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Aromatic and Aliphatic Apiuronides from the Bark of *Cinnamomum cassia*

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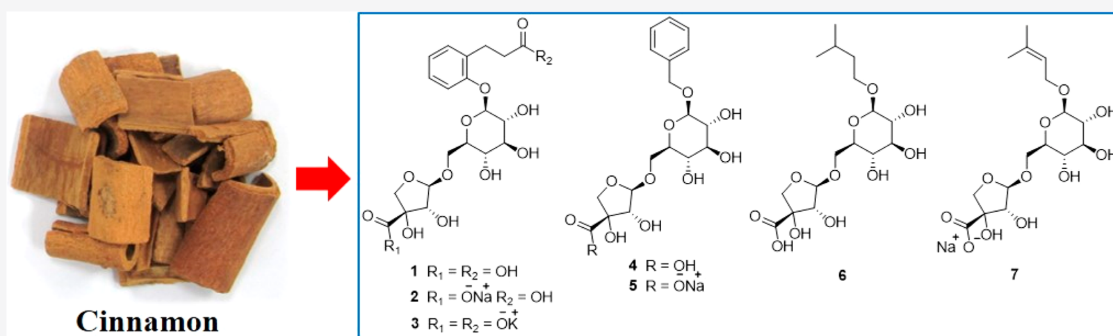

ABSTRACT: *Cinnamomum cassia* Presl (Cinnamon) has been widely cultivated in the tropical or subtropical areas, such as Yunnan, Fujian, Guandong, and Hainan in China, as well as India, Vietnam, Thailand, and Malaysia. Four new glycosides bearing apiuronic acid (**1**, **4**, **6**, and **7**) and their sodium or potassium salts (**2**, **3**, and **5**), together with 31 known compounds, were isolated from a hot water extract of the bark of *C. cassia* via repeated chromatography. The structures of the new compounds (**1**−**7**) were determined by NMR, IR, MS, and ICP-AES data and by acid hydrolysis and sugar analysis. This is the first report of the presence of apiuronic acid glycosides. Some of the isolates were evaluated for their analgesic effects on a neuropathic pain animal model induced by paclitaxel. Cinnzeylanol (**8**), cinnacaside (**9**), kelampayoside A (**10**), and syringaresinol (**11**) showed analgesic effects against paclitaxel-induced cold allodynia.

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A member of the Lauraceae family, *Cinnamomum cassia* Presl (Cinnamon) is a medium sized evergreen tree.[1] *C. cassia* originated in Southern China and can be widely cultivated in the Yunnan, Fujian, Guandong, and Hainan provinces of China, as well as in Thailand, Malaysia, Laos, India, Indonesia, and Vietnam.[2] Chinese cinnamon has been commonly used for centuries as a flavoring agent for desserts, pastries, and meat in Asia, Europe, and the Americas.[3] The bark of *C. cassia* (cinnamon) has been used as a traditional medicine to treat amenorrhea, gastrointestinal neurosis, cardiac palpitations diarrhea, edema, dysmenorrhea, rheumatoid arthritis, impotency, and diabetes.[4−6] The aqueous extract of cinnamon has been reported to exhibit potent anticomplement, immunosuppressive, antioxidant, antiproliferative, angiogenesis, antinociceptive, and anti-inflammatory activities and induced apoptosis.[7−13]

The bark of *C. cassia* represents one of the major sources of phenolic compounds, and there are various secondary metabolites such as diterpenoids,[14−16] phenylpropanoids,[17−19] flavonoids,[20] lignans,[20,21] sesquiterpenoids,[22] and other compounds.[23,24]

In a previous study, it was found that a hot water extract of the bark of *C. cassia* exhibited a relieving effect on cold allodynia induced by anticancer drugs. It was found that a major compound in *C. cassia*, coumarin, played a role in this analgesic effect.[25] Thus, we focused on secondary metabolites in the hot water extract with ameliorating effects against cold allodynia induced by paclitaxel in mice in the present work.

Four new glycosides bearing apiuronic acid moieties (**1**, **4**, **6**, and **7**) and their sodium or potassium salts (**2**, **3**, and **5**), together with 31 known compounds, were isolated from the hot water extract of the bark of *C. cassia* via repeated chromatography. The structures of the new compounds and their sodium or potassium salt forms were elucidated using spectroscopic [^{1}H NMR, ^{13}C NMR, 2D NMR, MS, IR, and inductively coupled plasma atomic emission spectroscopy (ICP-AES)] data, and the structures of the known compounds were identified by comparing spectroscopic data with

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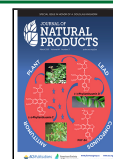

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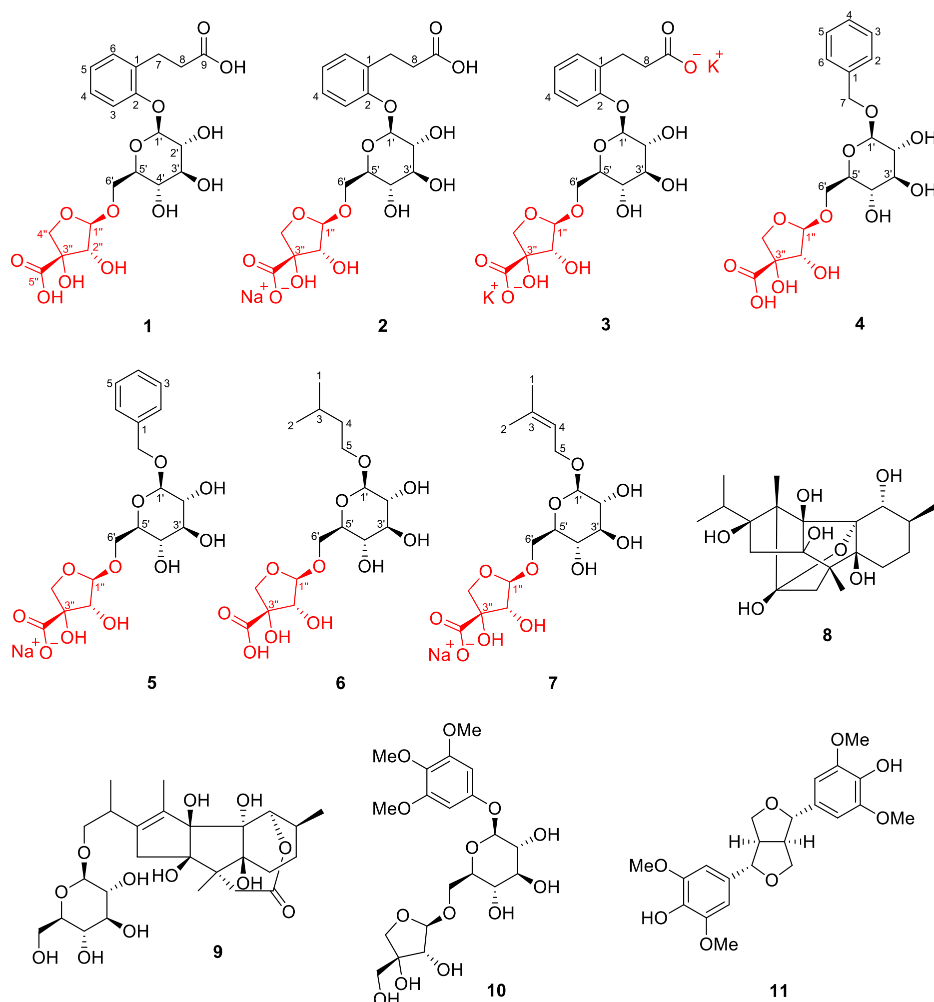

Figure 1. Key HMBC correlations of compounds **1**, **4**, and **6**.

published values. Thereafter, eight isolates with sufficient quantities for *in vivo* testing were evaluated for their analgesic effect against paclitaxel-induced cold allodynia.

■ RESULTS AND DISCUSSION

Structural Elucidation. Compound **1** was isolated as a white powder, for which the molecular formula was established as $C_{20}H_{26}O_{13}$ by HRQTOFMS (negative mode) m/z = 473.1301 $[M - H]^-$ (calcd for $C_{20}H_{25}O_{13}$ 473.1295). The UV spectrum showed absorption bands at 214 and 269 nm suggesting that **1** was a phenolic compound. The ^1H NMR spectrum of **1** showed signals including two methylene protons at δ_H 2.66 (2H, t, J = 7.0 Hz) and 2.93 (2H, ddd, J = 16.5, 14.0, 6.5 Hz) and four aromatic protons at δ_H 7.03 (1H, t, J = 7.5 Hz), 7.13 (1H, d, J = 8.0 Hz), 7.22 (1H, t, J = 7.0 Hz), and 7.24 (1H, d, J = 8.0 Hz), indicating that the aglycone of **1** is a phenylpropanoid. The ^1H NMR spectrum of **1** exhibited signals for a β-glucosyl moiety [δ_H 5.04 (1H, d, J = 7.5 Hz, Glc H-1′), 3.60 (1H, m, Glc H-2′), 3.57 (1H, m, Glc H-3′), 3.49 (1H, t, J = 9.0 Hz, Glc H-4′), 4.06 (1H, m, Glc H-6′a), and 3.76 (1H, m, Glc H-6′b)]. In addition, the spectrum displayed signals similar to those of a β-apiosyl moiety [δ_H 5.03 (1H, d, J = 3.5 Hz), 4.43 (1H, d, J = 4.0 Hz), 3.89 (1H, d, J = 10.0 Hz), and 4.28 (1H, d, J = 10.5 Hz)] except for the absence of a methylene group of apiose.[26,27] The ^{13}C NMR spectrum of **1** revealed 20 carbon signals, including aromatic ring signals (δ_C 154.7, 130.3, 130.0, 128.1, 123.3, and 115.4), two methylene signals (δ_C 34.0 and 22.2), and one hydroxycarbonyl carbon

(δ_C 178.2). The anomeric carbons of each sugar at δ_C 100.6 (Glc C-1′) and 108.6 (ApiA C-1″), eight methine or methylene carbons bearing oxygen in the sugars (δ_C 80.5, 80.3, 75.7, 74.8, 74.7, 72.9, 69.6, and 68.1), and an additional hydroxycarbonyl carbon (δ_C 174.6) were also observed in the ^{13}C NMR spectrum. From those spectra, the structure of **1** was assumed as a phenylpropanoid with two sugar moieties. The ^1H and ^{13}C NMR spectroscopic data of **1** were similar to those of cryptamygin C,[28] which was also isolated as one of the major compounds in this work. The major difference was that the methylene group of *β*-D-apiose in cryptamygin C was replaced by the hydroxycarbonyl group (δ_C 174.6, ApiA C-5″) in **1**. Based on the above results, it could be inferred that an apiuronic acid moiety is present in **1**. Apiuronic acid (3-carboxy-D-erythrose) was recently reported to be present as a constituent of marine polysaccharides for the first time,[29] but it has not been reported in the form of a glycoside. The locations of the sugar moieties and the hydroxycarbonyl group in **1** were defined by tracing the connectivity shown in the HMBC spectrum of **1** (Figure 1). The HMBC spectrum showed the correlations between the methylene protons (δ_H 2.66, 2H, t, J = 7.0 Hz, H-8) and hydroxycarbonyl carbon at δ_C 178.2 (C-9), which established the location of the hydroxycarbonyl group at C-9. The locations of the glucosyl and apiuronic acid moieties were also defined at C-2 and C-6′, respectively, by correlations between Glc H-1′ (δ_H 5.04) and C-2 (δ_C 154.7) and ApiA H-1″(δ_H 5.03) and Glc C-6′ (δc 68.1), similar to the disaccharide acuminose.[30] The D-configuration of *β*-glucose in **1** was defined by acid hydrolysis and sugar analysis with HPLC. The *β*-linkage of the apiuronic acid moiety in **1** was determined using ^1H and ^{13}C NMR data of the anomeric position [δ_H 5.03 (1H, d, J = 3.5 Hz); δ_C 108.6].[26,29] In order to determine the absolute configuration of the apiuronic acid, *α*/*β*-apiuronic acid was isolated from the acid hydrolysate of **1**. The ^1H and ^{13}C NMR spectroscopic data of the *α*/*β*-apiuronic acid were matched with those of *α*/*β*-(2*R*,3*R*)-apiuronic acid which has the corresponding orientation of hydroxy groups with D-apiose (Figures S1 and S2, Supporting Information).[29] Meanwhile, *α*/*β*-D-apiose was isolated from cryptamygin C, a major metabolite obtained in the present study and a homologue of **1** that differs only in the attachment of *β*-D-apiose instead of apiuronic acid. The D-configuration of the isolated *α*/*β*-apiose was confirmed by a positive specific rotation value of ($[\alpha]_D^{25}$ = +6).[30] The specific rotation of the *α*/*β*-apiuronic acid obtained was also positive ($[\alpha]_D^{25}$ = +9) like that of *α*/*β*-D-apiose, supporting the presence of D-apiuronic acid. Considering the above experimental results and biosynthetic aspects, the apiuronic acid moiety present in **1** was defined as *β*-D-apiuronic acid. Thus, the structure of cinnacassioside A (**1**) was defined as dihydrocoumaric acid 2-*O*-*β*-D-apiuronyl(1 → 6)-*β*-D-glucoside.

Compound **2** exhibited the same desodiated molecular ion at m/z = 473.1295 as **1** by HRQTOFMS data. The ^1H and ^{13}C NMR spectra of **2** closely resembled those of **1**, except for the chemical shifts of the apiuronic acid moiety (Table 1). In addition, the chemical shift of the hydroxycarbonyl carbon of apiuronic acid was deshielded from δ_C 174.6 to 176.3 (ApiA C-5″) (Table 2). Moreover, the IR spectrum of **2** showed changes in the intensities of the signal (Figure S3, Supporting Information). The IR signals at around 1700 cm^{-1} which indicate the hydrogen atom of a hydroxycarbonyl group decreased together with the increase of the signals at around 1500 cm^{-1} that indicates a carboxylate anion. These results suggest that an alkali metal ion was attached to a hydroxycarbonyl group in the apiuronic acid moiety of **2**. Therefore, inductively coupled plasma atomic emission

Table 1. ^1H NMR Spectroscopic Data of 1−3 (D_2O, 500 MHz)

	1	2	3
position[a]	δ_H multi (J in Hz)	δ_H multi (J in Hz)	δ_H multi (J in Hz)
1			
2			
3	7.24 d (8.0)	7.25 d (8.0)	7.28 t (7.0)
4	7.22 t (7.0)	7.23 t (7.0)	7.28 t (7.0)
5	7.03 t (7.5)	7.04 t (7.5)	7.09 t (7.5)
6	7.13 d (8.0)	7.15 d (8.0)	7.19 d (7.5)
7	2.93 ddd (16.5, 14.0, 6.5)	2.93 t (8.0)	2.94 ddd (16.5, 14.0, 6.5)
8	2.66 t (7.0)	2.61 td (8.0, 2.0)	2.47 ddd (17.5, 7.5, 2.0)
9			
Glc-1′	5.04 d (7.5)	5.05 d (7.5)	5.08 d (8.0)
Glc-2′	3.60 m	3.59−3.62 m	3.66 m
Glc-3′	3.57 m	3.56−3.59 m	3.61 m
Glc-4′	3.49 t (9.0)	3.52 t (9.5)	3.57 t (8.0)
Glc-5′	3.72 m	3.72−3.75 m	3.80 m
Glc-6′	4.06 d (8.5)	4.05 d (9.0)	4.11 d (9.0)
	3.76 m	3.75−3.77 m	3.80 d (9.0)
ApiA-1″	5.03 d (4.0)	4.97 d (5.0)	5.03 d (4.5)
ApiA-2″	4.43 d (4.0)	4.40 d (4.5)	4.44 d (4.5)
ApiA-3″			
ApiA-4″	4.28 d (10.5)	4.29 d (10.0)	4.35 d (10.0)
	3.89 d (10.0)	3.86 d (10.0)	3.92 d (10.0)
ApiA-5″			

[a]From HSQC, HMBC, and COSY results.

Table 2. ^{13}C NMR Spectroscopic Data of 1−3 (D_2O, 125 MHz)

	1	2	3
position[a]	δ_C	δ_C	δ_C
1	130.0	130.0	130.0
2	154.7	154.5	154.4
3	123.3	123.1	122.9
4	128.1	127.9	127.6
5	115.4	115.1	114.7
6	130.3	130.1	130.8
7	25.2	25.4	26.5
8	34.0	34.3	37.8
9	178.2	178.2	182.7
Glc-1′	100.6	100.4	100.3
Glc-2′	72.9	72.8	72.7
Glc-3′	75.7	75.5	75.4
Glc-4′	69.6	69.3	69.4
Glc-5′	74.8	74.7	74.7
Glc-6′	68.1	67.9	68.0
ApiA-1″	108.6	108.6	108.6
ApiA-2″	80.3	80.1	80.0
ApiA-3″	80.5	81.4	81.5
ApiA-4″	74.7	75.3	75.4
ApiA-5″	174.6	176.3	176.5

[a]From HSQC, HMBC, and COSY results.

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spectroscopy (ICP-AES) measurement was carried out to verify the metal ion.[31,32] Based on ICP-AES measurement, the metal ion in **2** was determined as sodium (Figure S4, Supporting Information). The D-configuration of *β*-glucose in **2** was confirmed by acid hydrolysis and sugar analysis with HPLC. The signals of the *α*/*β*-apiuronic acid in the ^{1}H NMR spectroscopic data of the acid hydrolysate of **2** were identical to those of *α*/*β*-(2*R*,3*R*)-apiuronic acid isolated from the acid hydrolysate of **1**. Therefore, the structure of cinnacassioside A 5″-sodium salt (**2**) was defined as dihydrocoumaric acid 2-*O*-*β*-D-apiuronyl(1 → 6)-*β*-D-glucoside 5″-sodium salt.

Compound **3** also showed the same deprotonated ion peak at *m*/*z* = 473.1297 as **1** and **2** by HRQTOFMS data. The ^{1}H and ^{13}C NMR spectra were highly similar to those of **2**, except for methylene proton and hydroxycarbonyl carbon chemical shifts of the aglycone moiety of **3**. The methylene proton signal was shifted from δ_H 2.61 to 2.47 (H-8), compared to the signal in the spectrum of **2** (Table 1). The hydroxycarbonyl carbon (C-9) of the aglycone was deshielded from δ_C 178.2 to 182.7, suggesting that alkali metal ions were attached to both hydroxycarbonyl groups of **3** (Table 2). The IR spectrum of **3** also showed similar changes in the intensities like **2**. The metal ion in **3** was determined as potassium by the ICP-AES analysis (Figure S4, Supporting Information). The presence of the *β*-D-glucosyl and *β*-D-apiuronic acid moieties in **3** was identified by the same methods as those described for **1** and **2**. Therefore, the structure of cinnacassioside A dipotassium salt (**3**) was defined as dihydrocoumaric acid 2-*O*-*β*-D-apiuronyl(1 → 6)-*β*-D-glucoside 5″,9-dipotassium salt.

Compound **4** was isolated as a white powder, for which the molecular formula was established as $C_{18}H_{24}O_{11}$ by HRQTOFMS data (*m*/*z* = 415.1246 [M − H]$^-$, calcd for $C_{18}H_{23}O_{11}$, 415.1240). The UV spectrum showed absorption bands at 209 and 258 nm indicating that **4** is also a phenolic compound. The ^{1}H NMR spectrum showed signals including methylene protons at δ_H 4.69 (1H, d, *J* = 11.5 Hz, H-7a) and 4.85 (1H, d, *J* = 12.0 Hz, H-7b) and five aromatic protons at δ_H 7.34−7.41 (5H, m). The anomeric protons resonated at δ_H 4.45 (1H, d, *J* = 8.0 Hz, Glc H-1′) and 5.06 (1H, d, *J* = 4.0 Hz, ApiA H-1″) and the remaining resonances of the glucosyl and apiuronic acid moieties at δ_H 3.25−4.48 ppm. The ^{13}C NMR spectrum of **4** revealed 18 carbon signals including six aromatic ring signals at δ_C 135.9, 128.2 × 2, 128.1 × 2, and 127.9 and one oxy-methylene signal (δ_C 71.6), indicating that the aglycone moiety is benzyl alcohol. The spectroscopic data of **4** were similar to those of icariside F_2 except for the sugar moiety.[33] Compound **4** had the same sugar moiety as in **1**. The HMBC spectrum exhibited the correlations between the anomeric proton of the glucosyl moiety and methylene carbon of the aglycone moiety [(δ_H 4.45, (1H, d, *J* = 8.0 Hz, Glc H-1′)/(δ_C 71.6, C-7)], which established the location of the glucopyranosyl unit. The location of the apiuronic acid moiety was also defined by the correlations between the resonances at δ_H 5.06 (1H, d, *J* = 4.0 Hz, ApiA H-1″) and δc 68.1 (Glc C-6′). The presence of the *β*-D-glucosyl and *β*-D-apiuronic acid units in **4** was identified by the above-mentioned methods. Collectively, the structure of cinnacassioside B (**4**) was defined as benzyl alcohol 7-*O*-*β*-D-apiuronyl(1 → 6)-*β*-D-glucoside.

Compound **5** showed the same deprotonated molecular ion at *m*/*z* = 415.1241 [M − Na]$^-$ (calcd for $C_{18}H_{23}O_{11}$, 415.1240) as **4** by HRQTOFMS data. The ^{1}H and ^{13}C NMR spectra closely resembled those of **4**, except for the chemical shifts of the apiuronic acid moiety. Compared to the appropriate signals in the spectra of **4**, the chemical shifts of the anomeric proton and carboxylic carbon of the apiuronic acid unit were changed as observed in **1** and **2** (Table 3). The IR spectrum of **5** also showed similar changes in the absorption intensities like **2**. These results showed that an alkali metal ion was bound to the carboxylic carbon of the apiuronic acid unit. The metal ion was determined as sodium by the ICP-AES data analysis (Figure S4, Supporting Information). The presence of the *β*-D-glucosyl and *β*-D-apiuronic acid moieties in **5** was identified via the above-mentioned methods. Thus, the structure of cinnacassioside B sodium salt (**5**) was defined as benzyl alcohol 7-*O*-*β*-D-apiuronyl(1 → 6)-*β*-D-glucoside 5″-sodium salt.

Table 3. ^{1}H and ^{13}C NMR Spectroscopic Data of 4 and 5 in D_2O

	4		5	
position[a]	δ_H multi (*J* in Hz)	δ_C	δ_H multi (*J* in Hz)	δ_C
1		135.6		136.4
2/6	7.34−7.41 m	128.8	7.34−7.42 m	128.6
3/5	7.34−7.41 m	128.9	7.34−7.42 m	128.7
4	7.34−7.41 m	127.5	7.34−7.42 m	128.3
7	4.85 d (12.0)	71.6	4.86 d (11.5)	71.6
	4.69 d (11.5)		4.69 d (11.5)	
Glc-1′	4.45 d (8.0)	101.2	4.45 d (8.0)	101.2
Glc-2′	3.25 d (8.5)	73.1	3.24 t (8.0)	72.9
Glc-3′	3.38 m	75.8	3.38 (1H, m)	75.6
Glc-4′	3.50 m	74.8	3.50 m	74.6
Glc-5′	3.39 m	69.7	3.40 m	69.4
Glc-6′	4.01 dd (11.5, 2.0)	68.1	4.01 dd (11.5, 1.5)	67.8
	3.71 dd (11.5, 6.5)		3.71 dd (11.5, 5.5)	
ApiA-1″	5.06 d (4.0)	108.6	4.99 d (4.5)	108.6
ApiA-2″	4.48 d (4.0)	80.3	4.39 d (4.5)	80.0
ApiA-3″		80.6		81.6
ApiA-4″	4.36 d (10.5)	74.8	4.37 d (10.0)	75.4
	3.95 d (10.0)		3.95 d (10.5)	
ApiA-5″		174.6		176.5

[a]From HSQC, HMBC, and COSY results. ^{1}H and ^{13}C NMR spectra were acquired at 500 and 125 MHz, respectively.

Compound **6** was isolated as a white amorphous powder, in which the molecular formula was established as $C_{16}H_{28}O_{11}$ by HRQTOFMS data (*m*/*z* = 395.1556 [M − H]$^-$, calcd for $C_{16}H_{27}O_{11}$, 395.1559). The ^{1}H NMR spectrum revealed the presence of two methylene groups at δ_H 1.46 (2H, dd, *J* = 14.0, 7.0 Hz, H-4), 3.66 (1H, dt, *J* = 10.5, 7.5 Hz, H-5a), and 3.89 (1H, dt, *J* = 13.5, 7.0 Hz, H-5b), a methyl-bearing methine proton at δ_H 1.63 (1H, sep, *J* = 7.0 Hz, H-3), and two methyl group protons at δ_H 0.84 (6H, d, *J* = 7.0 Hz, H-1 and 2). The ^{13}C NMR spectrum revealed 16 carbon signals, including isopentanol unit signals (δ_C 69.3, 37.6, 24.3, 21.8, and 21.8) (Table 4). The spectroscopic data indicated that the sugar moieties in **6** and **1** were identical (Tables 1, 2, and 4). The structure of **6** was confirmed by analysis of the HMBC spectrum that exhibited correlations between the anomeric proton of the glucosyl unit and the methylene carbon of the isopentanol moiety [δ_H 4.40 (1H, d, *J* = 8.0 Hz, Glc H-1′)/δ_C 69.3 (C-5)] and between the anomeric proton of the apiuronic acid unit and the methylene carbon of glucose [δ_H 5.05 (1H, d, *J* = 4.0 Hz, ApiA H-1″)/δc 68.2 (Glc C-6′)]. The presence of the *β*-D-glucosyl and *β*-D-apiuronic acid moieties in **6** was identified via the above-mentioned methods. Therefore, the

Table 4. ^{1}H and ^{13}C NMR Spectroscopic Data of 6 and 7 in D_2O

	6		7	
position[a]	δ_H multi (J in Hz)	δ_C	δ_H multi (J in Hz)	δ_C
1	0.84 d (7.0)	21.8	1.55 s	17.1
2	0.84 d (7.0)	21.8	1.61 s	24.8
3	1.63 sep (7.0)	24.3		141.3
4	1.46 dd (14.0, 7.0)	37.6	5.21 t (7.5)	118.2
5	3.89 td (13.5, 7.0)	69.3	4.15 m	65.5
	3.66 td (10.5, 7.0)			
Glc-1′	4.40 d (8.0)	102.4	4.30 d (8.0)	100.3
Glc-2′	3.19 t (8.0)	73.2	3.09 t (7.5)	72.9
Glc-3′	3.42 d (9.5)	75.9	3.42 m	75.7
Glc-4′	3.36 d (9.0)	74.7	3.29 t (7.5)	74.6
Glc-5′	3.54 ddd (10.5, 6.5, 2.0)	69.7	3.60 m	69.4
Glc-6′	4.02 dd (11.0, 1.5)	68.2	4.14 m	67.9
	3.71 dd (6.5, 2.0)		3.90 dd (11.5, 1.5)	
ApiA-1″	5.05 d (4.0)	108.6	4.88 d (4.5)	108.6
ApiA-2″	4.45 d (4.0)	80.3	4.27 d (4.5)	80.0
ApiA-3″		80.6		81.6
ApiA-4″	4.35 d (10.5)	74.9	4.18 d (10.5)	75.4
	3.94 d (10.0)		3.75 d (10.0)	
ApiA-5″		174.7		176.5

[a]From HSQC, HMBC, and COSY results. ^{1}H and ^{13}C NMR spectra were acquired at 500 and 125 MHz, respectively.

structure of cinnacassioside C (**6**) was elucidated as isopentanol 5-*O*-*β*-D-apiuronyl(1 → 6)-*β*-D-glucoside.

Compound **7** was isolated as a white amorphous solid, in which the molecular formula was established as $C_{16}H_{26}O_{11}$ by HRQTOFMS data (m/z = 393.1393 $[M - Na]^-$, calcd for $C_{16}H_{25}O_{11}$, 395.1397). The ^{1}H and ^{13}C NMR spectra were similar to those of **6**, except for the presence of an olefinic proton and the chemical shifts of the apiuronic acid moiety (Table 4). The ^{1}H NMR spectrum revealed two methyl group protons at δ_H 1.55 (3H, s, H-1) and 1.61 (3H, s, H-2), an olefinic proton at δ_H 5.21 (1H, t, J = 7.5 Hz, H-4), and two methylene protons at δ_H 4.15 (2H, m, H-5). The ^{13}C NMR spectrum revealed 16 carbon signals, including isopentenol unit signals at δ_C 141.3, 118.2, 65.5, 24.8, and 17.1. The ^{1}H and ^{13}C NMR spectroscopic data of the sugar moieties in **7** were highly similar to those of **2** and **5**. From these results, it was assumed that an alkali metal ion was bound to the carboxylate group of the apiuronic acid unit in **7**. The metal ion in **7** was determined as sodium by the ICP-AES data analysis (Figure S4, Supporting Information). The presence of the *β*-D-glucosyl and *β*-D-apiuronic acid moieties in **7** was identified by the above-mentioned methods. From these observations, the structure of cinnacassioside D sodium salt (**7**) was defined as isopentenol 5-*O*-*β*-D-apiuronyl(1 → 6)-*β*-D-glucoside 5″-sodium salt.

The new compounds **3** and **4** obtained in significant quantities were also identified in an EtOH extract of the bark of *Cinnamomum cassia* by UPLC-MS analysis, supporting that the apiuronic acid moieties are present as natural products and not as artifacts formed during the hot water extraction (Figures S111 and S112, Supporting Information).

By comparing the spectroscopic data (mainly ^{1}H and ^{13}C NMR) with reported data, the known compounds were identified as cinnzeylanol (**8**),[34] cinnacaside (**9**),[35] kelampayoside A (**10**),[23] syringaresinol (**11**),[36] cinnzeylanin,[34] anhydrocinnzeylanol,[37] anhydrocinnzeylanine,[38] 2-methoxycinnamaldehyde,[39] 2-hydroxycinnamaldehyde,[40] cinnamyl alcohol,[41] cinnamic acid,[32] 2-hydroxycinnamyl alcohol,[42] coniferyl aldehyde,[43] rosavin,[44] (2*E*)-3-phenyl-2propen-1-yl 6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranoside,[44] 1-phenyl-1,2,3-propanetriol,[42] guaiacylglycerol,[45] guaialcylglycerol 7-*O*-*β*-D-glucopyranoside,[46] *erythro*-syringylglycerol,[45] dihydrocinnacasside,[19] cryptamygin C,[29] 3,4-dimethoxyphenyl 1-*O*-D-apio-*β*-D-furanosyl-*β*-D-glucopyranoside,[23] canthoside C,[47] cinnacassosides C,[23] coumarin,[48] benzoic acid,[49] syringaldehyde,[50] (−)-lyoniresinol 2*α*-*O*-*β*-D-glucopyranoside,[51] (4*R*)-4-hydroxy-1,10-*seco*-muurol-5ene-1,10-dione,[52] decumbic acid,[53] and {[(6-*O*-(*β*-D-apiofuranosyl)-*β*-D-glucopyranosyl]oxy}propane (Figure S59, Supporting Information).[54]

Six phenylpropanoids [(2*E*)-3-phenyl-2propen-1-yl 6-*O*-*β*-D-xylopyranosyl-*β*-D-glucopyranoside, 1-phenyl-1,2,3-propanetriol, guaiacylglycerol, guaialcylglycerol 7-*O*-*β*-D-glucopyranoside, *erythro*-syringylglycerol, and cryptamygin C], a lignan glycoside [(−)-lyoniresinol 2*α*-*O*-*β*-D-glucopyranoside], a sesquiterpenoid [(4*R*)-4-hydroxy-1,10-*seco*-muurol-5-ene-1,10-dione], and a furanone (decumbic acid) were isolated from the bark of *C. cassia* for the first time in this study.

Analgesic Effects of the Isolates. Among the isolates from the bark of *C. cassia*, cinnzeylanol (**8**), cinnacaside (**9**), kelampayoside A (**10**), syringaresinol (**11**), cinnzeylanin, cryptamygin C, 3,4-dimethoxyphenyl 1-*O*-D-apio-*β*-D-furanosyl-*β*-D-glucopyranoside, and syringaldehyde were evaluated for their analgesic effects in a paclitaxel-induced cold allodynia mice model. Paclitaxel is an anticancer drug that has been applied on breast, ovarian, and lung cancers.[55−57] Despite its efficacy on cancer, it often induces peripheral sensory neurotoxicity, a dose-limiting factor.[58,59]

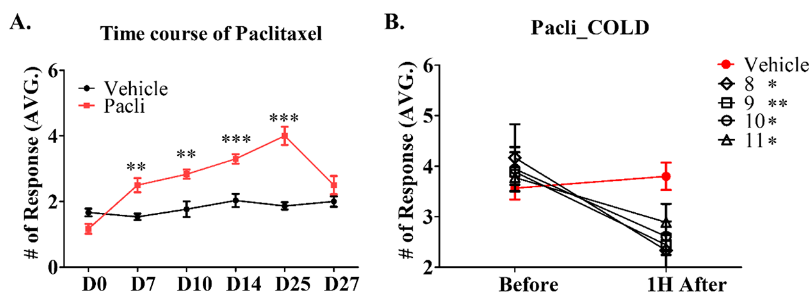

Figure 2. Time course of paclitaxel-induced cold allodynia and analgesic effects against paclitaxel-induced cold allodynia of isolates from the bark of *C. cassia*. Multiple injections of paclitaxel-induced cold allodynia from D7 to D25 (A) and analgesic effect against paclitaxel-induced cold allodynia of isolates **8**−**11** from the bark of *C. cassia* (B). Data are presented as mean ± SEM (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; two-way ANOVA followed by Bonferroni's multiple comparison test). Cinnzeylanol (**8**), cinnacaside (**9**), kelampayoside A (**10**), syringaresinol (**11**).

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Multiple injections (×4) of paclitaxel were delivered to mice intraperitoneally. A significant paclitaxel-induced cold allodynia was observed at D7 and lasted until D25 (Figure 2A). Thus, assessing the analgesic effects of the isolates was conducted from D7 to D25.

To evaluate the analgesic effects of the isolates from the bark of *C. cassia*, the compounds were given orally (10 mg/kg), and cold behavior test were conducted before and 1 h after treatment at D14 and D25. Among the eight compounds tested, cinnzeylanol (**8**), cinnacaside (**9**), kelampayoside A (**10**), and syringaresinol (**11**) showed significant analgesic effects against paclitaxel-induced cold allodynia, by lowering the response number (Figure 2B). Cinnzeylanin, cryptamygin C, 3,4-dimethoxyphenyl 1-*O*-D-apio-β-D-furanosyl-β-D-glucopyranoside, and syringaldehyde were not active in this experiment (Figure S58, Supporting Information). The analgesic effects of **8−11**, represented by decrease of paclitaxel-induced cold allodynia, were similar to the effect of 30 mg/kg gabapentin used as a positive control (i.p.) on paclitaxel-induced neuropathic pain mice model in the previous study.[60]

Cinnzeylanol (**8**) mostly found in *C. cassia*,[61−64] showed insecticidal effects on *Bombyx mori*,[65] whereas cinnacaside (**9**) is only found in the cinnamon tree. Kelampayoside A (**10**) and syringaresinol (**11**) are found in various plants.[66−71] Anti-inflammatory and antioxidant effects are well-known for syringaresinol (**11**).[71] However, their analgesic effects have not yet been reported. In this article, the antiallodynic effects of **8−11** on paclitaxel-induced cold allodynia are presented for the first time. Anti-inflammatory and antioxidant effects of phenolic compounds from natural sources are well-known.[72] Phenolic compounds showed suppressing inflammatory pain behavior.[73] Their pain modulation and inflammatory responses, such as proinflammatory cytokine release, were inhibited.[74] Based on this information, compounds **8−11** are worthy of further experiments for evaluation on their effects against anticancer drug-induced allodynia.

■ EXPERIMENTAL SECTION

General Experimental Methods. Melting points were measured on an MPA 100 instrument (Stanford research systems, Sunnyvale, CA, USA) in open capillary tubes. Optical rotations were measured on a Jasco P-2000 polarimeter (JASCO, Tokyo, Japan), using a 10 cm microcell. UV spectra were obtained on an Optizen pop (Mecasys, Daejeon, Korea) apparatus. HRMS spectra were obtained using a Q-TOF micro mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). NMR spectra were obtained using a JEOL 500 MHz spectrometer (JEOL, Tokyo, Japan) using TMS as an internal standard, and chemical shifts are expressed as δ values. ICP-AES analyses were performed on an Ultima Expert (Jobin Yvon, France) instrument, and IR spectra were obtained using an Agilent Cary 630 FTIR (Agilent Technologies) instrument. The operating conditions of the ICP-AES are as follows: RF generator = solid state, RF power output variable = 1.5 KW, RF frequency = 40.68 MHz, spectral range = 160−800 nm, spectrometer = thermally stabilized, 1 m focal length with 2400 g/mm grating used in the first and second order, and wavelength for analysis = K (766.490 nm) and Na (589.592 nm). TLC analysis were performed on silica gel 60 F_{254} (Merck, Kenilworth, NJ, USA) and RP-18 F_{254S} (Merck) plates. Compounds were visualized by dipping plates into 20% (v/v) H_2SO_4 reagent (Aldrich, St. Lois, MO, USA) and then heated at 110 °C for 5−10 min. Silica gel (Merck, 60A, 70−230 and 230−400 mesh ASTM), Sephadex LH-20 (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom), Diaion HP-20 (Mitsubishi, Tokyo, Japan), and reversed-phase silica gel (ODS-A 12 nm S-150 μm, YMC CO., Tokyo, Japan) were used for column chromatography. Flash chromatography was performed using the flash purification system (Combi Flash Rf, Teledyne Isco, Lincoln, NE, USA). Prepacked cartridges, Redi Sep-Silica (12, 24, and 40 g, Teledyne Isco), Redi Sep-C_{18} (13, 26, 43, and 130 g, Teledyne Isco), and SNAP Ultra C18 (30 g, Biotage, Uppsala, Sweden) were used for flash chromatography. HPLC was performed using the Gilson purification system (Gastorr BG-34 degasser, Gilson 321 pump, Gilson UV/vis-155 detector, Gilson UV/vis-159 detector, Gilson Inc., Middleton, WI, USA) with YMC Pack ODS-A column (250 × 20 mm^2 i.d., 5 μm, YMC Co.). All solvents used for the chromatographic separations were distilled before use.

Plant Material. The bark of *Cinnamomum cassia* were obtained from the MEDIFORUM (Seoul, Korea) in July 2017. The origin of the herbal material was identified by Prof. Dae Sik Jang and a voucher specimen (CICA1-2017) has been deposited in the Laboratory of Natural Product Medicine, College of Pharmacy, Kyung Hee University, Republic of Korea.

Extraction and Isolation. The dried plant material (6.0 kg) was extracted with 60 L of hot water twice at 100 °C in a water bath for 2 h, and the solvent was evaporated *in vacuo* at 45 °C. The hot water extract (150.0 g) was suspended in H_2O (1.0 L) and successively extracted with EtOAc (3 × 1 L), to give EtOAc-soluble (20.8 g) and water-soluble extracts (128.3 g), respectively. The EtOAc-soluble extract (20.8 g) was fractionated over silica gel (70−230 mesh, ϕ 5.0 × 100.0 cm) as stationary phase with a EtOAc-*n*-hexane gradient (from 0/1 to 1/0, v/v) as mobile phase to afford 13 fractions (EA1−EA13). Fraction EA4 was coumarin (2.0 g).

2-Methoxycinnamaldehyde (54.6 mg) was isolated from EA2 by a flash chromatography with a Redi Sep-Silica cartridge (40 g, CH_2Cl_2/*n*-hexane = 3/7 to 1/0, *v/v*). Fraction EA6 was fractionated using silica gel column chromatography (CC) (230−400 mesh, *n*-hexane/EtOAc = 9/1 to 8/2, v/v, ϕ 3.8 × 32.0 cm^2) to produce seven subfractions (EA6-1−EA6-7). 2-Hydroxycinnamaldehyde (22.5 mg), cinnamyl alcohol (5.4 mg), and cinnamic acid (212.3 mg) were obtained from fraction EA6-3 by flash chromatography with a Redi Sep-C_{18} cartridge (13 g, MeOH/H_2O = 3/7 to 6/4, v/v). Fraction EA10 was subjected to silica gel CC (230−400 mesh, ϕ 3.0 × 33.0 cm^2, CH_2Cl_2/acetone = 19/1 to 8/2, v/v) to obtain 2-hydroxycinnamyl alcohol (10.5 mg), coniferyl aldehyde (6.5 mg), and syringaldehyde (57.7 mg). Cinnzeylanin (136.8 mg), anhydrocinnzeylanine (10.3 mg), and (4*R*)-4-hydroxy-1,10-*seco*-muurol-5ene-1,10-dione (2.4 mg) were isolated from fraction EA12 by RP-HPLC with an YMC Pack ODS-A column (250 × 20 mm^2 i.d., 5 μm). Fraction EA13 was fractionated using Sephadex LH-20 CC with CH_2Cl_2/MeOH mixture (1/1, v/v) to afford 15 subfractions (EA13-1−EA13-15). Compounds **8** (38.1 mg) and **11** (38.6 mg) were obtained from subfraction EA13-1 and EA13-2 by flash chromatography with a Redi Sep-C_{18} cartridge (26 g, MeOH/H_2O = 4/6 to 5/5, v/v), respectively

The water-soluble extract (128.3 g) was fractionated using CC over Diaion HP-20 (ϕ 8.0 × 41.0 cm^2) and eluted with an acetone/H_2O gradient (from 0/1 to 1/0, v/v) to afford 11 fractions (W1−W11). Fraction W2 was further fractionated on Sephadex LH-20 (eluted with MeOH/H_2O = 2/8, v/v) to produce seven subfractions. Compound **1** (26.5 mg) and benzoic acid (6.2 mg) were isolated from subfraction W2-7 by a flash chromatographic system with a Redi Sep-C_{18} cartridge (26 g, MeOH/H_2O = 0/1 to 1/9, v/v). Fraction W3 was separated using Sephadex LH-20 eluted with (MeOH/H_2O = 3/7, v/v) to produce eight subfractions. Compound **3** (180.0 mg) was obtained by a flash chromatographic system with a SNAP Ultra C_{18} cartridge (30 g, MeOH/H_2O = 0/1 to 1/9, v/v) from subfraction W3-2. Decumbic acid (9.1 mg) was isolated using a flash chromatographic system with a Redi Sep-silica cartridge (24 g, EtOAc/MeOH/H_2O = 9/0.9/0.1 to 6/3.6/0.4, v/v/v) from subfraction W3-5. Fraction W6 was fractionated using Sephadex LH-20 eluted with (MeOH/H_2O = 3/7, v/v) to produce 11 subfractions. Subfraction W6-2 was subjected to a flash chromatographic system with a SNAP Ultra C_{18} cartridge [60 g, MeCN (with 0.1% formic acid)/H_2O (with 0.1% formic acid) = 0/1 to 2/8, v/v] to generate compounds **2** (19.0 mg), **4** (180.3 mg), **6** (9.9 mg), and **7**

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(19.0 mg). Subfraction W6-7 was fractionated using a flash chromatographic system with a SNAP Ultra C_{18} cartridge (30 g, $MeCN/H_2O$ = 0/1 to 2/8, v/v) to obtain *erythro*-syringylglycerol (7.0 mg) and canthoside C (25.9 mg). Guaialcylglycerol 7-*O*-β-D-glucopyranoside (36.4 mg) and {[(6-*O*-(β-D-apiofuranosyl)-β-D-glucopyranosyl]oxy}propane (24.7 mg) were isolated using a flash chromatographic system with a SNAP Ultra C_{18} cartridge (30 g, $MeCN/H_2O$ = 0/1 to 2/8, v/v) from W6-5. Guaiacylglycerol (10.8 mg) was obtained using a flash chromatographic system with a Redi Sep-silica cartridge (24 g, EtOAc/MeOH = 1/0 to 8/2, v/v) from subfraction W6-9. Fraction W7 was subjected to Sephadex LH-20 CC eluted with ($MeOH/H_2O$ = 3/7, v/v) to produce 13 subfractions. Subfraction W7-4 was subjected to silica gel CC (230−400 mesh, ϕ 3.2 × 32.0 cm^2, EtOAc/acetone/H_2O = 4/5/1 to 3/6/1, v/v/v) to give compound **5** (11.3 mg). 1-Phenyl-1,2,3-propanetriol (58.1 mg) and cinnacassoside C (5.0 mg) were obtained using a flash chromatographic system with a Redi Sep-silica cartridge (12 g, $CH_2Cl_2/MeOH/H_2O$ = 1/8.1/0.9 to 3/6.3/0.7, v/v/v) from subfraction W7-7. Fraction W8 was fractionated by Sephadex LH-20 CC (ϕ 4.8 × 46.5 cm^2) and eluted ($MeOH/H_2O$ = 8/2, v/v) to afford 14 subfractions. Compounds **9** (57.6 mg), **10** (38.9 mg), and 3,4-dimethoxyphenyl 1-*O*-apio-β-D-furanosyl-β-D-glucopyranoside (30.5 mg) were isolated using silica gel CC (230−400 mesh, ϕ 3.0 × 34.0 cm^2, EtOAc/acetone/H_2O = 4/5/1, v/v/v) and a flash chromatography system using a Redi Sep-C_{18} cartridge (26 g, $MeOH/H_2O$ = 2/8 to 4/6, v/v) from subfraction W8-7. Cryptamygin C (205.2 mg) and dihydrocinnacasside (18.6 mg) were separated by a flash chromatography system using a Redi Sep-silica cartridge (40 g, $CH_2Cl_2/MeOH/H_2O$ = 9/0.9/0.1 to 7/2.7/0.3, v/v/v) from fraction W9. Fraction W10 was further fractionated using Sephadex LH-20 CC (ϕ 4.8 × 46.5 cm^2) eluted with $MeOH/H_2O$ (7/3, v/v) to produce 16 subfractions. Subfraction W10-8 was subjected a flash chromatography system using a Redi Sep-C_{18} cartridge (13 g, $MeOH/H_2O$ = 2/8 to 4/6, v/v) and RP-HPLC with a YMC Pack ODS-A column (250 × 20 mm^2 i.d., 5 μm) to generate (−)-lyoniresinol 2α-*O*-β-D-glucopyranoside (5.5 mg). Rosavin (66.5 mg) and (2*E*)-3-phenyl-2propen-1-yl 6-*O*-β-D-xylopyranosyl-β-D-glucopyranoside (8.1 mg) were purified by a flash chromatography system using a Redi Sep-C_{18} cartridge (13 g, $MeOH/H_2O$ = 2/8 to 4/6, v/v) from subfraction W10-9. Anhydrocinnzeylanol (11.4 mg) was isolated from subfraction W10-11 by flash chromatography using a Redi Sep-C_{18} cartridge (26 g, $MeCN/H_2O$ = 2/8 to 5/5, v/v).

*Dihydrocoumaric Acid 2-O-β-D-Apiuronyl(1 → 6)-β-D-glucoside (**1**).* This compound is a white powder; mp 107.8 °C ; $[\alpha]_D^{25}$: −69 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 214 nm (4.07), 269 nm (3.38); IR (ATR) ν_{max} 3378, 2928, 2097, 1702, 1492, 1231, 1054 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRQTOFMS (negative mode) *m*/*z* = 473.1301 $[M - H]^-$ (calcd for $C_{20}H_{25}O_{13}$, 473.1295).

*Dihydrocoumaric Acid 2-O-β-D-Apiuronyl(1 → 6)-β-D-glucoside 5″-Sodium Salt (**2**).* This compound is a white powder; mp 137.8 °C ; $[\alpha]_D^{25}$: −67 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 214 nm (4.07), 269 nm (3.38); IR (ATR) ν_{max} 3213, 2922, 2101, 1703, 1587, 1491, 1397, 1231, 1050 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRQTOFMS (negative mode) *m*/*z* = 473.1295 $[M - Na]^-$ (calcd for $C_{20}H_{25}O_{13}$, 473.1295).

*Dihydrocoumaric Acid 2-O-β-D-Apiuronyl(1 → 6)-β-D-glucoside 5″,9-Dipotassium Salt (**3**).* This compound is a white powder; mp 137.8 °C ; $[\alpha]_D^{25}$: −64 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 214 nm (4.07), 269 nm (3.38); IR (ATR) ν_{max} 3071, 2129, 1561, 1490, 1392, 1228, 1054 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRQTOFMS (negative mode) *m*/*z* = 473.1297 $[M - 2K + H]^-$ (calcd for $C_{20}H_{25}O_{13}$, 473.1295).

*Benzyl Alcohol 7-O-β-D-apiuronyl(1 → 6)-β-D-glucoside (**4**).* This compound is a white powder; mp 97.6 °C; $[\alpha]_D^{25}$: − 86 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 209 nm (4.14), 258 nm (3.43); IR (ATR) ν_{max} 3391, 2927, 2885, 2114, 1719, 1230, 1039 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; HRQTOFMS (negative mode) *m*/*z* = 415.1246 $[M - H]^-$ (calcd for $C_{18}H_{23}O_{11}$, 415.1240).

*Benzyl Alcohol 7-O-β-D-Apiuronyl(1 → 6)-β-D-glucoside 5″-Sodium Salt (**5**).* This compound is a white powder; mp 102.6 °C; $[\alpha]_D^{25}$: −87 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 209 nm (4.14), 258 nm (3.43); IR (ATR) ν_{max} 3328, 2882, 2119, 1594, 1453, 1399, 1042 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; HRQTOFMS (negative mode) *m*/*z* = 415.1241 $[M - Na]^-$ (calcd for $C_{18}H_{23}O_{11,}$ 415.1240).

*Isopentanol 5-O-β-D-Apiuronyl(1 → 6)-β-D-glucoside (**6**).* This compound is a white amorphous powder; $[\alpha]_D^{25}$: −88 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 207 nm (3.86); IR (ATR) ν_{max} 3362, 2929, 2017, 1719, 1420, 1231, 1047 cm^{-1}; ^1H and ^{13}C NMR data, see Table 4; HRQTOFMS (negative mode) *m*/*z* = 395.1556 $[M - H]^-$ (calcd for $C_{16}H_{27}O_{11}$, 395.1559).

*Isopentenol 5-O-β-D-Apiuronyl(1 → 6)-β-D-glucoside 5″-Sodium Salt (**7**).* This compound is a white amorphous powder; $[\alpha]_D^{25}$: −24 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 207 nm (3.86); IR (ATR) ν_{max} 3323, 2926, 2122, 1591, 1400, 1229, 1011 cm^{-1}; ^1H and ^{13}C NMR data, see Table 4; HRQTOFMS (negative mode) *m*/*z* = 393.1393 $[M - Na]^-$ (calcd for $C_{16}H_{25}O_{11}$, 393.1397).

Sugar Identification. Each compound (**1**−**7**, 1.0 mg each) was heated at 80 °C with 2 M TFA (1 mL) for 24 h. The reaction mixture was evaporated *in vacuo* at 45 °C. The residues were dissolved in pyridine (0.5 mL), and L-cysteine methyl ester (5 mg) was added to the solution and heated for 1.5 h at 60 °C. *o*-Tolyl isothiocyanate (20 μL) was added, and the mixture was heated at 60 °C for 1.5 h. The products were analyzed directly by HPLC at 280 nm. Analytical HPLC was conducted on a Luna 5 μm C_{18} column (4.6 × 250 mm^2) at 25 °C using 0.1% TFA in CH_3CN:0.1% TFA in H_2O (25:75). D-Glucose [t_R, **1** (9.099 min), **2** (9.166 min), **3** (9.138 min), **4** (9.131 min), **5** (9.146 min), **6** (9.190 min), **7** (9.123 min)] was identified from each sample by comparing their retention times with those of the authentic D-glucose (t_R, 9.159 min) and L-glucose (t_R, 8.885 min).

Isolation of α/β-D-Apiuronic Acid. An acid hydrolysis of **1** (20 mg) was performed with 0.02 M trifluoroacetic acid (TFA) at 80 °C for 48 h. α/β-D-Apiuronic acid (5 mg) was isolated from the hydrolysate using silica gel CC with a $CHCl_3$/MeOH/water mixture (5/4/1).

α/β-D-Apiuronic Acid. This compound is a white amorphous powder; $[\alpha]_D^{25}$: +9 (*c* 0.1, H_2O); ^1H NMR (D_2O, 500 MHz) α-D-apiuronic acid = 5.32 (1H, d, *J* = 5.0 Hz, H-1), 4.31 (1H, overlapped, H-2), 4.16 (1H, d, *J* = 10.0 Hz, H, 3a), 3.97 (1H, d, *J* = 10.0 Hz, H-3b) and β-D-apiuronic acid = 5.16 (1H, d, *J* = 4.5 Hz, H-1), 4.32 (1H, d, *J* = 10.0 Hz, H-3a), 4.20 (1H, d, *J* = 4.5 Hz, H-2), 3.83 (1H, d, *J* = 9.5 Hz, H-3b); ^{13}C NMR (D_2O, 125 MHz) α-D-apiuronic acid = 179.3 (C-5), 96.4 (C-1), 80.0 (C-3), 76.5 (C-4), 75.8 (C-2) and β-D-apiuronic acid = 178.1 (C-5), 102.3 (C-1), 81.8 (C-3), 80.4 (C-2), 75.8 (C-4). The ratio of α- and β-D-apiuronic acids was 0.7:1 by peak integration of the ^1H NMR spectrum.

Isolation of α/β-D-Apiose. Cryptamygin C (10 mg) was hydrolyzed with 0.02 M TFA at 80 °C for 48 h. α/β-D-Apiose (1 mg) was isolated from the hydrolysate using silica gel CC with a $CHCl_3$/MeOH/water mixture (6/4/1). The apiose was identified by TLC comparing with authentic D-apiose (silica gel 60 F_{254}, $CHCl_3$/MeOH/water/HOAc = 6/4/1/0.1, R_f = 0.55). The isolated α/β-apiose exhibited a positive specific rotation value, $[\alpha]_D^{25}$: +6 (*c* 0.1, H_2O)], confirming D-configuration.[30]

Animals. Male C57BL/6 mice (6 weeks old) were obtained from DBL, Chungbuk, Korea. Mice were housed under SPF laboratory conditions with temperature (23 ± 2 °C), humidity (65 ± 5%), food and water available *ad libitum*, and a fixed 12 h light/dark cycle. All experimental protocols were approved by the Kyung Hee University Animal Care and Use Committee (KHUASP (SE)-19-011).

Feeding Experiments. All compounds were dissolved in 0.06% of Tween-80 solution (1 mg/mL) and were fed in an amount of 10 mg/kg per mice.

Paclitaxel Mice Model and Behavior Test. Paclitaxel (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 50% EtOH and 50% Cremophor EL solution. Paclitaxel (0.2 mg/mL, 2 mg/kg) was injected (i.p.) on days 0, 2, 4, and 6. The mice were caged in the

behavior set (an inverted clear plastic cage on a metal mesh floor) for 30 min prior to the behavior test. Acetone (10 μL, Daejung Chemical Ltd., Gyeonggi-do, Korea) was applied to the midplantar skin of both hind paws (three times). As a painful behavior, the frequencies of brisk withdrawal, licking, and shaking of the hind paw were counted after the acetone stimuli for 30 s. Counted number of the response of both hind paw was averaged and presented in Figure 2 as "no. of Response (AVG.)".

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c01062.

^{1}H NMR, ^{13}C NMR, FTIR, UV, HSQC, COSY, HMBC, and HRMS spectra and ACPAES results (PDF)

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Notes

The authors declare the following competing financial interest(s): J.H.L., S.K.K., and D.S.J. hold a patent application related to the contents of this article (10-2019-0118269 in Korea).

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DEDICATION

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