P-1010 polarimeter. UV spectra were recorded on a U-3000 spectrophotometer. ECD measurements were performed using a JASCO J-810 spectropolarimeter. 1D and 2D NMR spectra were recorded on a Varian Unity Inova 400 MHz FT-NMR instrument with tetramethylsilane (TMS) as internal standard. Mass spectrometry was performed on a Waters Acquity UPLC system

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coupled to a Micromass Q-Tof Micro mass spectrometer and Agilent 6220 Accurate-Mass TOF LC/MS system. Silica gel (230−400 mesh, Merck, Germany), RP-18 (YMC gel ODS-A, 120 Å, S-150 μm, YMC Co., Japan), and Sephadex LH-20 (GE Healthcare Bio-Science AB, Uppsala, Sweden) were used for column chromatography (CC). Thin-layer chromatographic (TLC) analysis was performed on Kieselgel 60 F_{254} (silica gel, 0.25 mm layer thickness, Merck, Germany) and RP-18 F_{254s} (Merck, Germany) plates, with visualization under UV light (254 and 365 nm) and 10% (v/v) sulfuric acid spray followed by heating (120 °C, 5 min). A YMC-Pack Pro C_{18} column (5 μm, 250 mm × 20 mm i.d.) was used for preparative HPLC, as performed on an Acme 9000 system (Young Lin, Korea). Analytical HPLC was conducted on a Phenomenex Luna C_{18} column (5 μm, 250 mm × 4.6 mm i.d.) equipped with a Waters 1525 binary pump and a Waters 996 photodiode array detector (Milford, MA, USA). D-Glucose, L-glucose, and D-apiose were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-Cysteine methyl ester hydrochloride and *o*-tolylisothiocyanate were purchased from TCI Chemicals (Tokyo, Japan).

Plant Material. The stem bark of *C. ovata* was collected at the Medicinal Plant Garden, College of Pharmacy, Ewha Womans University, in December 2013 and identified by Professor Je-hyun Lee (College of Oriental Medicine, Dongguk University). A voucher specimen (no. EA343) has been deposited at the Natural Product Chemistry Laboratory, College of Pharmacy, Ewha Womans University.

Extraction and Isolation. The dried and chopped stem bark of *C. ovata* (8 kg) was extracted with MeOH (4 × 18 L) at room temperature overnight. After removing the solvent under reduced pressure, the concentrated MeOH extract (2.6 kg) was suspended in distilled H_2O (2 L) and then partitioned with hexanes (5 × 3 L), EtOAc (10 × 3 L), and BuOH (5 × 3 L), sequentially, to afford hexanes- (30 g), EtOAc- (250 g), and BuOH (1.3 kg)-soluble extracts with a residual aqueous extract (1 kg). The EtOAc extract was subjected to CC over silica gel by elution with gradient mixtures of CH_2Cl_2−MeOH (999:1 → 1:4) to afford eight fractions (F1−F8). Fraction F4 (14 g), eluted with CH_2Cl_2−MeOH (23:1), was subjected to RP-18 CC using a MeOH−H_2O gradient solvent system (1:4 → 7:3) to yield 15 subfractions (F4.01−F4.15). Subfraction F4.12 (500 mg) was further purified by RP-18 CC (MeOH−H_2O, 3:2) and Sephadex LH-20 CC (MeOH 100%), sequentially, to afford **29** (1 mg). Fraction F5 (30 g), eluted with CH_2Cl_2−MeOH (9:1) from the first separation, was chromatographed over silica gel using gradient mixtures of $CHCl_3$−MeOH (49:1 → 1:1) to obtain 15 subfractions (F5.01−F5.15). Subfraction F5.08 (500 mg) was subjected to RP-18 CC, eluted with a MeOH−H_2O gradient solvent system (2:3 → 11:9), to yield 19 subfractions (F5.08.01−F5.08.19). Compound **19** (3 mg) was purified from subfraction F5.08.17 (4 mg) by preparative HPLC with MeOH−H_2O (4:1, 2 mL/min, t_R 36.8 min). Subfraction F5.09 (300 mg) was fractionated by RP-18 CC using gradient mixtures of MeOH−H_2O (2:3 → 3:2) as eluent, affording 15 subfractions (F5.09.01−F5.09.15). Subfraction F5.09.11 (12 mg) was further purified using preparative HPLC with MeOH−H_2O (3:2, 2 mL/min) to obtain **18** (4 mg, t_R 45.8 min). Subfraction F5.10 (2 g) was subjected to RP-18 CC, eluted by a MeOH−H_2O gradient solvent system (2:3 → 9:1), to yield **1** (100 mg) and **2** (180 mg) with 24 subfractions (F5.10.01−F5.10.24). Subfraction F5.10.06 (400 mg) was chromatographed over Sephadex LH-20 using MeOH (100%) and then purified by separation on preparative HPLC with MeOH−H_2O (11:9, 2 mL/min) to give **22** (17 mg, t_R 34.5 min). Subfraction F5.10.08 (100 mg) was chromatographed using Sephadex LH-20 with MeOH (100%) to yield **20** (30 mg). Subfraction F5.10.13 (50 mg) was separated by Sephadex LH-20 CC (MeOH 100%) and then further purified by preparative HPLC with MeOH−H_2O (3:2, 2 mL/min) to afford **23** (10 mg, t_R 42.8 min). A part of subfraction F5.10.16 (70 mg) was subjected to preparative HPLC with MeOH−H_2O (3:2, 2 mL/min) to afford **17** (5 mg, t_R 53.9 min). Subfraction F5.12 (4 g) was separated by RP-18 CC with a MeOH−H_2O gradient solvent system (2:3 → 1:1) as eluent to give **5** (240 mg), **9** (50 mg), and **21** (9 mg) with 15 subfractions (F5.12.01−F5.12.15). Subfraction F5.12.13 (1.4 g) was purified by separation over RP-18 CC with MeOH−H_2O (3:2) to yield **7** (80 mg). Fraction F6 (130 g), eluted with CH_2Cl_2−MeOH (4:1) from the first separation, was subjected to silica gel CC using a $CHCl_3$−MeOH−H_2O gradient solvent system (90:10:1 → 15:10:2) for elution to obtain 13 subfractions (F6.01−F6.13). A part (5 g) of subfraction F6.08 (40 g) was fractionated by RP-18 CC with gradient mixtures of MeOH−H_2O (1:4 → 1:1) as eluent to give **3** (2.4 g) and **8** (2 mg) with 17 subfractions (F6.08.01−F6.08.17). Subfraction F6.08.08 (5 mg) was further purified by separation on preparative HPLC with MeOH−H_2O (11:9, 2 mL/min) to yield **10** (2 mg, t_R 39.6 min). Subfraction F6.11 (5 g) was chromatographed over an RP-18 column by elution with a MeOH−H_2O gradient solvent system (1:9 → 1:1), affording **6** (120 mg) with 12 subfractions (F6.11.01−F6.11.12). Subfraction F6.08.11.06 (200 mg) was further separated by RP-18 CC using MeOH−H_2O (11:9) as eluent to give **12** (60 mg) and **15** (1 mg). Subfraction F6.08.11.08 (160 mg), eluted with MeOH−H_2O (1:1), was subjected to RP-18 CC by elution with MeOH−H_2O (1:1) to afford **4** (18 mg) and two additional subfractions, which were further purified using preparative HPLC to yield **13** (2 mg, MeOH−H_2O, 11:9; 2 mL/min, t_R 29.5 min) and **27** (20 mg, MeOH−H_2O, 2:3; 2 mL/min, t_R 37.1 min), respectively. Subfraction F6.12 (1.4 g) was separated by RP-18 CC using a MeOH−H_2O gradient solvent system (1:9 → 1:1) as eluent, affording 18 subfractions (F6.12.01−F6.12.18). Subfractions F6.12.04 (13 mg) and F6.12.05 (12 mg) were chromatographed over Sephadex LH-20 with MeOH (100%) to give **25** (6 mg) and **28** (5 mg), respectively. Compounds **11** (1 mg) and **14** (3 mg) were separated from subfraction F6.12.07 (17 mg) by preparative HPLC with MeOH−H_2O (1:1, 2 mL/min, t_R **11**: 49.9, **14**: 52.8 min). Subfractions F6.12.08 (30 mg), F6.12.12 (100 mg), and F6.12.15 (80 mg) were further purified by preparative HPLC using MeOH−H_2O (11:9, 2 mL/min) to afford **16** (5 mg, t_R 36.9 min), **26** (70 mg, t_R 32.3 min), and **24** (20 mg, t_R 40.9 min), respectively.

*6,10-O-Di-trans-feruloyl catalpol (**1**):* light yellow, amorphous powder; $[\alpha]^{22}_D$ −135 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.48), 236 (4.40), 298 (4.49), 327 (4.64) nm; ^{1}H NMR (400 MHz, methanol-d_4) and ^{13}C NMR (100 MHz, methanol-d_4) data, see Table 1; HRESIMS *m*/*z* 715.2242 $[M + H]^+$ (calcd for $C_{35}H_{39}O_{16}$, 715.2233).

*6,6′-O-Di-trans-feruloyl catalpol (**2**):* light yellow, amorphous powder; $[\alpha]^{22}_D$ −132 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.47), 237 (4.39), 298 (4.46), 327 (4.63) nm; ^{1}H NMR (400 MHz, methanol-d_4) and ^{13}C NMR (100 MHz, methanol-d_4) data, see Table 1; HRESIMS *m*/*z* 715.2233 $[M + H]^+$ (calcd for $C_{35}H_{39}O_{16}$ 715.2233).

*3,4-Dihydro-6-O-trans-feruloyl catalpol (**10**):* light yellow, amorphous powder; $[\alpha]^{22}_D$ −90 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.24), 234 (4.14), 297 (4.17), 327 (4.33) nm; ^{1}H NMR (400 MHz, methanol-d_4) and ^{13}C NMR (100 MHz, methanol-d_4) data, see Table 1; HRESIMS *m*/*z* 563.1729 $[M + Na]^+$ (calcd for $C_{25}H_{32}O_{13}Na$, 563.1735).

*(8R,7′S,8′R)-Laricresinol 9′-O-β-D-(6-O-trans-feruloyl)-glucopyranoside (**17**):* light yellow, amorphous gum; $[\alpha]^{22}_D$ +12 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.33), 230 (4.33), 287 (4.13), 327 (4.21) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 239 (−3.25), 289 (−2.86) nm; ^{1}H NMR (400 MHz, methanol-d_4) and ^{13}C NMR (100 MHz, methanol-d_4) data, see Table 2; HRESIMS *m*/*z* 721.2473 $[M + Na]^+$ (calcd for $C_{36}H_{42}O_{14}Na$, 721.2467).

*Ovatoside A (**18**):* colorless, amorphous gum; $[\alpha]^{22}_D$ −102 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.45), 290 (4.26), 329 (4.49) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 231 (+1.50), 242 (−2.36), 288 (+2.58) nm; ^{1}H NMR (400 MHz, methanol-d_4) and ^{13}C NMR (100 MHz, methanol-d_4) data, see Table 2; HRESIMS *m*/*z* 717.2159 $[M + Na]^+$ (calcd for $C_{36}H_{38}O_{14}Na$, 717.2154).

*Ovatoside B (**19**):* colorless, amorphous gum; $[\alpha]^{22}_D$ −33 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.15), 281 (3.91), 320 (3.79) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 230 (−1.80), 239 (+1.53), 290 (−1.89) nm; ^{1}H NMR (400 MHz, methanol-d_4) and ^{13}C NMR (100 MHz, methanol-d_4) data, see Table 2; HRESIMS *m*/*z* 733.2471 $[M + Na]^+$ (calcd for $C_{37}H_{42}O_{14}Na$, 733.2467).

*Ovatoside C (**20**):* light yellow, amorphous powder; $[\alpha]^{22}_D$ −58 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.34), 231 (4.26), 295

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(4.18), 326 (4.31) nm; ^{1}H NMR (400 MHz, methanol-d_4) and ^{13}C NMR (100 MHz, methanol-d_4) data, see Table 3; HRESIMS m/z 501.1367 $[M + Na]^+$ (calcd for $C_{23}H_{26}O_{11}Na$, 501.1367).

Ovatoside D (21): white, amorphous powder; $[\alpha]^{22}_D$ −38 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.22), 231 (4.16), 285 (3.98), 298 (4.03), 326 (4.19) nm; ^{1}H NMR (400 MHz, methanol-d_4) and ^{13}C NMR (100 MHz, methanol-d_4) data, see Table 3; HRESIMS m/z 515.1526 $[M + Na]^+$ (calcd for $C_{24}H_{28}O_{11}Na$, 515.1524).

Ovatoside E (22): white, amorphous powder; $[\alpha]^{22}_D$ −15 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.12), 234 (4.04), 298 (4.06), 325 (4.20) nm; ^{1}H NMR (400 MHz, methanol-d_4) and ^{13}C NMR (100 MHz, methanol-d_4) data, see Table 3; HRESIMS m/z 519.1830 $[M + Na]^+$ (calcd for $C_{24}H_{32}O_{11}Na$, 519.1837).

Ovatoside F (24): white, amorphous gum; $[\alpha]^{22}_D$ −56 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.39), 224 (4.43), 314 (4.50) nm; ^{1}H NMR (400 MHz, methanol-d_4) and ^{13}C NMR (100 MHz, methanol-d_4) data, see Table 2; HRESIMS m/z 601.1897 $[M + Na]^+$ (calcd for $C_{28}H_{34}O_{13}Na$, 601.1892).

Acid Hydrolysis and Absolute Configuration Determination of Sugar Moieties. The absolute configurations of sugar moieties were determined using a HPLC-UV-based method.[28−31] Compounds **1**, **2**, **10**, **17**−**22**, and **24** (each 1−2 mg) were hydrolyzed in the presence of 2 M HCl (dioxane−H_2O, 1:1) at 80 °C for 2 h. The dioxane was removed from each reaction mixture under vacuum, and then EtOAc was used for extraction. The aqueous layer was neutralized with Amberlite IRA-67 (OH^- form), dried under a vacuum evaporator, and dissolved in anhydrous pyridine (0.5 mL) with addition of L-cysteine methyl ester hydrochloride (2 mg). After the reaction mixture was heated at 60 °C for 1.5 h, *o*-tolylisothiocyanate (50 μL) was added and the mixture was kept at 60 °C for 1 h. The reaction product was directly analyzed by RP-18 HPLC (MeCN−H_2O, 1:3; 1 mL/min). The monosaccharides D-glucose and D-apiose in **1**, **2**, **10**, **17**−**22**, and **24** were identified based on comparison of the retention times with those of authentic samples (t_R: D-glucose 20.9 min, L-glucose 19.0 min, and D-apiose 36.7 min).

Measurement of $ONOO^-$-Scavenging Activity. The $ONOO^-$-scavenging ability was measured by monitoring the oxidation of dihydrorhodamine 123 (DHR 123). The method reported by Kooy et al. was slightly modified.[43] A stock solution of DHR 123 (5 mM) in dimethylformamide was purged with N_2 and stored at −80 °C. A working solution of DHR 123 (5 μM) was diluted from the stock solution and immediately placed on ice and kept in the dark before use. The buffer was composed of 90 mM NaCl, 50 mM Na_3PO_4, 5 mM KCl (pH 7.4), and 100 μM diethylenetriaminepentaacetic acid. All materials for the buffer were prepared with high-quality deionized H_2O, purged with N_2, and kept on ice. Oxidation of DHR 123 was measured on an FL 500 microplate fluorescence reader (Bio-Tek Instruments Inc., Winooski, VT, USA) with excitation and emission wavelengths of 480 and 530 nm, respectively. DHR 123 was oxidized gradually by decomposition of SIN-1. The background and final fluorescent intensities were measured 5 min after treatment with and without SIN-1 (10 μM). The results are expressed as means ± SD of three independent experiments for the final fluorescence intensity minus background fluorescence. The effects were described by the percent inhibition of DHR 123 oxidation, and DL-penicillamine was used as a positive control.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00139.

1D and 2D NMR and HRESIMS spectra of compounds **1**, **2**, **10**, **17**−**22**, and **24** (PDF)

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Notes

The authors declare no competing financial interest.

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