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PAPER

Dammarane-type Triterpenoids as 11β-HSD1 Inhibitors from *Homonoia riparia*†

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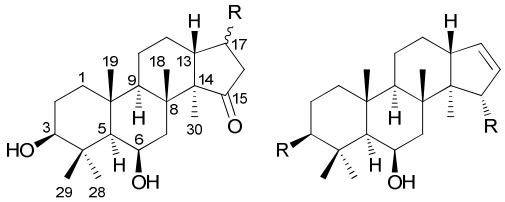
An exploration for 11β-HSD1 inhibitors from *Homonoia riparia* returned eight new dammarane-type triterpenoids, horipenoids A–H (**1**–**8**), and a known oleanane-type triterpenoid (**9**). Their structures were elucidated on the basis of comprehensive analysis of spectroscopic data, and the absolute configuration of horipenoid E (**5**) was established by single crystal X-ray crystallography. Compounds **1**–**4** represent a rare class of octanortriterpenoids. Horipenoids C (**3**) and E (**5**) showed potent inhibition against mouse 11β-HSD1 with IC_{50} values of 0.810 ± 0.058 and 0.898 ± 0.215 μM, respectively.

Introduction

Homonoia (Euphorbiaceae) is a small genus of shrub or arbor mainly distributed in the south and southwest of Asia.[1] *H. riparia* is the only species that grows in the south of China, such as Hainan, Guangdong and Guangxi Provinces.[1] The roots of *H. riparia* have been used in Chinese folk medicine to treat hepatitis, diarrhea, bellyache and scald.[2] Previous studies of this plant resulted in the isolation of fatty acids, flavone glycosides, steroids and triterpenoids,[3-6] some of which exhibited cytotoxic activity[3] and inhibitory effect on vascular permeability[4]. Recently, 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) has emerged as a promising target for the treatment of metabolic diseases such as abdominal adiposity, insulin resistance and hyperglycemia dyslipidemia.[7-9] Natural products have been demonstrated as an important source of 11β-HSD1 inhibitors.[10-12] In our continuing efforts for 11β-HSD1 inhibitors from medicinal plants,[13] eight new dammarane-type triterpenoids, horipenoids A–H (**1**–**8**), along with a known oleanane-type analogue, 2α,3β-dihydroxyl-oleana-12-en-28-oic acid (**9**)[14,15], were isolated from the ethanolic extract of *H. riparia*. Compounds **1**–**4** are a rare class of octanordammaranes. Their structures were assigned on the basis of spectroscopic data, and that of **5** was confirmed by single crystal X-ray diffraction. All compounds except **2** were assessed for the inhibitory effects against both human and mouse 11β-HSD1. Most of the tested compounds showed moderate inhibition against the two types of enzymes, and horipenoids C (**3**) and E (**5**) exhibited strong activities against mouse 11β-HSD1 with IC_{50} values of 0.810 ± 0.058 and 0.898 ± 0.215 μM, respectively. This paper describes the isolation and structure elucidation of these triterpenoids as well as their biological evaluations.

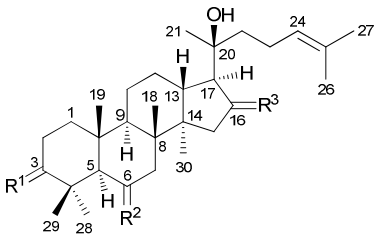
Results and discussion

Compound **1** was obtained as a white amorphous powder. HR-ESI(−)MS analysis showed a *quasi*-molecular ion at *m/z*



1 R = α-OH
2 R = β-OH

3 R = OH
4 R = OAc



	R^1	R^2	R^3
5	H, β-OH	H, α-OH	H, β-OH
6	O	H, α-OH	H, β-OH
7	H, β-OH	O	H, β-OH
8	H, β-OH	H, α-OH	O

363.2528 $[M - H]^-$ (calcd 363.2535), corresponding to a molecular formula of $C_{22}H_{36}O_4$ with five indices of hydrogen deficiency. The IR spectrum revealed the presence of hydroxy (3546, 3475 and 3438 cm^{-1}) and carbonyl (1722 cm^{-1}) functionalities. The ^{13}C NMR spectrum with DEPT data exhibited 22 resonances including five methyl, six methylene, six methine (three oxygenated) and five quaternary carbons (one carbonyl). The only carbonyl accounted for one of five indices of hydrogen deficiency, and the remaining four required **1** to be tetracyclic. Comprehensive analysis of 1D and 2D NMR data allowed the establishment of the planar structure of **1** as an

octanordammarane triterpenoid without the C-17 side chain. The ^{1}H-^{1}H COSY data (Fig. 1A) enabled the construction of three proton-bearing fragments, **a** (C-1 to C-3), **b** (C-5, C-6(OH) and C-7) and **c** (C-9, C-11 to C-13, C-17 and C-16) as drawn in bold bonds. The **a**, **b** and **c** fragments were connected through four quaternary sp^3 carbons and one carbonyl via analysis of HMBC data (Fig. 1A), where the correlations from H_3-28/H_3-29 to C-3, C-4 and C-5; H_3-19 to C-1, C-5, C-9 and C-10; H_3-18 to C-7, C-8, C-9 and C-14; H_3-30 to C-8, C-13, C-14 and C-15, and H-17 to C-15 were evidenced.

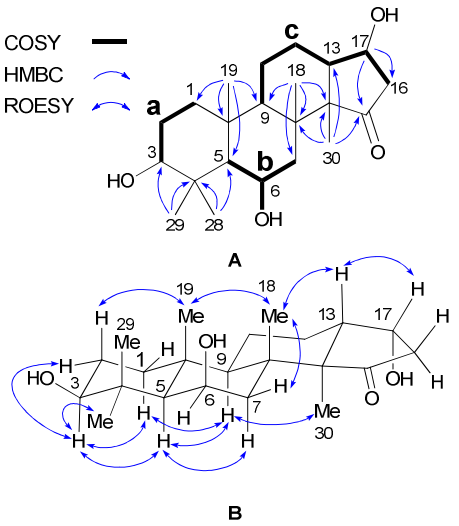


Fig. 1 Key ^{1}H-^{1}H COSY, HMBC (**A**) and ROESY (**B**) correlations for **1**.

The relative configuration of **1** was established by proton couplings and analysis of ROESY spectrum (Fig. 1B). The coupling constant of $J_{2,3}$ (11.6 Hz) revealed that H-3 was axially bonded, and was assigned to be α-oriented randomly. Subsequently, H-1α, H-2α, H-5, H-7α, H-9 and H_3-30 were fixed in an α-orientation by the ROESY interactions of H-3 with H-1α, H-2α, H-5; H-5 with H-7α and H-9; and H-9 with H_3-30. The small value of $J_{5,6}$ (2.6 Hz) indicated that H-6 was equatorially located and thus β-oriented. The ROESY correlations of H-2β/H_3-19, H_3-19/H_3-18 and H_3-18/H-13 revealed that H-2β, H_3-18, H_3-19 and H-13 were β-oriented. H-17 was assigned to be β-oriented by its strong ROESY correlation with H-13. The structure of **1** was thus characterized as shown.

Compound **2** was obtained as a white amorphous powder. The HR-EIMS ion at *m/z* 346.2512 $[M - H_2O]^+$ (calcd 346.2508) and NMR data assigned a molecular formula of $C_{22}H_{36}O_4$ to **2**, which was supported by the molecular ion peak at *m/z* 364 (12%) in the LR-EIMS spectrum. Comprehensive analysis of the NMR data (Table 1) of **2** revealed that it also featured an octanordarmmarane skeleton with the C-17 side chain absent. Further comparison of its NMR data (Table 1), particularly the coupling constants, with those of **1** revealed that it was likely a stereoisomer of **1** with a reversed C-17 configuration. This was confirmed by the analysis of its 2D NMR data (see ESI Figures S12–S14), especially the ROESY spectrum, in which the key correlation of H_3-30/H-17 was observed.

Compound **3**, a white amorphous powder, had a molecular formula of $C_{22}H_{36}O_3$ as determined by the HR-ESI(–)MS ion peak at *m/z* 393.2635 $[M + HCO_2]^-$ (calcd 393.2641). The IR absorption bands at 3382 cm^{-1} and 1641 cm^{-1} revealed the presence of hydroxy and olefinic functionalities, respectively. Analysis of its 1D NMR data (Table 1) revealed that compound **3** possessed the same octanordarmmarane backbone as **1**. The main differences occurred at the D-ring, where an oxygenated methine at δ_C 79.0 was assigned to be CH-15 bearing a hydroxy group by the key HMBC correlation of H_3-30/C-15 (see ESI Figure S22); and a disubstituted Δ^{16} double bond was assigned on the basis of correlations of H-13/H-17, H-15/H-16, and H-16/H-17 in the ^{1}H-^{1}H COSY spectrum (see ESI Figure S20). The 15-OH was assigned to be β-oriented by the ROESY cross-peak between H-13 and H-15 (see ESI Figure 23).

Compound **4** was obtained as a white amorphous powder. The molecular formula of $C_{26}H_{40}O_5$ was established by the HR-ESI(–)MS ion peak at *m/z* 477.2853 $[M + HCO_2]^-$ (calcd 477.2852). The NMR data (Table 1) of **4** showed high similarities to those of **3**, except for the presence of two additional acetyl groups (both resonated at δ_H 2.06), and meanwhile H-3 and H-15 in the ^{1}H NMR data were downfield shifted by $\Delta\delta_H$ 1.31 and 1.00 ppm respectively, indicating that two acetyloxy groups were attached to C-3 and C-15, respectively. Compound **4** was thus assigned to be the 3,15-*O*-diacetylated derivative of **3**. This conclusion was corroborated by the HMBC correlations from H-3 (δ_H 4.43) and H-15 (δ_H 5.92) to the corresponding acetyl carbonyls.

Compound **5**, colorless crystals from methanol (mp 149–150°C), had a molecular formula of $C_{30}H_{52}O_4$ as determined by HR-ESI(–)MS experiment at *m/z* 521.3843 $[M + HCO_2]^-$ (calcd 521.3842), corresponding to five indices of hydrogen deficiency. The IR absorption bands showed the presence of hydroxy (3489 cm^{-1}) and olefinic (1656 cm^{-1}) functionalities. The ^{13}C NMR spectrum exhibited 30 carbons that were classified via the aid of DEPT experiment as eight methyl, eight methylene, eight methine (three oxygenated and one olefinic) and six quaternary carbons (one oxygenated and one olefinic). Two methyls at δ_H 1.66 and 1.71, together with two carbon resonances at δ_C 126.4 (methine) and δ_C 130.1 (quaternary carbon), suggested the presence of a terminal –CH=C(CH$_3$)$_2$ group. This accounted for one of five indices of hydrogen deficiency, and the remaining four required the presence of four rings in **5**. Comprehensive analysis of the ^{1}H-^{1}H COSY and HMBC data (Fig. 2A) delineated the planar structure of **5** as drawn with a dammarane skeleton. In details, the ^{1}H-^{1}H COSY data revealed four proton-bearing fragments (**a**–**d**) as drawn with bold bonds (Fig. 2A), which were connected through quaternary carbons by the HMBC correlations from H_3-28/H_3-29 to C-3, C4 and C-5; H_3-19 to C-1, C-5, C-9 and C-10; H_3-18 to C-7, C-8, C-9 and C-14; H_3-30 to C-8, C-13, C-14 and C-15; H_3-21 to C-17, C-20 and C-22; and H_3-26/H_3-27 to C-24 and C-25. The existence of 3-OH, 6-OH, 16-OH, and 20-OH were assigned on the basis of the elemental composition, and the chemical shifts of the corresponding carbons C-3 (δ_C 78.7), C-6(δ_C 67.9), C-16 (δ_C 74.2) and C-20 (δ_C 75.1).

The relative configuration of **5** was mainly established by analysis of the ROESY spectrum (Fig. 2B). The β-orientation of 3-OH was assigned by the chemical shift and coupling pattern of H-3 at δ_H 3.57 (dd, 11.6, 4.9 Hz). This was followed by the establishment of an α-orientation for H-1α, H-5, H-7α, H-9, H_3-30, H-12α, H-16 and H-17 based on the ROESY correlation network of H-3/H-1α and H-5, H-5/H-7α, H-7α/H-9, H-9/H_3-30, and H_3-30/H-12α, H-16 and H-17. The large coupling constant of $J_{5,6}$ (10.2 Hz) indicated that H-6 was axially bonded and β-oriented. Subsequently, the ROESY correlations of H-6/H_3-18 and H_3-29, H_3-29/H_3-19, H_3-19/H_3-

Table 1. ^{1}H and ^{13}C NMR data of compounds **1**–**4**.

	1[a]		**2**[b]		**3**[c]		**4**[c]	
No.	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C
1α	1.08 (*td*, 13.1, 3.8)	42.0	0.99 (*m*)	40.8	0.94 (*m*)	41.0	1.06 (*m*)	40.7
1β	1.72 (*m*)		1.69 (*m*)		1.66 (*m*)		1.66 (*m*)	
2	α 1.96 (*m*)	28.9	α 1.58 (*dt*, 13.6, 4.1)	26.8	1.64 (*m*)	27.4	1.73 (*m*)	23.9
	β 2.11 (*m*)		β 1.70 (*m*)		1.65 (*m*)		1.74 (*m*)	
3	3.51 (*dd*, 11.6, 4.2)	78.9	3.07 (*dd*, 11.7, 4.2)	78.5	3.13 (*m*)	79.0	4.43 (*dd*, 11.6, 4.4)	80.8
4		41.0		39.5		39.7		38.8
5	1.01 (*d*, 2.6)	57.2	0.74 (*d*, 2.6)	56 .0	0.73 (*d*, 2.5)	56.2	0.84 (*d*, 2.3)	56.3
6	4.90 (*brs*)	68.1	4.45 (*q*, 3.1)	67.2	4.51 (*td*, 4.0, 2.5)	68.7	4.49 (*m*)	68.6
7α	2.05 (*m*)	42.0	1.66 (*m*)	41.0	1.72 (*m*)	43.9	1.43 (*m*)	43.4
7β	2.97 (*dd*, 14.4, 2.8)		2.32 (*dd*, 11.5, 3.1)		1.85 (*dd*, 14.5, 3.8)		1.80 (*dd*, 14.5, 4.0)	
8		40.7		39.2		39.5		39.6
9	1.52 (*m*)	52.9	1.26 (*m*)	51.7	1.45 (*m*)	51.6	1.47 (*m*)	51.5
10		38.0		36.8		36.9		36.9
11	1.82 (*m*)	22.3	1.74 (*m*)	20.9	α 1.62 (*m*)	21.8	α 1.64 (*m*)	21.7
	1.53 (*m*)		1.42 (*m*)		β 1.34 (*qd*, 12.5, 4.3)		β 1.37 (*m*)	
12	α 1.87 (*m*)	23.4	α 1.18 (*m*)	22.1	1.69 (*m*)	24.3	1.66 (*m*)	23.9
	β 2.02 (*m*)		β 1.99 (*dq*, 8.8, 3.3)		1.41 (*m*)		1.50 (*m*)	
13	2.42 (*ddd*, 13.0, 5.0, 3.0)	44.7	2.18 (*ddd*, 12.8, 9.9, 3.6)	47.0	2.68 (*m*)	45.0	2.75 (*m*)	44.8
14		56.4		58.3		58.9		58.3
15		221.6		218.5	4.92 (*td*, 3.3, 1.8)	79.0	5.92 (*td*, 3.3, 1.6)	80.3
16	α 2.76 (*d*, 18.9)	49.6	α 2.72 (*dd*, 18.9, 7.4)	46.7	5.66 (*dt*, 5.8, 1.5)	134.8	5.76 (*dt*, 5.9, 2.0)	130.2
	β 2.61 (*dd*, 18.9, 6.6)		β 1.90 (*dd*, 18.9, 7.3)					
17	4.65 (*dd*, 6.6, 5.0)	68.7	4.04 (*ddd*, 9.9, 7.4, 7.3)	69.1	5.49 (*ddd*, 5.8, 3.3, 1.5)	133.5	5.48 (*ddd*, 5.9, 3.3, 1.5)	135.9
18	1.75 (*s*, 3H)	19.6	1.39 (*s*, 3H)	17.8	1.43 (*s*, 3H)	19.1	1.45 (*s*, 3H)	19.0
19	1.55 (*s*, 3H)	18.2	1.19 (*s*, 3H)	16.5	1.20 (*s*, 3H)	16.8	1.24 (*s*, 3H)	17.7
28	1.45 (*s*, 3H)	28.6	1.03 (*s*, 3H)	26.6	1.06 (*s*, 3H)	27.4	0.94 (*s*, 3H)	27.5
29	1.78 (*s*, 3H)	18.3	1.15 (*s*, 3H)	16.0	1.15 (*s*, 3H)	17.7	1.24 (*s*, 3H)	18.1
30	1.67 (*s*, 3H)	15.5	0.92 (*s*, 3H)	11.2	0.97 (*s*, 3H)	9.8	1.04 (*s*, 3H)	11.2
6-OH	5.90 (*brs*)							
OAc-3							2.06 (*s*, 3H)	21.5
								171.0
OAc-15							2.06 (*s*, 3H)	21.4
								171.0

[a] Measured in pyridine-d_5; [b] Measured in methanol-d_4; [c] Measured in $CDCl_3$.

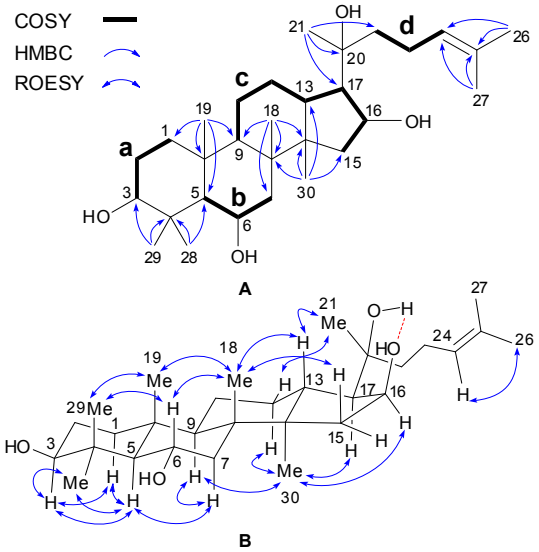


Fig. 2 Key ^{1}H-^{1}H COSY, HMBC (**A**), and ROESY (**B**) correlations for **5**.

18, and H_3-18/H-13 and H-15β revealed that H_3-29, H_3-19, H_3-18, H-13 and H-15β were β-oriented. In addition, the correlation between H-24 and H_3-26 helped to distinguish C-26 (δ_C 26.2) and C-27 (18.1). The establishment of the C-20 configuration proved challenging because of the likely rotatory nature of the side chain. Previous assignments in similar structures based on CD and NMR data did not seem convincing

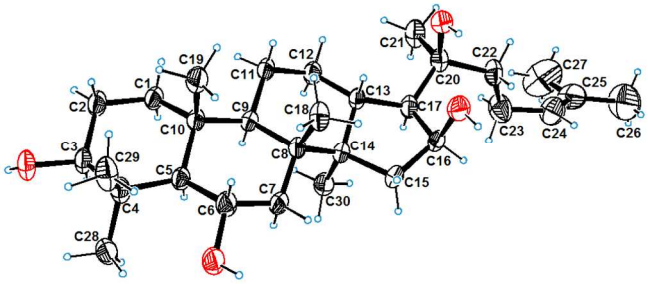


Fig. 3 Single-crystal X-ray structure of **5**.

due to the improper selection of model compounds.[16,17] In order to unambiguously assign the C-20 configuration of **5**, we finally resorted to single crystal X-ray crystallography (Fig. 3) which established the absolute configuration of **5** as 3*S*, 5*R*, 6*S*, 8*R*, 9*R*, 10*R*, 13*R*, 14*R*, 16*S*, 17*R*, 20*S* [absolute structure parameter –0.08(13)].[18] The X-ray data also showed that the H-bonding between 16-OH and 20-OH allowed the formation of a *pseudo* six-membered ring in a chair-like conformation, which was in agreement with the ROESY correlations observed for H_3-21 with both H-12β and H-13. Thus compound **5** was identified and named horipenoid E.

Compound **6**, a white amorphous powder, possessed a molecular formula of $C_{30}H_{50}O_4$ as evidenced by the HR-ESI(–)MS experiment displaying a *quasi*-molecular ion at *m/z* 519.3687 $[M + HCO_2]^-$ (calcd 519.3686). Comparison of the NMR data (Table 2) with those of **5** revealed that they were structural analogues. The main differences were the chemical shifts of protons and carbons in the A-ring, where the

Table 2. ^{1}H and ^{13}C NMR data of compounds **5**–**8** in pyridine-d_5.

	5		**6**		**7**		**8**	
Position	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C	δ_H (multi, *J* in Hz)	δ_C
1α	1.07 (*td*, 13.1, 4.1)	39.8	1.60 (*m*)	40.3	1.28 (*td*, 13.2, 4.5)	40.6	1.05 (*td*, 13.0, 4.2)	39.6
1β	1.68 (*m*)		1.74 (*m*)		1.73 (*m*)		1.66 (*m*)	
2	1.94 (*m*)	28.5	α 2.35 (*ddd*, 14.0, 9.8, 2.9)	33.8	α 1.88 (*m*)	28.5	1.95 (*m*)	28.6
	1.93 (*m*)		β 2.86 (*ddd*, 14.0, 12.1, 6.6)		β 1.92 (*m*)		1.94 (*m*)	
3	3.57 (*dd*, 11.6, 4.9)	78.7		219.1	3.44 (*brd*, 10.4)	78.1	3.58 (*dd*, 11.4, 5.1)	78.8
4		40.7		48.1		38.8		40.8
5	1.26 (*d*, 10.2)	62.1	1.94 (*d*, 10.6)	59.4	2.39 (*s*)	66.3	1.25 (*m*)	62.2
6	4.42 (*td*, 10.5, 3.8)	67.9	4.21 (*td*, 10.4, 4.1)	67.1		212.3	4.41 (*td*, 10.5, 3.7)	67.9
7α	1.99 (*m*)	48.3	1.91 (*m*)	46.1	2.74 (*d*, 11.3)	53.7	1.93 (*m*)	48.4
7β	1.92 (*dd*, 12.1, 3.8)		1.88 (*dd*, 12.3, 4.1)		1.99 (*d*, 11.3)		1.80 (*dd*, 12.2, 3.7)	
8		41.9		41.2		47.3		40.8
9	1.53 (*dd*, 12.3, 3.0)	50.6	1.58 (*dd*, 12.9, 3.3)	49.4	2.04 (*m*)	51.1	1.60 (*dd*, 12.6, 2.9)	50.9
10		39.7		38.6		44.5		39.9
11α	1.60 (*m*)	22.0	1.49 (*brd*, 12.4)	22.8	1.63 (*m*)	22.6	1.62 (*m*)	22.0
11β	1.31 (*td*, 12.8, 4.6)		1.26 (*td*, 12.9, 6.2)		1.34 (*dt*, 13.2, 5.0)		1.31 (*m*)	
12α	1.42 (*qd*, 12.6, 4.2)	27.7	1.39 (*qd*, 12.6, 3.9)	27.8	1.47 (*qd*, 12.7, 4.4)	27.4	1.56 (*qd*, 12.8, 4.1)	28.0
12β	2.04 (*m*)		2.02 (*m*)		2.09 (*m*)		2.13 (*brd*, 13.9)	
13	2.46 (*m*)	41.0	2.44 (*m*)	41.2	2.44 (*m*)	41.3	2.56 (*td*, 11.8, 3.5)	41.2
14		48.3		48.1		48.4		45.8
15α	1.81 (*dd*, 12.7, 7.1)	44.6	1.82 (*dd*, 12.7, 7.0)	44.6	1.72 (*m*)	44.5	1.97 (*d*, 15.8)	50.5
15β	1.97 (*m*)		1.92 (*m*)		1.88 (*dd*, 12.2, 4.9)		2.49 (*d*, 15.8)	
16	4.87 (*q*, 6.2)	74.2	4.88 (*q*, 6.5)	74.2	4.86 (*m*)	73.9		220.6
17	2.07 (*dd*, 11.8, 7.1)	52.2	2.10 (*dd*, 12.4, 7.2)	52.1	2.07 (*m*)	52.5	2.34 (*d*, 10.6)	58.5
18	1.23 (*s*, 3H)	17.8	1.15 (*s*, 3H)	16.8	1.15 (*s*, 3H)	16.7	1.16 (*s*, 3H)	18.0
19	1.00 (*s*, 3H)	18.8	0.81 (*s*, 3H)	18.1	0.95 (*s*, 3H)	17.6	1.01 (*s*, 3H)	17.9
20		75.1		75.2		75.1		74.6
21	1.62 (*s*, 3H)	27.0	1.62 (*s*, 3H)	27.1	1.62 (*s*, 3H)	27.1	1.50 (*s*, 3H)	27.0
22	2.04 (*m*)	44.3	2.05 (*m*)	44.3	2.07 (*m*)	44.2	2.01 (*m*)	41.3
	2.03 (*m*)		2.04 (*m*)		2.06 (*m*)		1.97 (*m*)	
23	2.48 (*m*)	23.6	2.48 (*m*)	23.6	2.48 (*m*)	23.6	2.43 (*m*)	23.6
	2.41 (*m*)		2.41 (*m*)		2.42 (*m*)		2.34 (*m*)	
24	5.34 (*brt*, 7.0)	126.4	5.34 (*brt*, 6.6)	126.4	5.35 (*brt*, 6.9)	126.4	5.32 (*brt*, 6.8)	125.9
25		131.1		131.2		131.2		131.5
26	1.71 (*brs*, 3H)	26,.2	1.71 (*brs*, 3H)	26.2	1.71 (*brs*, 3H)	26.2	1.69 (*brs*, 3H)	26.2
27	1.66 (*brs*, 3H)	18.1	1.66 (*brs*, 3H)	18.1	1.67 (*brs*, 3H)	18.1	1.66 (*brs*, 3H)	18.1
28	2.02 (*s*, 3H)	32.4	1.70 (*s*, 3H)	32.5	1.44 (*s*, 3H)	28.5	2.02 (*s*, 3H)	32.4
29	1.49 (*s*, 3H)	16.9	1.72 (*s*, 3H)	20.4	1.15 (*s*, 3H)	16.6	1.48 (*s*, 3H)	16.9
30	1.01 (*s*, 3H)	18.2	1.01 (*s*, 3H)	18.7	1.06 (*s*, 3H)	18.9	1.00 (*s*, 3H)	17.5
20-OH					5.69 (*s*, 3H)			

hydroxymethine (δ_H 3.57, δ_C 78.7, CH-3) in **5** was replaced by a keto carbonyl (δ_C 219.1) in **6**. This was further supported by a strong absorption band at 1691 cm^{-1} in the IR spectrum.Compound **6** was thus identified to be an oxidative derivative of **5**, which was also confirmed by 2D NMR data (see ESI Figures S47–S49).

Compound **7**, a white amorphous power, showed a molecular formula of $C_{30}H_{50}O_4$ as determined by HR-ESI(–)MS at *m/z* 519.3689 $[M + HCO_2]^-$ (calcd 519.3686). Comparison of the NMR data (Table 2) of **7** with those of **5** revealed that the structures of these two compounds were closely related, with the only difference being the oxidative patterns at C-6. A carbonyl (δ_C 212.3, C-6) and a singlet proton (H-5, δ_H 2.39) signals were observed in **7** instead of those of an oxymethine (δ_H 3.57, δ_C 78.7, CH-6) and a doublet proton (H-5, δ_H 1.26) in **5**, indicating the presence of a keto group at C-6 in compound **7**. Comprehensive analysis of its 2D NMR spectra (see ESI Figures S56–S58) confirmed the structure of **7** as shown.

Compound **8**, a white amorphous powder, was assigned a molecular formula of $C_{30}H_{50}O_4$ based on the HR-ESI(–)MS ion peak at *m/z* 519.3679 $[M + CO_2H]^-$ (calcd 519.3686). Analysis of the NMR data (Table 2) showed that it was a regio-isomer of **6** and **7**, being also an oxidative analogue of **5.** The presence of a carbonyl resonance (δ_C 220.6, C-16) in **8** and the absence of proton and carbon signals for CH-16 (δ_H 4.87, δ_C 74.2) in **5**, allowed the location of a keto group at C-16 in compound **8**. The structure of **8** was further confirmed by 2D NMR spectra (see ESI S65–S67).

Preliminary testing of the pure entities at 10 μM using the scintillation proximity assay (SPA)[19,20] revealed >50% inhibition of compounds **1** and **4**–**8** against human 11β-HSD1 and of **3**–**8** against mouse 11β-HSD1. (see ESI Tables S1 and S2). Further bioassays demonstrated that horipenoids A (**1**) and E (**5**) exhibited mild inhibitory activity against human 11β-HSD1 with IC_{50} values of 6.23 ± 1.32 and 4.11 ± 0.53 μM, respectively, while horipenoids C (**3**) and E (**5**) displayed much stronger inhibition against mouse 11β-HSD1 with IC_{50} values of 0.810 ± 0.058 and 0.898 ± 0.215 μM, respectively. The IC_{50} of other tested compounds with >50% inhibitory activity at 10 μM were not acquired due to poor solubility in the assay buffer.

Conclusions

In summary, during the course of our search for 11β-HSD1 inhibitors from Chinese plant resources, the chemical investigation of the extract of *H. riparia* yielded eight new dammarane-type triterpenoids (**1**–**8**) and a known oleanane-type triterpenoid (**9**). The structure characterization of **1**–**9** was accomplished via spectroscopic analyses with that of **5** being confirmed by X-ray data. The establishment of the C-20 configuration in **5** also provided new reference for later assignments of similar structural fragments. Following our report of tirucallane-type triterpenoids as 11β-HSD1 inhibitors,[13] compounds **1**–**9** represent two extra types of analogues that exhibit inhibitory effects against both human and mouse 11β-HSD1, demonstrating the tremendous potential of triterpenoids in the discovery of new drug leads for the treatment of 11β-HSD1 involved diseases.

Experimental section

General experimental procedures

Optical rotations were detected on a Perkin-Elmer 341 polarimeter at room temperature. IR spectra were recorded on a Perkin-Elmer 577 spectrometer using KBr disks. NMR spectra were measured on a Bruker AM-500 spectrometer with TMS as internal standard. ESI(±)MS and HRESI(±)MS analysis were carried out on a Bruker Daltonics Esquire 3000 plus LC-MS instrument and a waters Q-TOF Ultima mass spectrometer, respectively. EIMS (70 eV) and HREIMS were carried out on a Finnigan MAT 95 mass spectrometer. Semipreparative HPLC was carried out on a Waters 1525 binary pump system with a Waters 2489 detector (210 nm) using a YMC-Pack ODS-A column (250×10 mm, S-5 μm). Silica gel (200–300 esh, Qingdao Haiyang Chemical Co. Ltd), C_{18} reversed-phase silica gel (150–200 mesh, Merck) and MCI gel (CHP20P, 75–150 μM, Mitsubishi Chemical Industries, Ltd.), D101-macroporous absorption resin (Shanghai Huangling Resin Co. Ltd.) were used for column chromatography (CC). Pre-coated silica gel GF254 plates (Qingdao Haiyang Chemical Co. Ltd.) were used for TLC detection. All solvents used for CC were of analytical grade (Shanghai Chemical Reagents Co. Ltd.) and solvents used for HPLC were of HPLC grade (J & K Scientific Ltd.).

Extraction and isolation

The air-dried powdered stems and leaves (5 kg) of *H. riparia* were extracted with 95% ethanol at room temperature three times. After evaporation of the solvent under reduced pressure, the residue (120 g) was dissolved in 1 L water and then partitioned with EtOAc (1 L×3). The combined EtOAc layer was evaporated under reduced pressure to give a dark material (50 g) which was then subjected to column chromatography over macroporous resin D-101 (EtOH/H_2O, 30%, 50%, 80% and 95%, v/v) to get three fractions. The 80% EtOH elution was separated on a column of MCI gel (MeOH/water, 50% to 100%, v/v) to get three further fractions and the second fraction was then chromatographed on a silica gel column eluted with petroleum ether/acetone (15:1 to 1:3, v/v) to get four major sub-fractions A–D. Fraction C was fractionated on silica gel CC eluted with $CHCl_3$/MeOH to afford two fractions C1 and C2. Fraction C2 was chromatographed on a reversed-phase C18 silica gel column using MeOH/water system (40% to 100%, v/v) to get eight major fractions C2-1 to C2-8. Faction C2-1 was purified with semipreparative HPLC with 50% acetonitrile/water to yield compounds **1** (21.6 mg) and **2** (1.5 mg). Upon a silica gel column chromatography eluted with petroleum/acetone (8:1 to 4:1, v/v), fraction C2-5 yielded compounds **3** (50 mg) and **4** (4.0 mg). Via the similar procedure as fraction C2-5, C2-7 furnished compounds 5 (34.5 mg) and a mixture, and the latter was then purified with semipreparative HPLC with 70% acetonitrile/water to yield compounds **6** (6.0 mg), **7** (3.9 mg) and **8** (4.2 mg). Fraction C2-8 was chromatographed on a silica gel column eluted with petroleum/acetone (6:1 to 3:1, v/v) to afford compound **9** (10 mg).

Horipenoid A (1): white amorphous power; $[\alpha]^{22}_D$ 16.4 (*c* 0.34, EtOH); IR (KBr) ν_{max} 3438, 2941, 1722, 1444, 1377, 1265, 1228, 1022, 954 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; LR-ESIMS *m/z* 387.3 $[M + Na]^+$, 751.3 $[2M + Na]^+$; HR-ESI(–)MS *m/z* 363.2528 $[M - H]^-$ (calcd for $C_{22}H_{35}O_4$, 363.2535).

Horipenoid B (2): white amorphous power; $[\alpha]^{22}_D$ 25.0 (*c* 0.04, EtOH); IR (KBr) ν_{max} 3429, 2935, 2873, 1732, 1448, 1387, 1234, 1090, 1028, 993 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; LR-EIMS *m/z* $[M]^+$ 364(8), 346(10), 328(15), 313(12), 285(7), 224(22), 207(89), 196(24), 187(41), 151(26), 135(23), 123(100), 109(43), 95(31), 81(32), 69(20), 54(25); HR-EIMS *m/z* 346.2512 $[M - H_2O]^+$ (calcd for $C_{22}H_{34}O_3$, 346.2508) .

Horipenoid C (3): white amorphous power; $[\alpha]^{22}_D$ –33.8 (*c* 0.37, EtOH); IR (KBr) ν_{max} 3382, 2941, 1641, 1444, 1371, 1124, 1061, 1028, 985, 769 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; LR-ESIMS *m/z* 371.2 $[M + Na]^+$, 393.3 $[M + HCO_2]^-$; HR-ESI(–)MS *m/z* 393.2635 $[M + HCO_2]^-$ (calcd for $C_{23}H_{37}O_5$, 393.2641).

Horipenoid D (4): white amorphous power; $[\alpha]^{22}_D$ –32.0 (*c* 0.20, EtOH); IR (KBr) ν_{max} 3506, 2939, 1734, 1373, 1244, 1028, 984 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; LR-ESIMS *m/z* 455.3 $[M + Na]^+$; HR-ESI(–)MS *m/z* 477.2853 $[M + HCO_2]^-$ (calcd for $C_{27}H_{41}O_7$, 477.2852).

Horipenoid E (5): colorless crystals; mp 149–150°C; $[\alpha]^{22}_D$ 52.0 (*c* 0.26, EtOH); IR (KBr) ν_{max} 3334, 2945, 1448, 1388, 1298, 1024, 982 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; LR-ESIMS *m/z* 499.4 $[M + Na]^+$, 521.7 $[M + HCO_2]^-$; HR-ESI(–)MS *m/z* 521.3843 $[M + HCO_2]^-$ (calcd for $C_{31}H_{53}O_6$, 521.3842).

Horipenoid F (6): white amorphous power; $[\alpha]^{22}_D$ 344.0 (*c* 0.09, EtOH); IR (KBr) ν_{max} 3386, 2968, 1691, 1626, 1421, 1380, 1045, 987 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; LR-ESIMS *m/z* 497.3 $[M + Na]^+$, 519.6 $[M + HCO_2]^-$; HR-ESI(–)MS *m/z* 519.3687 $[M + HCO_2]^-$ (calcd for $C_{31}H_{51}O_6$, 519.3686).

Horipenoid G (7): white amorphous power; $[\alpha]^{22}_D$ 28.3 (*c* 0.12, EtOH); IR (KBr) ν_{max} 3332, 2952, 1712, 1450, 1390, 1282, 1051, 952 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; LR-ESIMS *m/z* 497.3 $[M + Na]^+$, 519.5 $[M + HCO_2]^-$; HR-ESI(–)MS *m/z* 519.3689 $[M + HCO_2]^-$ (calcd for $C_{31}H_{51}O_6$, 519.3686).

Horipenoid H (8): white amorphous power; $[\alpha]^{22}_D$ –10.0 (*c* 0.11, EtOH); IR (KBr) ν_{max} 3433, 2935, 1720, 1450, 1385, 1036,

981 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; LR-ESIMS *m/z* 971.8 $[2M + Na]^+$, 519.8 $[M + HCO_2]^-$; HR-ESI(–)MS *m/z* 519.3679 $[M + HCO_2]^-$ (calcd for $C_{31}H_{51}O_6$, 519.3686).

X-ray diffraction analysis

Horipenoid E (**5**) was crystallized in methanol with a little water at room temperature. The X-ray crystallographic data of them were obtained on a Bruker SMART CCD detector employing graphite monochromated Cu-Kα radiation (λ = 1.54178 Å) at 296(2) K (operated in the ϕ-ω scan mode). The structures were solved by direct method using SHELXS-97 (Sheldrick 2008) and refined with full-matrix least-squares calculations on F2 using SHELXL-97 (Sheldrick 2008). All non-hydrogen atoms were refined anisotropically. The hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms.

Crystallographic data for **5** (key parameters see Table S3 in ESI) have been deposited at the Cambridge Crystallographic Data Centre (Deposition No.: CCDC 982484). Copies of these data can be obtained free of charge via the internet at www.ccdc.cam.ac.uk/conts/retriving.html or on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Tel: (+44) 1223-336-408; Fax: (+44) 1223-336-033; E-mail: deposit@ccdc.cam.ac.uk].

Bioassays

As previously reported.[13]

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Notes and references

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† Electronic Supplementary Information (ESI) available: IR, MS, 1D and 2D NMR spectra of compounds **1**–**8**, together with the preliminary assay results, are provided. See DOI: 10.1039/b000000x/

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Graphical and textual abstract for

Dammarane-type Triterpenoids as 11β-HSD1 Inhibitors from *Homonoia riparia*†

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The chemistry and bioactivity of a series of dammarane-type triterpenoids from *Homonoia riparia* were reported in this article.

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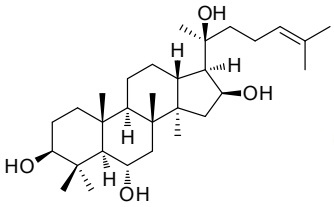

Horopenoid E (**5**)