ORGANIC LETTERS

2012
Vol. 14, No. 7
1772–1775

Houttuynoids A–E, Anti-Herpes Simplex Virus Active Flavonoids with Novel Skeletons from *Houttuynia cordata*

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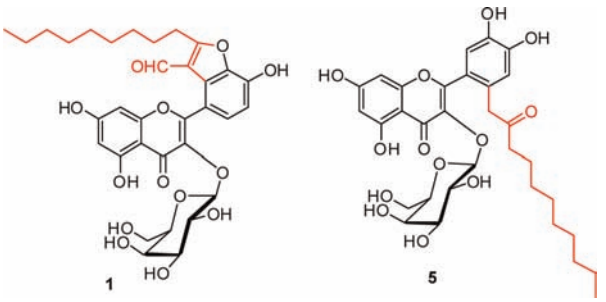
Received February 15, 2012

ABSTRACT



Houttuynoids A–E (1–5), a new type of flavonoid with houttuynin tethered to hyperoside, and their presumed biosynthetic precursor hyperoside (6) were isolated from the whole plant of *Houttuynia cordata*. Their structures were elucidated by analysis of 1D and 2D NMR. A hypothetical biogenetic pathway for houttuynoids A–E was proposed. Compounds 1–5 exhibited potent anti-HSV (herpes simplex viruses) activity.

Natural products play an important role in organic chemistry and pharmaceutical science for their various skeletons and diverse bioactivities, which not only broaden the strategies of synthetic chemists but also provide numerous candidates for drug discovery.[1] Plants are one of the most important sources of natural products.

Houttuynia cordata THUNB., belonging to the family of Saururaceae, is well-known in China under the name "Yu-Xing-Cao", a plant medicine to relieve fever, to reduce swelling, to drain pus, and to promote urination.[2] Recently, *H. cordata* has been used for the treatment of herpes simplex virus type 1 (HSV-1), influenza virus, and human immunodeficiency virus type 1 (HIV-1).[3,4] At the time of the Severe Acute Respiratory Syndrome (SARS) outbreak, *H. cordata* was one of the ingredients included in the SARS prevention formula recognized by the Health Ministry of China. Phytochemical investigations of *H.*

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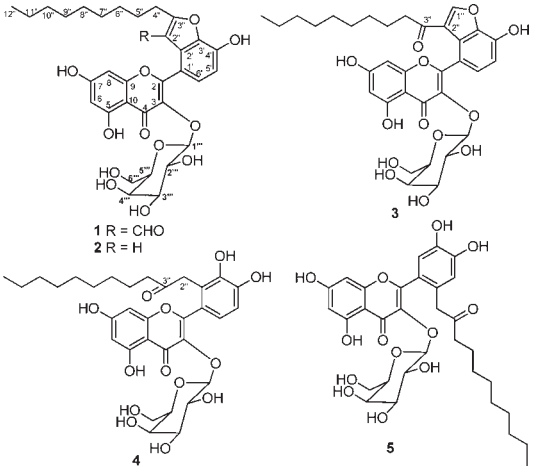
10.1021/ol300017m
Published on Web 03/13/2012

cordata, ongoing for many years, have revealed that this herb was rich in volatile oils,[5−7] flavonoids,[8,9] and alkaloids.[9−11]

In a recent research effort aimed at the discovery of anti-HSV active compounds from *H. cordata*, we investigated the 50% aq. EtOH extract of the whole plant of *H. cordata*. Bioassay-guided fractionation of the extract led to the isolation of houttuynoids A−E (**1**−**5**), five novel flavonoids with unprecedented carbon skeletons, and their presumably biosynthetic precursor hyperoside (**6**). Houttuynoids A−E (**1**−**5**) are a new type of flavonoid with houttuynin (3-oxododecanal) tethered to hyperoside. Moreover, houttuynoids A−C (**1**−**3**) contain a stable furano-type junction. In addition, they exhibited potent anti-HSV activity *in vitro*. Below, we describe the isolation, structure elucidation, and a possible biogenetic pathway of these flavonoids, as well as their anti-HSV activity *in vitro*.



The air-dried powder of the whole plant (5.0 kg) of *Houttuynia cordata* was refluxed with 50% (v/v) aqueous ethanol to afford 556 g of crude extract, and the extract was separated continuously by column chromatography over macroporous resin AB-8, ODS, HW-40, Sephadex LH-20 and preparative HPLC to yield compounds **1** (132.4 mg), **2** (6.4 mg), **3** (83.2 mg), **4** (7.2 mg), **5** (7.8 mg), and **6** (42.5 mg).

Houttuynoid A (**1**) was obtained as a brown amorphous powder ($[\alpha]_D^{25}$ −1.1 (c = 0.50, CH_3OH)). It had the molecular formula $C_{33}H_{38}O_{13}$ determined by using HR-ESI-MS (m/z 665.2197 $[M + Na]^+$, calcd. for 665.2205). HPLC analysis of products obtained from acid hydrolysis and derivatization reactions by L-cysteine methyl ester and *o*-tolyl isothiocyanate[12] revealed that **1** contained a D-galactose moiety. Analysis of ^{13}C NMR spectroscopic data revealed that **1** possessed 33 carbons, including an aldehyde [δ_H 9.90 (1H, s); δ_C 185.1], two tetrasubstituted benzene rings [δ_H 6.23, 6.43 (each 1H, d, J = 1.9 Hz), 6.73 and 8.05 (each 1H, d, J = 8.3 Hz); δ_C 164.5, 161.3, 156.7, 144.8, 142.3, 128.9, 124.6, 113.7, 111.0, 104.5, 98.7, and 93.7], a hexose moiety [δ_H 5.23 (1H, d, J = 7.7 Hz) and 3.25−3.63 (6H, m); δ_C 102.2, 75.5, 73.1, 71.1, 67.7, and 60.0], and a nonyl group [δ_H 3.15 (2H, br. s), 1.74 (2H, m), 1.25−1.31 (12H, m), and 0.83 (3H, t, J = 6.7 Hz); δ_C 31.2, 28.8, 28.6 (×3), 27.7, 26.6, 22.1, and 13.9]. The ^1H and ^{13}C NMR resonances associated with fragment A (Supporting Information) were very similar to those of hyperoside (**6**)[13] (Scheme 1).

According to the degree of unsaturation, the molecular formula information, and the obvious downfield at C-3″, the two remaining quaternary carbons (δ_C 170.9 and 117.4) could be assigned as a bridge between 3′-*O* and C-2′ as part of a benzofuran ring system. In the HMBC spectrum, correlations that existed between H-1″ and C-2′/C-3″/C-2″ and between H-4″ and C-2″/C-3″ (see Supporting Information) revealed the aldehyde was linked to C-2″ and the nonyl was attached to C-3″. Therefore, the structure of **1** was determined as a 5,7,4′-trihydroxy-2″-nonyl-3″-carboxaldehyde-[2″,3″:2′,3′]-3-*O*-β-D-galactopyranosyl furanoflavonoid, and its assignment of the ^1H and ^{13}C NMR signals (Table 1) was aided by the results of $^1H-^1H$ COSY, HSQC, and HMBC experiments.

Houttuynoid B (**2**) was isolated as a brown amorphous powder ($[\alpha]_D^{25}$ −1.3 (c = 0.50, CH_3OH)). The molecular formula of **2** was established as $C_{32}H_{38}O_{12}$ by its HR-ESI-MS (m/z 637.2253 $[M + Na]^+$, calcd for 637.2255). **2** also contained a D-galactose moiety as evidenced by HPLC analysis of products obtained from acid hydrolysis and derivatization reactions by L-cysteine methyl ester and *o*-tolyl isothiocyanate.[12] The NMR spectroscopic data of **2** were similar to those of **1**, except for the lack of a signal associated with an aldehyde group, which was supported by the HMBC correlations between H-2″ and C-3″/C-2′/C-3′. Thus, the structure of **2** was assigned as a 5,7,4′-trihydroxy-2″-nonyl-[2″,3″:2′,3′]-3-*O*-β-D-galactopyranosyl furanoflavonoid based on the results of 2D NMR experiments.

Houttuynoid C (**3**) was obtained as a brown amorphous powder ($[\alpha]_D^{25}$ −1.7 (c = 0.50, CH_3OH)). The molecular formula of **3** was assigned as $C_{33}H_{38}O_{13}$ based on HR-ESI-MS (m/z 665.2199 $[M + Na]^+$, calcd for 665.2205). It is an isomer of **1**. HPLC analysis of products obtained from acid hydrolysis and derivatization reactions by L-cysteine methyl ester and *o*-tolyl isothiocyanate[12] indicated that **3** contained a D-galactose moiety. The similarity of the ^1H and ^{13}C NMR spectroscopic data of **3** to those of **1** indicated that **3** possessed the same furanoflavonoid skeleton with an anellated furan ring at the C-2′/C-3′ positions and a nonyl group. **3** contained a ketone (δ_C 195.0) rather

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Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data of **1**–**5** in DMSO-d_6 (J in Hz)[a]

	1		**2**		**3**		**4**		**5**	
no.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2		158.6		157.1		158.7		158.7		158.7
3		134.3		133.4		133.9		134.8		134.9
4		178.0		177.4		178.1		177.8		177.7
5	12.61 (s)	161.3	12.67 (s)	161.3	12.58 (s)	161.3	12.61 (s)	161.3	12.61 (s)	161.2
6	6.22 (d, 1.8)	98.7	6.23 (d, 1.9)	98.8	6.19 (d, 1.8)	98.6	6.19 (d, 1.8)	98.7	6.20 (s)	98.8
7		164.5		164.3		164.5		164.6		164.6
8	6.16 (d, 1.8)	93.7	6.43 (d, 1.9)	93.5	6.13 (s)	93.5	6.13 (d, 1.8)	93.4	6.20 (s)	93.6
9		156.7		156.3		156.2		156.5		156.7
10		104.5		104.0		104.3		104.2		104.2
1′		113.7		112.7		114.2		122.5		120.9
2′		124.6		129.7		124.1		122.2	7.03 (s)	118.0
3′		142.3		142.5		144.3		143.7		143.3
4′		144.8		144.9		145.4		146.9		147.2
5′	6.86 (d, 8.3)	111.0	6.73 (d, 8.3)	109.5	6.86 (d, 8.3)	111.0	6.76 (d, 8.3)	113.1	6.67 (s)	118.3
6′	7.71 (d, 8.3)	128.9	8.05 (d, 8.3)	127.2	7.71 (d, 8.3)	129.0	7.09 (d, 8.3)	121.9		125.9
1″	9.90 (s)	185.1			8.93 (s)	152.7				
2″		117.4	6.79 (s)	103.2		123.3	a 3.65 (d, 15.3) b 3.57 (d, 15.3)	41.1	a 3.61 (d, 15.3) b 3.51 (d, 15.3)	46.3
3″		170.9		160.4		195.0		207.2		207.9
4″	3.15 (br.s)	26.6	2.81 (t, 7.4)	27.6	2.82 (t, 7.2)	40.6	2.36 (t, 7.3)	41.0	2.27 (t, 7.3)	41.3
5″	1.74 (m)	27.7	1.70 (m)	28.2	1.39 (m)	23.9	1.40 (m)	23.2	1.32 (m)	23.2
6″	1.31 (m)	28.6	1.32 (m)	28.9	1.22	28.7	1.15 (m)	28.8	1.15	28.8
7″	1.25	28.8	1.21	27.7	1.19	28.9	1.21	28.8	1.15	28.8
8″	1.25	28.6	1.21	28.7	1.19	28.8	1.21	28.6	1.15	28.6
9″	1.25	28.6	1.21	28.6	1.19	28.5	1.21	28.5	1.15	28.5
10″	1.25	31.2	1.21	31.3	1.19	31.3	1.21	31.2	1.15	31.2
11″	1.25	22.1	1.21	22.1	1.19	22.1	1.21	22.0	1.20	22.0
12″	0.83 (t, 6.7)	13.9	0.83 (t, 6.7)	13.9	0.84 (t, 6.7)	13.9	0.85 (t, 6.7)	13.9	0.84 (t, 6.7)	13.9
1‴	5.23 (d, 7.7)	102.2	5.42 (d, 7.6)	101.5	5.23 (d, 7.7)	102.2	5.07 (d, 7.2)	101.8	4.94 (d, 7.0)	102.6
2‴	3.28	71.1	3.47	71.1	3.31	71.1	3.28 (m)	70.9	3.27	71.0
3‴	3.28	73.1	3.35 (m)	73.1	3.30	73.2	3.28 (m)	73.1	3.26	73.2
4‴	3.63 (m)	67.7	3.66 (m)	67.8	3.64 (m)	67.7	3.64 (m)	67.8	3.58 (m)	67.6
5‴	3.26	75.5	3.31	75.7	3.28	75.5	3.28 (m)	75.6	3.26	75.6
6‴	a 3.43 (dd, 9.5, 5.4) b 3.25	60.0	a 3.44 b 3.33	60.1	a 3.44 (dd, 9.5, 5.4) b 3.21 (m)	60.0	a 3.49 (m) b 3.32	60.1	a 3.46 (m) b 3.27	60.0

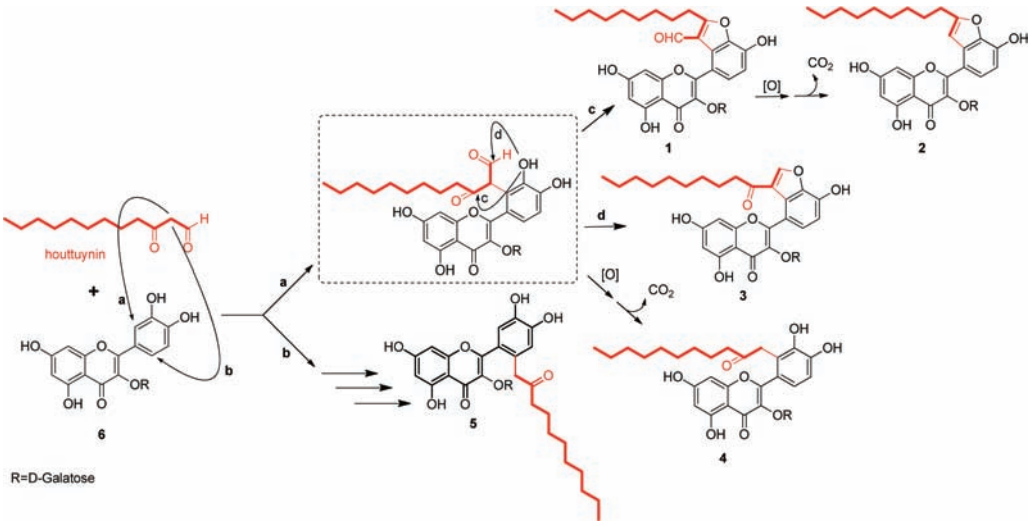
[a] Overlapped signals are reported without designating multiplicity.

than an aldehyde group as in the case of **2**. The HMBC correlation that existed between H_2-4″ and C-3″ illustrated the ketone carbon in **3** was connected to the nonyl group directly. The resulting decanoyl was located at C-2″ as a consequence of the HMBC correlations between H-1″ and C-2′/C-3′ and the obvious downfield of H-1″ at δ_H 8.93 (1H, s). Hence, the structure of **3** was determined as a 5,7,4′-trihydroxy-3″-decanonyl-[1″,2″:2′,3′]-3-*O*-*β*-D-galactopyranosyl furanoflavonoid.

Houttuynoid D (**4**) was isolated as a brown amorphous powder ($[\alpha]_D^{25}$ −2.2 (c = 0.50, CH_3OH)). Its molecular formula $C_{32}H_{40}O_{13}$ was assigned by using HR-ESI-MS (m/z 655.2367 $[M + Na]^+$, calcd for 655.2361) showed that **4** possessed one degree of unsaturation less than **2**. **4** also contained a D-galactose moiety as evidenced by HPLC analysis of products obtained from acid hydrolysis and derivatization reactions by L-cysteine methyl ester and *o*-tolyl isothiocyanate.[12] The ^{1}H and ^{13}C NMR spectroscopic data of **4** resembled those of **2** (Table 1), except for resonances associated with C-2″ and C-3″. Notably, the sp^2 methine at C-2″ of **2** was replaced by an sp^3 methylene [δ_H 3.65, 3.57 (each 1H, d, J = 15.3 Hz); δ_C 41.1] in **4**, and the oxygenated quaternary carbon at C-3″ has become a ketone carbon (δ_C 207.2), which suggested that the bond of 3′-*O*−C-3″ was broken. This proposal was further confirmed by the HMBC correlations that existed between H_2-4″ and C-3″, between H_2-2″ and C-3″, and between H_2-2″ and C-1′/C-3′. Therefore, the structure of **4** was determined as a 5,7,3′,4′-tetrahydroxy-2′-(2-undecanonyl)-3-*O*-*β*-D-galactopyranosyl flavonoid.

The molecular formula of houttuynoid E (**5**) obtained as a brown amorphous powder was established as $C_{32}H_{40}O_{13}$

Scheme 1. A Hypothetical Biogenetic Route of **1**–**5**



on the basis of its HR-ESI-MS ion peak (*m*/*z* 655.2346 $[M + Na]^+$, calcd for $C_{32}H_{40}O_{13}Na$: 655.2361) ($[\alpha]_D^{25}$ −1.8 (*c* = 0.50, CH_3OH)). HPLC analysis of products obtained from acid hydrolysis and derivatization reactions by L-cysteine methyl ester and *o*-tolyl isothiocyanate[12] indicated that **5** contained a D-galactose moiety. It is an isomer of **4** and had similar ^1H and ^{13}C NMR spectroscopic data to those of **4** (Table 1). Two singlet aromatic proton resonances (δ_H 7.03 and 6.67) in the ^1H NMR spectrum suggested that the 2-undecanonyl group was located at C-6′ in **5**, not at C-2′ as in **4**. The correlations between H_2-4″ and C-1′/C-5′ displayed in the HMBC spectrum, together with the correlations between H-2′ and C-4′/C-6′, H-5′ and C-1′/C-3′, further illustrated the location of the 2-undecanonyl at C-6′. Accordingly, the structure of **5** was identified as a 5,7,3′,4′-tetrahydroxy-6′-(2-undecanonyl)-3-*O*-*β*-D-galactopyranosyl flavonoid, and its ^1H and ^{13}C NMR data (Table 1) were obtained by comprehensive analysis of ^1H–^1H COSY, HSQC and HMBC experiments.

As mentioned above, earlier efforts had shown that *H. cordata* was rich in flavonoids and volatile oils. Besides, quercetin glycosides were identified as primary constituents of the flavonoids while houttuynin and 2-undecanone were the main constituents of the volatile oils.[14] Houttuynin was observed to be unstable and easily degraded to form 2-undecanone.[14,15] Compounds **4** and **5** possess a 2-undecanone moiety tethered to C-2′ or C-6′ of a hyperoside unit. As such, it appeared that hyperoside and houttuynin were biosynthetic precursors of houttuynoids A–E, generated by the series of reactions given in Scheme 1.

The inhibitory effects of houttuynoids A–E (**1**–**5**) and hyperoside (**6**) on the herpes simple virus (HSV) were examined employing acyclovir (ACV) as a positive control (IC_{50} = 46.56 ± 4.95 μM).[16] The results showed that **1**–**5** exhibited potent inhibitory activities against HSV, with respective IC_{50} values of 23.50 ± 1.82, 57.71 ± 8.03, 50.75 ± 11.07, 59.89 ± 6.63, and 42.03 ± 10.22 μM. In contrast, **6** did not display anti-HSV activity. The selective index (SI) of anti-HSV activity of **1**–**5** was 7.08, 3.15, 10.47, 3.02, and 3.21 respectively.

Acknowledgment. This research was financially supported by the State Key Development Program of Basic Research of China (2009CB522300), by the Program for New Century Excellent Talents in University (NCET-10-0120) from the Ministry of Education of China, by Key Sci-tech Research Projects of Guangdong Province (No. 8351063201000003), and by the Fundamental Research Funds for the Central Universities (21611203).

Supporting Information Available. Experimental procedures; ^1H and ^{13}C NMR data (in tables); a listing of UV, IR, HR-ESI-MS, NMR spectra for **1**–**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.