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Hupehenols A–E, Selective 11β-Hydroxysteroid Dehydrogenase Type 1 (11β-HSD1) Inhibitors from *Viburnum hupehense*

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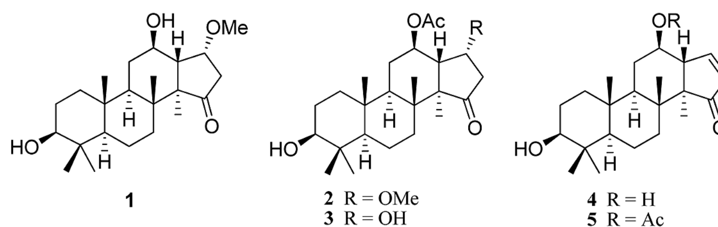
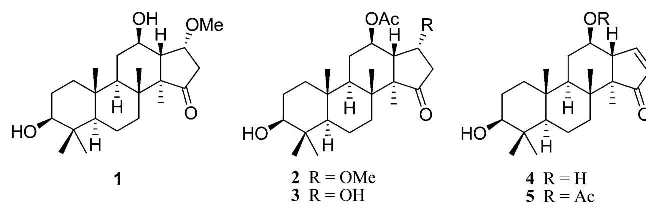
Supporting Information



ABSTRACT: Five selective 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) competitive inhibitors, hupehenols A–E (**1**–**5**), were isolated from *Viburnum hupehense*. The structure elucidation indicated that compounds **1**–**5** are new 20,21,22,23,24,25,26,27-octanordammarane triterpenoids. Their structures were established on the basis of NMR spectroscopic and mass spectrometric analysis. Hupehenols A–E (**1**–**5**) showed inhibition against human 11β-HSD1, with hupehenols B (**2**) and E (**5**) having IC_{50} values of 15.3 and 34.0 nM, respectively. Moreover, hupehenols C (**3**) and D (**4**) are highly selective inhibitors of human 11β-HSD1 when compared to murine 11β-HSD1.

11β-Hydroxysteroid dehydrogenase (11β-HSD) regulates glucocorticoid action in glucocorticoid and mineralocorticoid target tissues.[1] It has two distinct isoenzymes, 11β-HSD1 and 11β-HSD2. 11β-HSD1 catalyzes the transformation of inactive 11-ketoglucocorticoids (cortisone/prednisone in humans, dehydrocorticosterone in rodents) into active 11β-hydroxylated forms (cortisol/prednisolone in humans, corticosterone in rodents) in metabolically relevant tissues, mainly in the liver and adipose tissue. Inhibition of 11β-HSD1 has been proposed to be of potential therapeutic benefit to persons with metabolic syndrome and obesity-related disorders.[2] Many natural products have been reported as 11β-HSD1 inhibitors.[3] In contrast, 11β-HSD2 catalyzes the transformation of active 11β-hydroxy ketoglucocorticoids to their inactive form in the kidney. It has been reported that reduced 11β-HSD2 function may result in sodium retention, hypokalemia, and hypertension.[4] Therefore, selective inhibition of 11β-HSD1 could be a potential treatment for diabetes and/or metabolic syndrome.[5] Until now, only a few natural products have been reported as selective 11β-HSD1 inhibitors.[6]

Viburnum hupehense Rehder, belonging to the family Caprifoliaceae, is distributed mainly in the Hubei, Sichuan, and Yunnan Provinces of the People's Republic of China.[7] As part of a research program on naturally occurring bioactive metabolites from this genus,[8] a 70% aqueous acetone extract of the aerial parts of *V. hupehense* was investigated, which led to the isolation of five new 20,21,22,23,24,25,26,27-octanordammarane triterpenoids, hupehenols A–E (**1**–**5**), along with three known triterpenoids, 2α,3β-dihydroxy-12-ursen-28-oic acid,[9] lupeol,[10] and serratagenic acid.[11] Herein the isolation, structural elucidation, and inhibitory effects on 11β-HSD of **1**–**5** are described.



Compound **1** was obtained as a white, amorphous powder. Its molecular formula, $C_{23}H_{38}O_4$, was established on the basis of positive HRESIMS ($[M + Na]^+$, m/z 401.2671, calcd 401.2667), indicating five degrees of unsaturation. The IR spectrum of **1** showed the presence of hydroxy (3431 cm^{-1})

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and carbonyl (1732 cm^{-1}) groups. The ^1H NMR spectrum exhibited the signals of six singlet methyls [δ_H 0.73, 0.83, 0.93 (6H), 1.19, 3.30] and three oxygenated CH group functionalities (δ_H 3.12, dd, J = 13.0, 5.0 Hz; 3.92, m; 4.07, dd, J = 6.0, 4.8 Hz) (Table 1). The ^{13}C NMR and DEPT spectra revealed 23 resonances that could be ascribed to six methyl groups (including one methoxy group), six methylenes, six methines, and five quaternary carbons (including one ketone and four sp^3 carbon atoms) (Table 2). The degrees of unsaturation (including one C═O) indicated the skeleton of **1** to be that of a degraded tetracyclic triterpenoid. Taking into account the NMR data combined with the molecular formula, **1** could be assigned with the skeleton of a 20,21,22,23,24,25,26,27-octanordammarane triterpenoid.[12] $^1H-^1H$ COSY correlations revealed three spin systems, namely, H_2-1/H_2-2/H-3, H-5/H_2-6/H_2-7, and H-9/H_2-11/H-12/H-13/H-17/H_2-16, which indicated that C-3, C-12, and C-17 are the three oxygenated CH signals resonating at δ_C 79.6, 67.1, and 76.5, respectively. The HMBC correlation from OMe (δ_H 3.30) to C-17 (δ_C 76.5, s) suggested that the methoxy group is connected to C-17. Additionally, the HMBC cross-peaks of H-16 (δ_H 2.27, dd, J = 20.0, 6.0 Hz; 2.55, d, J = 20.0 Hz), H-17 (δ_H 4.07, dd, J = 6.0, 4.8 Hz), and Me-30 (δ_H 1.19, s) with the carbonyl (δ_C 222.4) suggested that the C═O group is located at C-15 (Figure 1). Detailed analysis of the HMBC and COSY NMR spectra clearly demonstrated the planar structure of **1** as shown in Figure 1.

Table 1. ^1H NMR Data of Hupehenols A−E (1−5) in $CDCl_3$

position	1	2	3	4	5
1a	1.73 (m)	1.64 (m)	1.68 (m)	1.67 (m)	1.63 (m)
1b	1.03 (m)	1.04 (m)	1.02 (m)	1.00 (m)	1.03 (m)
2a	1.57 (m)	1.63 (m)	1.66 (m)	1.66 (m)	1.63 (m)
2b	1.63 (m)	1.55 (m)	1.58 (m)	1.59 (m)	1.56 (m)
3	3.12 (dd, 13.0, 5.0)	3.20 (dd, 11.8, 3.9)	3.16 (dd, 14.5, 5.5)	3.19 (dd, 14.0, 4.5)	3.18 (dd, 14.5, 4.5)
5	0.75 (m)	0.74 (m)	0.77 (m)	0.76 (m)	0.79 (m)
6a	1.60 (m)	1.58 (m)	1.62 (m)	1.62 (m)	1.62 (m)
6b	1.46 (m)	1.42 (m)	1.46 (m)	1.48 (m)	1.46 (m)
7a	2.10 (m)	2.10 (m)	2.09 (m)	2.17 (m)	2.23 (m)
7b	1.52 (m)	1.51 (m)	1.52 (m)	1.58 (m)	1.47 (m)
9	1.35 (m)	1.37 (m)	1.42 (m)	1.50 (m)	1.55 (m)
11a	1.95 (m)	2.09 (m)	1.92 (m)	2.01 (m)	2.05 (m)
11b	1.24 (m)	1.20 (m)	1.52 (m)	1.21 (m)	1.27 (m)
12	3.92 (m)	5.12 (td, 10.9, 5.1)	5.05 (td, 13.1, 5.3)	3.91 (m)	5.00 (td, 10.6, 4.6)
13	2.16 (dd, 11.2, 4.8)	2.40 (dd, 11.7, 5.1)	2.17 (dd, 11.2, 4.8)	3.02 (m)	3.17 (m)
16a	2.55 (d, 20.0)	2.56 (d, 19.5)	2.53 (d, 19.6)	5.95 (dd, 5.6, 2.8)	5.94 (dd, 6.0, 3.0)
16b	2.27 (dd, 20.0, 6.0)	2.25 (dd, 19.5, 6.5)	2.34 (dd, 19.6, 6.8)		
17	4.07 (dd, 6.0, 4.8)	3.96 (t, 5.76)	4.28 (dd, 6.8, 4.8)	7.73 (dd, 5.6, 1.2)	7.45 (dd, 6.0, 1.5)
18	0.83 (s)	0.83 (s)	0.85 (s)	0.87 (s)	0.85 (s)
19	0.93 (s)[a]	0.98 (s)[a]	0.97 (s)	1.05 (s)	1.05 (s)
28	0.93 (s)[a]	0.98 (s)[a]	0.98 (s)	0.98 (s)	0.97 (s)
29	0.73 (s)	0.77 (s)	0.77 (s)	0.78 (s)	0.77 (s)
30	1.19 (s)	1.26 (s)	1.38 (s)	1.18 (s)	1.23 (s)
OAc		2.07 (s)	2.14 (s)		2.10 (s)
OMe	3.30 (s)	3.23 (s)			

[a]Signals overlapped.

The relative configuration of **1** was revealed by the analysis of the ROESY spectrum and the ^1H spectroscopic coupling constants. The coupling constant $J_{2,3}$ (13.0 Hz) revealed H-3 to be axially bonded and established the α-orientation of H-3,[13] which was confirmed by the ROESY correlation of H-3/H-5. The ROESY correlations of H-5/H-9, H-9/H-12, H-12/Me-30, and H-5/Me-28 suggested H-5, H-9, H-12, Me-28, and Me-30 are also α-oriented. In turn, the β-orientations of H-13, Me-18, Me-19, and Me-29 were deduced from the ROESY correlations of H-13/Me-19, Me-18/Me-19, and Me-18/Me-29. The H-17 proton was determined to be axially oriented based on the small coupling constant between H-17 and the axial H-16b [dd, J = 20.0 ($^3J_{H16a-H16b}$), 6.0 ($^3J_{H16b-H17}$) Hz)] and the small coupling constant between H-17 and equatorial H-16a [d, J = 20.0 ($^3J_{H16a-H16b}$) Hz].[13] These observations were verified by the strong ROESY correlation of H-13/H-17. Therefore, the structure of **1** was elucidated as 3β,12β-dihydroxy-17α-methoxy-15-oxo-20,21,22,23,24,25,26,27-octanordammarane and has been named hupehenol A.

The molecular formula of hupehenol B (**2**) was established as $C_{25}H_{40}O_5$ on the basis of its positive HRESIMS ($[M + Na]^+$, m/z 443.2767, calcd 443.2773). The ^1H and ^{13}C NMR spectra of **2** closely resembled those of **1** (Tables 1 and 2). The only difference was that the hydroxy group at C-12 in **1** was replaced by an acetyl group in **2**, as deduced from the correlation of δ_H 2.07 (s, OAc) with δ_C 69.8 (d, C-12) in the HMBC spectrum. The relative configuration of **2** was deduced as being identical to that of **1** according to the ROESY spectrum. Consequently, hupehenol B was determined as 12β-acetoxy-3β-hydroxy-17α-methoxy-15-oxo-20,21,22,23,24,25,26,27-octanordammarane.

Hupehenol C (**3**), a white, amorphous powder, gave a molecular formula of $C_{24}H_{38}O_5$, as inferred from its HRESIMS ($[M + Na]^+$, m/z 429.2620, calcd 429.2616). The ^1H and ^{13}C NMR spectra of **3** were very similar to those of **2** except for the absence of signal for a methoxy group, suggesting **3** to be an analogue of **2** with an OH group substituted at C-17 instead of an OCH_3 group in **2**, which was supported by the upfield chemical shift of C-17 (δ_C 71.2) and by the mass spectrum. Further 2D NMR analysis verified the planar structure of **3**. The ROESY correlation of H-12/H-30 indicated that H-12 is in an α-orientation. Also, the OH moiety at C-17 was assigned as α-oriented due to the ROESY correlation of H-13/H-17 and the coupling constants of H-16a (d, J = 19.6 Hz) and H-16b (dd, J = 19.6, 6.8 Hz). The β-orientation of the hydroxy group at C-3 was inferred from the chemical shift and coupling constants of H-3 (J = 14.5, 5.5 Hz).[13] Therefore, the structure of **3** was elucidated as 12β-acetoxy-3β,17α-dihydroxy-15-oxo-20,21,22,23,24,25,26,27-octanordammarane.

Hupehenol D (**4**) was obtained as a white, amorphous powder. Its molecular formula, $C_{22}H_{34}O_3$, was established on the basis of the HRESIMS ($[M + Na]^+$, m/z 369.2412, calcd 369.2405). The IR spectrum showed the presence of OH (3430 cm^{-1}) and C═O (1700 cm^{-1}) groups. Analysis of the ^1H NMR, ^{13}C NMR, and DEPT data revealed the presence of five methyl groups, five methylene carbons, three methine carbons, four sp^3 quaternary carbons, two oxymethine carbons [δ_C 78.7 (d, C-3); δ_C 67.3 (d, C-12)], one double bond [δ_C 159.4 (d, C-17), 132.2 (d, C-16)], and one carbonyl group [δ_C 212.4 (s, C-15)] in **4** (Tables 1 and 2). Comparison of its spectroscopic data with those of **1** suggested **4** is also a 20,21,22,23,24,25,26,27-octanordammarane triterpenoid. The $^1H-^1H$ COSY correlations indicated the two oxymethine carbons are located at C-3 and C-12, while the double bond could be located between C-16 and C-17. Additionally, the carbonyl group was placed at C-15 as inferred from the HMBC

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Table 2. ^{13}C NMR Data of Hupehenols A–E (1–5) in $CDCl_3$

position	1	2	3	4	5
1	40.3 CH_2	38.8 CH_2	38.8 CH_2	39.0 CH_2	38.8 CH_2
2	28.0 CH_2	27.1 CH_2	27.1 CH_2	27.2 CH_2	27.1 CH_2
3	79.6 CH	78.6 CH	78.5 CH	78.7 CH	78.5 CH
4	40.0 qC	38.9 qC	38.9 qC	38.9 qC	38.8 qC
5	57.1 CH	55.6 CH	55.6 CH	56.0 CH	56.0 CH
6	19.2 CH_2	17.8 CH_2	17.8 CH_2	18.0 CH_2	17.9 CH_2
7	34.6 CH_2	33.1 CH_2	32.8 CH_2	33.3 CH_2	33.4 CH_2
8	41.3 qC	39.8 qC	39.8 qC	39.0 qC	39.0 qC
9	51.8 CH	50.2 CH	50.1 CH	51.0 CH	50.8 CH
10	38.7 qC	37.5 qC	37.4 qC	37.5 qC	37.7 qC
11	32.8 CH_2	28.0 CH_2	27.8 CH_2	33.6 CH_2	29.0 CH_2
12	67.1 CH	69.8 CH	65.3 CH	67.3 CH	70.5 CH
13	50.1 CH	48.0 CH	49.8 CH	53.8 CH	50.6 CH
14	57.8 qC	56.3 qC	56.3 qC	59.5 qC	59.7 qC
15	222.4 qC	218.6 qC	218.5 qC	212.4 qC	211.4 qC
16	45.4 CH_2	44.1 CH_2	46.4 CH_2	132.2 CH	132.2 CH
17	76.5 CH	74.9 CH	71.2 CH	159.4 CH	158.6 CH
18	19.8 CH_3	15.9 CH_3	15.8 CH_3	16.0 CH_3	15.9 CH_3
19	17.0 CH_3	18.7 CH_3	18.8 CH_3	19.7 CH_3	20.4 CH_3
28	29.1 CH_3	28.0 CH_3	28.0 CH_3	28.0 CH_3	28.0 CH_3
29	16.5 CH_3	15.3 CH_3	15.4 CH_3	15.3 CH_3	15.2 CH_3
30	16.2 CH_3	14.9 CH_3	15.0 CH_3	20.8 CH_3	19.7 CH_3
OAc		170.3 qC	173.1 qC		170.8 qC
		21.2 CH_3	21.3 CH_3		21.2 CH_3
OMe	58.2 CH_3	57.6 CH_3			

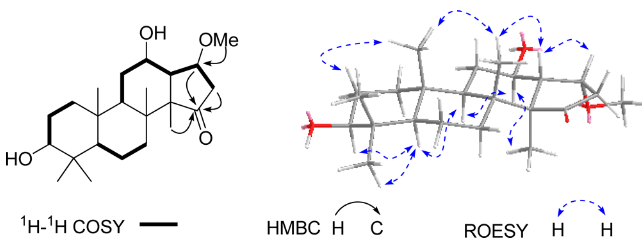

Figure 1. Selected 2D NMR correlations for compound 1.

cross-peaks of H-16 (δ_H 5.95, dd, J = 5.6, 2.8 Hz), H-17 (δ_H 7.73, dd, J = 5.6, 1.2 Hz), and Me-30 (δ_H 1.18, s) with the ketone carbon signal (δ_C 212.4). Further HMBC correlations established the planar structure as shown. The relative configuration of **4** was inferred from a ROESY experiment, in which a correlation between H-12 and Me-30 suggested the C-12 OH to be β-oriented. Also, similarities of the coupling constants and the chemical shift of H-3 to those of **1** implied that OH-3 is β-oriented.[13] Thus, the structure of hupehenol D was elucidated as 3β,12β-dihydroxy-15-oxo-16-en-20,21,22,23,24,25,26,27-octanordammarane.

The HRESIMS of hupehenol E (**5**) exhibited a quasimolecular ion peak at m/z 411.2520 $[M + Na]^+$, suggesting a molecular formula of $C_{24}H_{36}O_4$, with seven degrees of unsaturation. Detailed comparison of the NMR data of **5** with those of **4** indicated these compounds to be structural analogues (Tables 1 and 2). The only differences were that the hydroxy group at C-12 in **4** is replaced by an acetoxy group in **5**, as deduced from the HMBC correlation of H-12 (δ_H 5.00, td, J = 10.6, 4.6 Hz) with the carboxyl carbon (δ_C 170.8, s). The relative configuration of **5** was determined to be consistent with that of **4** on the basis of analysis of the ROESY spectrum. Hence, the structure of hupehenol E was established as 12β-acetoxy-3β-hydroxy-15-oxo-16-en-20,21,22,23,24,25,26,27-octanordammarane.

It has been reported that glycyrrhetinic acid (GA) and a variety of endogenous steroids possess 11β-HSD1 inhibitory properties.[14] However, they also inhibit 11β-HSD2 and hence show nonselectivity in this regard. As **1**–**5** share a similar core skeleton to the steroids, their potential activities to inhibit human and murine 11β-HSD1 were examined. The results showed that hupehenols A–E (**1**–**5**) all inhibit human 11β-

Table 3. Inhibitory Effects of Hupehenols A–E (1–5) on Human and Murine 11β-HSD

	human (IC_{50})			mouse (IC_{50})		
compound	11β-HSD1	11β-HSD2	HSD2/HSD1	11β-HSD1	11β-HSD2	HSD2/HSD1
1	194.5 nM	25.5 μM	131.1	2.8 μM	291.6 μM	104.5
2	15.3 nM	40.7 μM	2660.6	3.3 μM	155.4 μM	47.5
3	75.5 nM	299.6 μM	3967.0	4.7 μM	>1.0 mM	>213.7
4	395.2 nM	283.6 μM	717.7	2.9 μM	>1.0 mM	>340.1
5	34.1 nM	11.3 μM	330.4	7.2 μM	216.6 μM	29.9
GA[a]	29.1 nM	1.2 nM	0.1	40.2 nM	ND[b]	ND[b]

[a]GA: glycyrrhetinic acid, which was used as a positive control. [b]ND: not determined.

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HSD1, with hupehenols B (**2**) and E (**5**) having IC_{50} values of 15.3 and 34.0 nM, respectively. Hupehenols C (**3**) and D (**4**) were highly selective inhibitors for human 11β-HSD1 when compared to murine 11β-HSD1 (Table 3).

As obesity and associated metabolic syndrome disorders are becoming some of the greatest health threats to modern society, the development of selective 11β-HSD1 inhibitors has become an interest.[15] Up to now, a number of potent and selective 11β-HSD1 inhibitors were reported, some of which are progressing to clinical trial.[16] However, most of the inhibitors are synthetic chemicals.[17] Naturally derived inhibitors of this type are still scarce.[6] To the best of our knowledge, this is the first report of such highly selective 11β-HSD1 inhibitors as natural products.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO-20C digital polarimeter. IR spectra were obtained on a Tenor 27 spectrometer with KBr pellets. ^{1}H and ^{13}C NMR spectra were performed on a Bruker AM-400 or DRX-500 spectrometer with TMS as internal standard. EIMS and HRESIMS were taken on a VG Auto Spec-3000 mass spectrometer. Column chromatography was performed using silica gel (200−300 mesh, Qingdao Marine Chemical Co., Ltd., Qingdao, People's Republic of China) or silica gel H (10−40 μm, Qingdao Marine Chemical Co. Ltd.), Lichroprep RP-18 gel (40−63 μm, Merck, Darmstadt, Germany), and Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden). All solvents were distilled before use.

Plant Material. The aerial parts of *V. hupehense* (7.5 kg) were collected at Caojian, Yunnan Province, People's Republic of China, in July 2006. The sample was identified by one of the authors (X. Cheng). A voucher specimen (20060715) was deposited at Kunming Institute of Botany.

Extraction and Isolation. The dried and powdered aerial parts of *V. hupehense* (7.5 kg) were extracted with 70% aqueous acetone (3 × 35 L) for 24 h each time at room temperature. The extract was evaporated to dryness under reduced pressure to afford a residue (600 g). The residue was suspended in H_2O (1000 mL) and then partitioned with ethyl acetate to afford a dried ethyl acetate extract (180 g). This extract was subjected to silica gel column chromatography eluted with gradient mixtures of petroleum ether−acetone (1:0 → 0:1), to give six fractions, F_1−F_6. Fraction F_3 (20.3 g) was further purified over silica gel eluted with petroleum ether−acetone (9:1) to provide five subfractions ($F_{3.1}$−$F_{3.5}$). Subfraction $F_{3.1}$ (1.8 g) was separated over silica gel eluted with petroleum ether−acetone (10:1) then recrystallized to afford 2α,3β-dihydroxy-12-ursen-28-oic acid.[9] Subfraction $F_{3.2}$ (4.1 g) was subjected to passage over Sephadex LH-20 ($CHCl_3$−MeOH, 1:1) and then further chromatographed on a silica gel column using $CHCl_3$−MeOH (150:1) as eluent to yield **2** (150 mg) and **3** (32 mg). Fraction F_4 (11.7 g) was applied to a silica gel column that was eluted with petroleum ether−acetone (8:2) to yield five other subfractions, $F_{4.1}$−$F_{4.5}$. Subfraction $F_{4.3}$ (2.8 g) was further purified by elution over Sephadex LH-20 with $CHCl_3$−MeOH (1:1), followed by chromatography over silica gel using $CHCl_3$−MeOH (100:1) as solvent, to afford **1** (10 mg), **4** (11 mg), and **5** (120 mg). Fraction F_5 (10.7 g) was subjected to separation over RP-18, eluted with MeOH−H_2O (1:2 → 1:0), to provide five additional subfractions ($F_{5.1}$−$F_{5.5}$). Subfraction $F_{5.4}$ (1.6 g, eluted from 1:1) was purified over silica gel eluted with $CHCl_3$−MeOH (100:1) to yield lupeol[10] (20 mg) and serratagenic acid[11] (45 mg).

*Hupehenol A (**1**):* white, amorphous powder; $[\alpha]^{24}_D$ +19 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3431, 2944, 2876, 1732, 1628, 1462, 1378, 1033 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 378 [M]$^+$; HRESIMS *m/z* 401.2671 [M + Na]$^+$ (calcd for $C_{23}H_{38}O_4Na$, 401.2667).

*Hupehenol B (**2**):* white, amorphous powder; $[\alpha]^{24}_D$ +21 (*c* 0.15, MeOH); IR (KBr) ν_{max} 3538, 2965, 2924, 1735, 1712, 1264, 1035 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 420 [M]$^+$; HRESIMS *m/z* 443.2767 [M + Na]$^+$ (calcd for $C_{25}H_{40}O_5Na$, 443.2773).

*Hupehenol C (**3**):* white, amorphous powder; $[\alpha]^{24}_D$ +9 (*c* 0.16, MeOH); IR (KBr) ν_{max} 3451, 2945, 2876, 1735, 1629, 1376, 1276, 1033 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 406 [M]$^+$; HRESIMS *m/z* 429.2620 [M + Na]$^+$ (calcd for $C_{24}H_{38}O_5Na$, 429.2616).

*Hupehenol D (**4**):* white, amorphous powder; $[\alpha]^{24}_D$ −191 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3430, 2942, 2873, 1700, 1639, 1461, 1389, 1094, 1027 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 346 [M]$^+$; HRESIMS *m/z* 369.2412 [M + Na]$^+$ (calcd for $C_{22}H_{34}O_3Na$, 369.2405).

*Hupehenol E (**5**):* white, amorphous powder; $[\alpha]^{24}_D$ −103 (*c* 0.16, MeOH); IR (KBr) ν_{max} 3439, 2942, 2874, 1736, 1709, 1636, 1456, 1371, 1240, 1031 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 388 [M]$^+$; HRESIMS *m/z* 411.2520 [M + Na]$^+$ (calcd for $C_{24}H_{36}O_4Na$, 411.2511).

11β-HSD1 and 11β-HSD2 Inhibition Assays. Inhibition of compounds on human or mouse 11β-HSD1 and 11β-HSD2 enzymatic activities was determined by a scintillation proximity assay (SPA) using microsomes containing 11β-HSD1 or 11β-HSD2, according to a previous report.[18] Briefly, the full-length cDNAs of human or murine 11β-HSD1 and 11β-HSD2, obtained from cDNA libraries (NIH Mammalian Gene Collection), were cloned into the pcDNA3 expression vector. HEK-293 cells were transfected with the pcDNA3-derived expression plasmid and selected by cultivation in the presence of G418 (700 μg/mL). The microsomal fraction overexpressing 11β-HSD1 or 11β-HSD2, which was prepared from the HEK-293 cells stably transfected with either 11β-HSD1 or 11β-HSD2, was used as the enzyme source for the SPA. Microsomes containing human or mouse 11β-HSD1 were incubated with NADPH, [^{3}H] cortisone, and each compound. The product, [^{3}H] cortisol, was specifically captured by a monoclonal antibody coupled to protein A-coated SPA beads. The 11β-HSD2 screening was performed by incubating 11β-HSD2 microsomes with [^{3}H] cortisol, NAD$^+$, and each compound. 18β-Glycyrrhetinic acid was used as positive control. IC_{50} values were calculated by using GraphPad Prism software (version 4).

■ ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR spectroscopic data of hupehenols A−E (**1**−**5**) are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on the Web on January 15, 2015, with errors in the graphics for compound **5**. The corrected version was reposted on February 6, 2015.