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Houttuynoid M, an Anti-HSV Active Houttuynoid from *Houttuynia cordata* Featuring a Bis-houttuynin Chain Tethered to a Flavonoid Core

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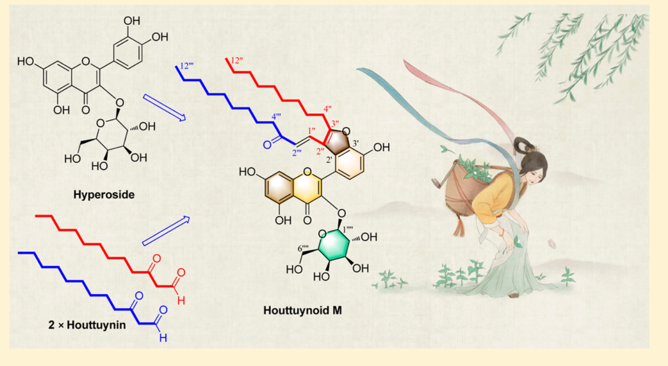
Supporting Information

ABSTRACT: Houttuynoid M (**1**), a new houttuynoid, and the related known compound houttuynoid A (**2**) were isolated from *Houttuynia cordata*. Their structures were defined using NMR data analysis, HR-MS^n experiment, and chemical derivatization. Houttuynoid M is the first example of a houttuynoid with a bis-houttuynin chain tethered to a flavonoid core. A putative biosynthetic pathway of houttuynoid M (**1**) is proposed. The anti-herpes simplex virus (anti-HSV) activities of **1** and **2** (IC_{50} values of 17.72 and 12.42 μM, respectively) were evaluated using a plaque formation assay with acyclovir as the positive control.



Houttuynia cordata (Saururaceae) is mainly distributed in East and Southeast Asia.[1] The dried aerial parts or fresh whole plant of *H. cordata* has a long history of being used as traditional Chinese medicine (TCM) as Yu Xing Cao (Houttuyniae Herba). It is commonly used in relieving fever, detoxifying, dissolving carbuncles, and draining pus.[2−4] Modern pharmacology research showed that it possesses diverse bioactivities, including anti-inflammatory, antibacterial, antiviral, antidiabetic, and cytotoxic activities.[1,5]

Because *H. cordata* is one of the common and important TCMs, a large number of phytochemical investigations have been carried out, and more than 160 compounds have been identified, including flavonoids,[6−14] phenylpropanoids,[7,15−18] alkaloids,[1,7,19−24] steroids,[1,18,25] terpenoids,[1,25] volatile oils,[6] and fatty acids.[24,26] A new type of flavonoid, named houttuynoids,[12−14] was discovered from *H. cordata*. Houttuynoids are composed of a flavonoid core and a houttuynin chain and have anti-herpes simplex virus (HSV) activities.[12−14] Although houttuynoids are rare and only 12 analogues have been reported, they have already attracted much attention due to their structural novelty and promising antiviral activity. The total synthesis of houttuynoid B has been achieved in 2016.[27]

To search for additional houttuynoids from nature, the DAD and MS charateristics of the peaks in the HPLC-DAD-MS chromatogram of the extract of *H. cordata* were analyzed and showed peaks with the DAD characteristics of hyperoside and with 330−350 Da higher molecular weights (molecular weight of about two houttuynin units) than hyperoside, which indicated that there may be new houttuynoids with two houttuynin units in *H. cordata*. Subsequent chemical investigation on *H. cordata* led to the isolation of houttuynoid M (**1**) and its biosynthetic related known compound houttuynoid A (**2**). Houttuynoid M (**1**) is the first example of a houttuynoid with a bis-houttuynin chain tethered to a flavonoid core. In addition, houttuynoid M (**1**) exhibited anti-HSV activity in a plaque formation assay.

RESULTS AND DISCUSSION

The air-dried aerial parts of *H. cordata* were extracted with EtOH−H_2O (60:40, v/v). The extract was separated by continuously different column chromatography to yield houttuynoid M (**1**) and its related known compound, houttuynoid A (**2**).

Houttuynoid M (**1**) was obtained as a brown amorphous powder. The molecular formula of **1** was established as $C_{44}H_{58}O_{13}$ (16 indices of hydrogen deficiency) from its HRESIMS data (m/z 795.3954 $[M + H]^+$, calcd for $C_{44}H_{59}O_{13}$: 795.3956). In the HR-MS^2 experiment (Figure S1, Supporting Information), the ion at m/z 817.3752 $[M + Na]^+$ gave the positive fragment at m/z 655.3218 $[M + Na - 162]^+$, indicating the presence of a hexose unit in **1**. The ^{13}C

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NMR spectrum showed 44 carbon signals. Combined with the DEPT 135 experiment, these signals were classified as two carbonyls (δ_C 198.7 and 177.6), 18 aromatic or olefinic carbons [including six sp^2 methines (δ_C 132.5, 128.3, 128.2, 110.2, 98.9, and 93.9)], 16 sp^3 nonoxygenated methylenes, two methyls (δ_C 13.94 and 13.90), and six hexosyl carbons (δ_C 101.7, 75.6, 73.0, 71.0, 67.5, and 59.7) (Table 1).

The $^1H-^1H$ COSY and ^1H NMR spectra of **1** revealed the presence of four fragments, including C-5′−C-6′, C-4″−C-12″, C-1″−C-2‴, and C-4‴−C-12‴ (Figure 1). The HMBC correlations (Figure 1) from H-6 to C-5/C-7/C-8/C-10, H-8 to C-6/C-7/C-9/C-10, H-5′ to C-1′/C-3′, H-1″ to C-2′/C-2″/C-3″/C-2‴/C-3‴, H-4″ to C-2″/C-3″, H-5″ to C-3″/C-4″, H-12″ to C-10″/C-11″, H-2‴ to C-3‴/C-4‴, Hb-4‴ to C-3‴/C-5‴/C-6‴, H-5‴ to C-3‴/C-4‴/C-6‴, H-12‴ to C-10‴/C-11‴, and 5-OH to C-5/C-6/C-10 revealed the partial structure of the aglycone moiety. The geometry of the $\Delta^{1''}$ double bond was determined as *E* by the value of $^3J_{H-1''/H-2'''}$ (16.3 Hz). Considering **1** from the similar biosynthetic pathway to houttuynoid A (**2**), the length of the alkyl chain at C-3″ should be the same as that of houttuynoid A (**2**). Furthermore, the aglycone ion at *m/z* 655.3218 ($[M + Na - C_6H_{10}O_5]^+$) in the HR-$MS^2$ spectrum showed an ion at *m/z* 483.1765 ($[M + Na - C_6H_{10}O_5 - C_{10}H_{20}O_2]^+$) in the HR-$MS^3$ spectrum (Figure S1, Supporting Information), which confirmed the above deduction. On the basis of the comparison of 1D NMR data with those of houttuynoid A (**2**),[12] the molecular formula, the indices of hydrogen deficiency, and the above analyses, the structure of the aglycone of **1** was established. The NMR data are collated in Table 1.

Based on comparison with the ^{13}C NMR data of galactopyranose,[12] the hexose moiety of **1** was identified as galactopyranose. After acid hydrolysis and derivatization (Figure S2, Supporting Information), the absolute configuration of the galactose was determined as D according to the procedure developed by Tanaka et al.[28] The galactopyranose unit in **1** was attached to the aglycone via a *β*-linkage based on the coupling constant of the anomeric proton (δ_H 5.24, d, J = 6.3 Hz, H-1⁗) and the observed NOESY correlations between H-1⁗ and H-3⁗/H-5⁗. The location of the galactopyranose moiety to the aglycone was deduced as C-3 based on the NOESY correlation between Ha-6⁗ and 5-OH and the comparison of the ^{13}C NMR data of **1** with houttuynoid A (**2**).[12] Therefore, the structure of houttuynoid M (**1**) was elucidated as 5,7-dihydroxy-2-[7-hydroxy-2-nonyl-3-{(*E*)-3-oxododec-1-en-1-yl}benzofuran-4-yl]-3-[{(2*S*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl}oxy]-4*H*-chromen-4-one.

Houttuynoid A (**2**) was identified by comparison of its reported 1D NMR data.[12]

Houttuynoids are a rare type of flavonoid. Houttuynoid M (**1**) is the first case of a houttuynoid with a bis-houttuynin chain tethered to the quercetin flavonoid core. Houttuynoid A (**2**) may be the putative biosynthetic precursor of **1**. The additional houttuynin moiety, 3-oxododecanal, may be tethered to houttuynoid A (**2**) at C-1″ by aldol condensation and further be oxidized and decarboxylated to form **1** (Scheme 1).

The known houttuynoids exhibited anti-HSV (HSV-1/Blue) activities in an in vitro screening model of an HSV immediate−early promoter-directed reporter system.[12−14,29] The isolated compounds were evaluated for their inhibitory effects against HSV (HSV-1/F) using a classical plaque formation assay with acyclovir (ACV) as the positive control [IC_{50} = 0.15 μM, SI (selectivity index, CC_{50}/IC_{50}) > 1333]. The result showed that both houttuynoid M (**1**) (IC_{50} = 17.72 μM, SI > 11.29) and houttuynoid A (**2**) (IC_{50} = 12.42 μM, SI > 16.10) displayed anti-HSV-1 activity (Table 2).

Table 1. NMR Data of 1 in DMSO-d_6 (600 MHz for ^1H; 150 MHz for ^{13}C)

no.	δ_C, mult	δ_H (J in Hz)[e]	$^1H-^1H$ COSY	HMBC	NOESY
2	157.5, C				
3	134.3, C				
4	177.6, C				
5	161.4, C				
6	98.9, CH	6.16, d (1.3)	8	5, 7, 8, 10	
7	164.7, C				
8	93.9, CH	6.09, d (1.3)	6	6, 7, 9, 10	
9	156.1, C				
10	104.2, C				
1′	112.9, C				
2′	126.9, C				
3′	142.5, C				
4′	144.8, C				
5′	110.2, CH	6.82, d (8.2)	6′	1′, 3′	
6′	128.2, CH	7.67, br s	5′		
1″	132.5, CH	7.28, d (16.3)	2‴	2′, 2″, 3″, 2‴, 3‴	4″, 4‴a, 4‴b
2″	112.7, C				
3″	160.7, C				
4″	26.6, CH_2	2.91, t (7.5)	5″	2″, 3″	1″, 2‴
5″	27.5, CH_2	1.71	4″, 6″	3″, 4″	
6″	28.88,[a] CH_2	1.03−1.31	5″		
7″	28.66,[a] CH_2	1.03−1.31			
8″	28.63,[a] CH_2	1.03−1.31			
9″	28.55,[a] CH_2	1.03−1.31			
10″	31.30,[b] CH_2	1.23			
11″	22.09,[c] CH_2	1.25	12″		
12″[d]	13.90, CH_3	0.84, t (7.0)	11″	10″, 11″	
2‴	128.3, CH	6.04, d (16.3)	1″	2″, 3″, 3‴, 4‴	4″
3‴	198.7, C				
4‴	38.9, CH_2	a: 2.06	4‴b, 5‴		1″
		b: 1.99	4‴a, 5‴	3‴, 5‴, 6‴	1″
5‴	23.3, CH_2	1.19	4‴a, 4‴b	3‴, 4‴, 6‴	
6‴	28.86,[a] CH_2	1.03−1.31			
7‴	28.85,[a] CH_2	1.03−1.31			
8‴	28.59,[a] CH_2	1.03−1.31			
9‴	28.53,[a] CH_2	1.03−1.31			
10‴	31.23,[b] CH_2	1.23			
11‴	22.07,[c] CH_2	1.25	12‴		
12‴[d]	13.94, CH_3	0.87, t (6.9)	11‴	10‴, 11‴	
1⁗	101.7, CH	5.24, d (6.3)	2⁗		3⁗, 5⁗
2⁗	71.0, CH	3.33	1⁗, 3⁗	1⁗, 3⁗	
3⁗	73.0, CH	3.32	2⁗, 4⁗	2⁗	1⁗
4⁗	67.5, CH	3.63, br s	3⁗, 5⁗	2⁗, 3⁗	
5⁗	75.6, CH	3.30	4⁗, 6⁗a, 6⁗b	1⁗, 4⁗, 6⁗	1⁗
6⁗	59.7, CH_2	a: 3.39	5⁗, 6⁗b		5-OH
		b: 3.23	5⁗, 6⁗a		
5-OH		12.53, s		5, 6, 10	6⁗a

[a−d]Assignment may be interchanged in each group. [e]Indiscernible signals from overlap or complex multiplicity are reported without designating multiplicity.

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Figure 1. Stucture of **1** and key ^{1}H−^{1}H COSY, HMBC, and NOESY correlations of **1**.

Scheme 1. Putative Biosynthetic Pathway of Compound 1

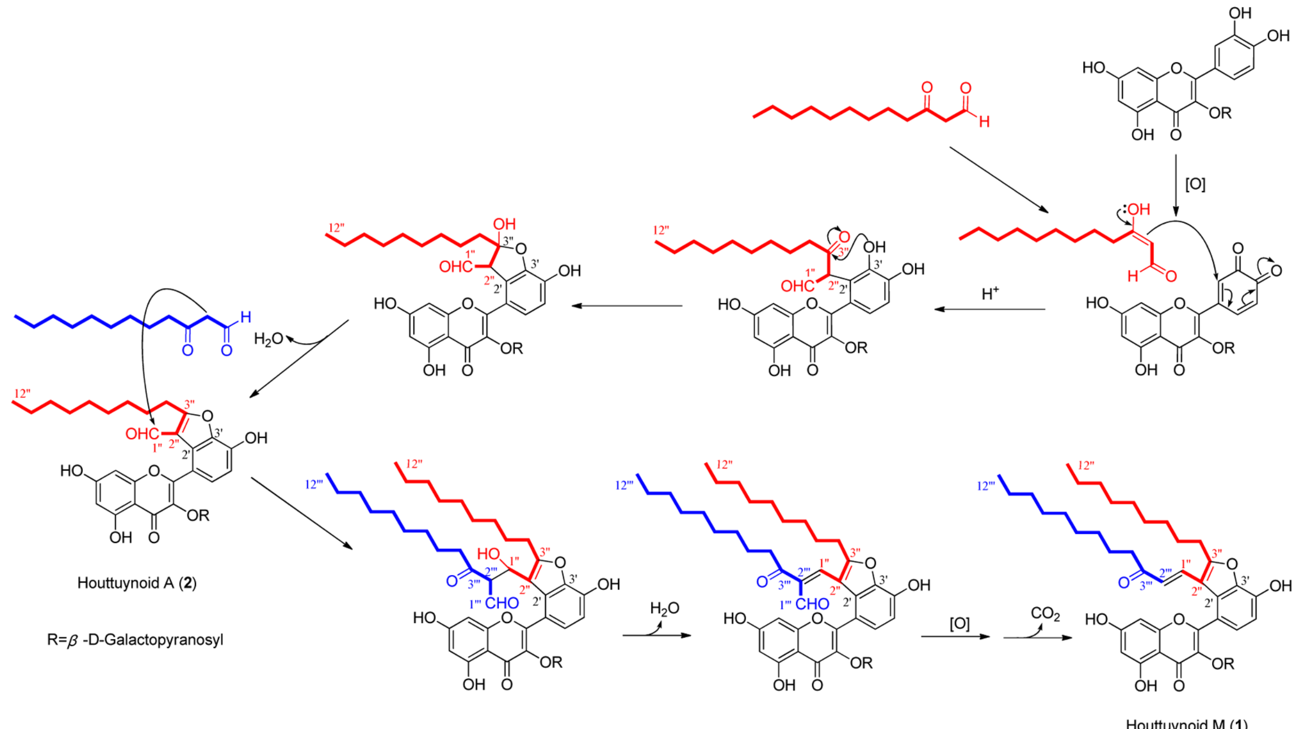

Table 2. Anti-HSV Activities of Compounds 1 and 2

no.	CC_{50} (μM)	IC_{50} (μM)	SI[a]
1	>200	17.72	>11.29
2	>200	12.42	>16.10
ACV	>200	0.15	>1333

[a]SI (selectivity index) = CC_{50}/IC_{50}.

■ EXPERIMENTAL SECTION

General Experimental Procedures. The details of the reagents and the instruments used in this work are provided in the Supporting Information.

Plant Material. The plant material was purchased from the Qingping market of traditional Chinese medicine, Guangdong Province, China, in September 2014. In addition, it was identified as the dried aerial parts of *H. cordata* by Dr. Ying Zhang, College of Pharmacy, Jinan University.

Extraction and Isolation. The dried aerial parts (50.0 kg) of *H. cardata* were extracted twice with 600 L of EtOH−H_2O (60:40, v/v, 2 h each). After filtration and evaporation in vacuo, 7.47 kg of crude extract was obtained. The crude extract was separated by an AB-8 macroporous resin column (20 × 130 cm) with EtOH−H_2O (10:90, 44:66, 95:5, v/v) to give three fractions (F1−F3). Fraction F3 (177.4 g) was separated by silica gel CC (8 × 50 cm) with $CHCl_3$−MeOH (95:5, 90:10, 85:15, 80:20, 70:30, 50:50, 0:100, v/v) to afford fractions 3.1−3.5. Fraction 3.3 (35.2 g) was subjected to MPLC on ODS CC (3 × 35 cm) eluted with a gradient of MeOH−H_2O (68:32 to 100:0, v/v) to afford fractions 3.3.1−3.3.7.

Fraction 3.3.2 (6.6 g) was separated by preparative HPLC with a Cosmosil Packed C_{18} column [eluted with MeOH−H_2O−HCOOH (70:30:0.1, v/v/v), 8 mL/min] to yield fractions 3.3.2.1−3.3.2.10. Fraction 3.3.2.4 (872.7 mg) was subjected to preparative HPLC with a Cosmosil Packed C_{18} column [eluted with CH_3CN−H_2O−HCOOH (40:60:0.1, v/v/v), 8 mL/min] to afford **2** (t_R: 83.3 min, 584.5 mg).

Fraction 3.3.5 (4.1 g) was subjected to Sephadex LH-20 CC (11 × 96 cm) eluted with MeOH to afford fractions 3.3.5.1−3.3.5.8. Fraction 3.3.5.3 (385.0 mg) was separated by preparative HPLC with a Phenomenex Kinetex C_8 column [eluted with CH_3CN−H_2O−HCOOH (56:44:0.1, v/v/v), 8 mL/min] to give **1** (t_R: 55.6 min, 19.2 mg).

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Houttuynoid M (1): brown amorphous powder; $[\alpha]^{29}_{D}$ −34 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.91), 256 (3.78), 305 (3.53) nm; IR (KBr) ν_{max} 3421, 2922, 2854, 2362, 1651, 1606, 1498, 1461, 1359, 1303, 1195, 1072, 1025, 996 cm^{-1}; ESIMS (positive) *m*/*z* 1611 $[2 M + Na]^+$, 817 $[M + Na]^+$; HRESIMS (positive) *m*/*z* 795.3954 $[M + H]^+$ (calcd for $C_{44}H_{59}O_{13}$, 795.3956); ^1H and ^{13}C NMR data see Table 1.

Cytotoxicity Assay of 1 and 2. Vero cells were grown in 96-well plates. After 24 h, the cells were continuously cultured in fresh Dulbecco's modified Eagle's medium (DMEM) treated with either various concentrations of compounds or equivalent solvent. The Vero cell viabilities were assessed by the CellTiter-Glo Luminescent cell viability assay (Promega) according to the manufacturer's protocol at 24 h. The luminescent signal was measured using an Envison 2102 multilabel reader (PerkinElmer). The 50% cytotoxic concentration (CC_{50}) for each compound was calculated from these dose−response curves using Graphpad 5.0 Prism. The results are presented as mean values with standard deviations (n = 3).

Plaque Formation Assay of 1 and 2. Vero cells were grown in 24-well plates to 80−90% confluence. After 24 h, the cells were treated with various concentrations of compounds (with ACV as the positive control) and infected with HSV-1 strain F (from Wuhan Institute of Virology, Chinese Academy of Sciences). At 24 h postinfection, the supernatants were used to reinfect the Vero cells seeded in the 24-well plates. The supernatants were removed and replaced with fresh DMEM medium after 2 h of incubation. The cultures were stained with 1% crystal violet at 24 h post-reinfection, and subsequently plaques were counted. By reference to the number of plaques observed in virus control monolayers (untreated cultures), the concentration of test compounds that inhibited plaque numbers by 50% (IC_{50}) was determined from dose−response curves. The results are presented as mean values with standard deviations (n = 3).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00620.

Details of general experimental procedures, HR-MS^n experiment of **1**, acid hydrolysis, and NMR spectra of **1** and **2** (PDF)

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Author Contributions

⊥J.-J. Li and G.-D. Chen contributed equally to this work.

Notes

The authors declare no competing financial interest.

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