

# Cycloartane and Friedelane Triterpenoids from the Leaves of *Caloncoba glauca* and Their Evaluation for Inhibition of $11\beta$ -Hydroxysteroid Dehydrogenases

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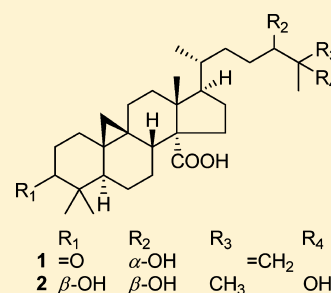
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## Supporting Information

**ABSTRACT:** Five new triterpenoids, caloncobic acids A and B (**1** and **2**), caloncobalactones A and B (**3** and **4**), and glaucalactone (**5**), along with the known compounds  $3\beta,21\beta$ -dihydroxy-30-nor-(D:A)-friedo-olean-20(29)-en-27-oic acid (**6**) and acetyltrichadenic acid B (**7**), were isolated from the leaves of *Caloncoba glauca*. The structures of **1**–**5** were elucidated using spectroscopic methods. Compounds **1**–**7** were evaluated for their inhibitory activities against two isozymes of  $11\beta$ -hydroxysteroid dehydrogenase ( $11\beta$ -HSD1 and  $11\beta$ -HSD2). Compounds **1** and **2** exhibited strong inhibitory activities against mouse ( $EC_{50}$  132 and 13 nM) and human ( $EC_{50}$  105 and 72 nM)  $11\beta$ -HSD1.

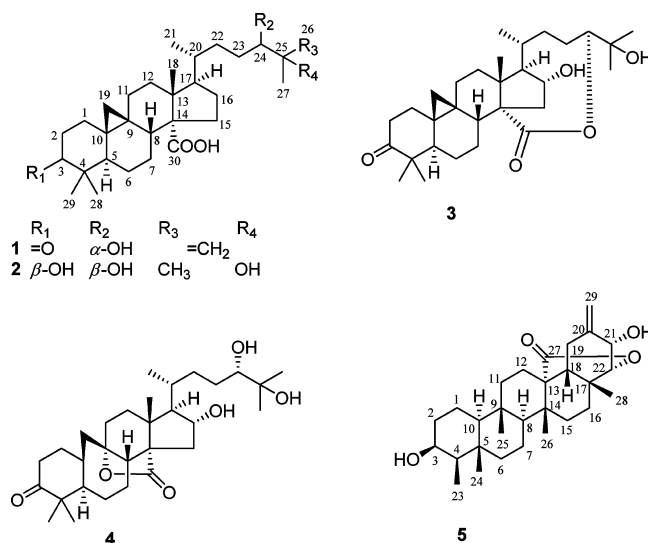


*Caloncoba glauca* (P. Beauv.) Gilg. (Flacourtiaceae) is a tree common in the tropical rain forests of Western Cameroon.<sup>1,2</sup> Information collected directly from traditional healers has indicated that the leaves are used as a purgative, while the fruits, leaves, and stem bark are used to treat inflammation and skin diseases. Previous phytochemical investigations of the stem bark of this species have resulted in the isolation and characterization of some friedelane-type triterpenoids.<sup>1,3</sup> There is no prior report on the constituents from the leaves of *C. glauca*. In this paper, we report the isolation and structure elucidation of five new triterpenoids (**1**–**5**) from this plant, as well as their inhibitory activities against two isozymes of  $11\beta$ -hydroxysteroid dehydrogenase.

## RESULTS AND DISCUSSION

The MeOH extract from the leaves of *C. glauca* was fractionated on a silica gel column to give several fractions. These were further purified by repeated column chromatography over MCI gel CHP20P, silica gel, ODS, and Sephadex LH-20 to afford five new triterpenoids (**1**–**5**) and the known  $3\beta,21\beta$ -dihydroxy-30-nor-(D:A)-friedo-olean-20(29)-en-27-oic acid (**6**)<sup>3</sup> and acetyltrichadenic acid B (**7**).<sup>4</sup>

Compound **1** was obtained as a white powder. The molecular formula was determined as C<sub>30</sub>H<sub>46</sub>O<sub>4</sub> by positive-ion HRESIMS, which showed a pseudomolecular ion peak at  $m/z$  493.3305 [M + Na]<sup>+</sup>, in conjunction with the NMR data. The IR spectrum showed absorptions for hydroxy (3425 cm<sup>-1</sup>) and carbonyl (1708 and 1693 cm<sup>-1</sup>) groups. The <sup>1</sup>H NMR spectrum (Table 1) showed singlets for four tertiary methyl groups at  $\delta_H$  0.99, 1.09, 1.10, and 1.67, a secondary methyl at



$\delta_H$  0.89 (3H, d,  $J$  = 6.2 Hz), a terminal methylene at  $\delta_H$  4.77 (1H, brs) and 4.86 (1H, brs), and characteristic cyclopropane methylene proton signals at  $\delta_H$  0.51 (1H, d,  $J$  = 4.2 Hz) and 0.86 (1H, overlapped), indicating a cycloartane-type skeleton.<sup>5</sup> The signals at  $\delta_H$  2.74 (1H, td,  $J$  = 13.8, 6.4 Hz) and 2.23 (1H, m) were attributable to the deshielded protons of a ketomethylene group.<sup>6</sup> The <sup>1</sup>H NMR spectrum also showed a signal of an oxymethine proton at  $\delta_H$  3.92 (1H, t,  $J$  = 6.4 Hz).

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Table 1. <sup>1</sup>H NMR Data of Compounds 1–4 ( $\delta$  in ppm,  $J$  in Hz)

position	1	2	3 <sup>a</sup>	4 <sup>a</sup>
1ax	1.61 m	1.40 m	2.04 <sup>b</sup>	2.03 <sup>b</sup>
1eq	1.85 dd (13.8, 4.0)	1.73 <sup>b</sup>	1.57 m	1.66 <sup>b</sup>
2ax	2.74 td (13.8, 6.4)	1.28 <sup>b</sup>	2.74 td (13.8, 6.3)	2.37 dt (15.5, 4.7)
2eq	2.23 <sup>b</sup> m	1.84 <sup>b</sup> m	2.35 brd (14.2)	2.45 brm
3		3.36 <sup>b</sup>		
4				
5	1.69 <sup>b</sup>	1.44 <sup>b</sup>	1.80 dd (12.3, 3.8)	1.84 dd (13.4, 7.0)
6ax	1.48 <sup>b</sup> m	1.69 m	1.00 brd (13.9)	1.23 m
6eq	1.02 <sup>b</sup> m	0.98 m	1.53 m	1.97 m
7ax	1.16 m	1.24 <sup>b</sup>	1.13 <sup>b</sup>	1.32 m
7eq	1.47 <sup>b</sup> m	1.56 <sup>b</sup>	1.41 <sup>b</sup>	1.47 <sup>b</sup>
8	1.78 <sup>b</sup>	1.82 <sup>b</sup>	1.69 dd (12.1, 5.2)	2.08 dd (12.0, 2.7)
9				
10				2.68 m
11ax	2.27 <sup>b</sup> m	2.40 m	2.43 brt (12.0)	1.71 <sup>b</sup>
11eq	1.32 <sup>b</sup> m	1.54 <sup>b</sup>	1.28 <sup>b</sup>	1.69 <sup>b</sup>
12ax	1.81 <sup>b</sup>	1.94 m	1.86 m	1.69 <sup>b</sup>
12eq	1.73 <sup>b</sup>	1.85 <sup>b</sup> m	1.47 m	1.79 <sup>b</sup>
13				
14				
15ax	2.11 <sup>b</sup>	2.28 <sup>b</sup>	2.15 <sup>b</sup>	1.66 <sup>b</sup>
15eq	1.21 m	1.35 <sup>b</sup> m	2.01 <sup>b</sup>	2.04 <sup>b</sup>
16ax	2.10 <sup>b</sup>	1.37 <sup>b</sup> m		
16eq	1.24 m	1.51 m	4.10 brt (8.1)	4.01 m
17	1.50 <sup>b</sup> m	1.67 <sup>b</sup> m	1.84 <sup>b</sup>	1.61 dd (11.2, 5.6)
18	1.10 s	1.24 s	1.15 s	0.93 s
19 $\alpha$	0.51 d (4.2)	0.40 d (4.0)	0.53 d (4.1)	1.89 brd (15.5)
19 $\beta$	0.86 <sup>b</sup>	0.76 d (3.9)	0.86 d (4.0)	2.18 dd (15.3, 9.5)
20	1.44 m	1.65 <sup>b</sup> m	1.93 m	1.71 <sup>b</sup>
21	0.89 d (6.2)	1.04 d (5.5)	0.96 d (6.5)	0.88 d (6.4)
22 $\alpha$	1.06 m	1.42 m	1.44 <sup>b</sup>	1.80 <sup>b</sup> m
22 $\beta$	1.35 <sup>b</sup> m	1.63 m	2.20 <sup>b</sup> m	1.66 <sup>b</sup> m
23 $\alpha$	1.56 m	1.72 <sup>b</sup>	2.17 <sup>b</sup>	1.37 brm
23 $\beta$	1.38 <sup>b</sup> m	2.26 <sup>b</sup>	1.62 m	1.44 <sup>b</sup>
24	3.92 t (6.4)	3.37 <sup>b</sup>	4.77 dd (10.1, 2.4)	3.28 brd (10.3)
25				
26	4.86 brs 4.77 brs	1.28 <sup>b</sup>	1.21 s	1.16 s
27	1.67 s	1.28 <sup>b</sup>	1.40 s	1.12 s
28	0.99 s	1.07 s	1.07 s	1.13 s
29	1.09 s	0.93 s	1.11 s	1.06 s

<sup>a</sup>Run in CDCl<sub>3</sub>. Other spectra run in CDCl<sub>3</sub>–MeOD. <sup>b</sup>Overlapped signals within a column.

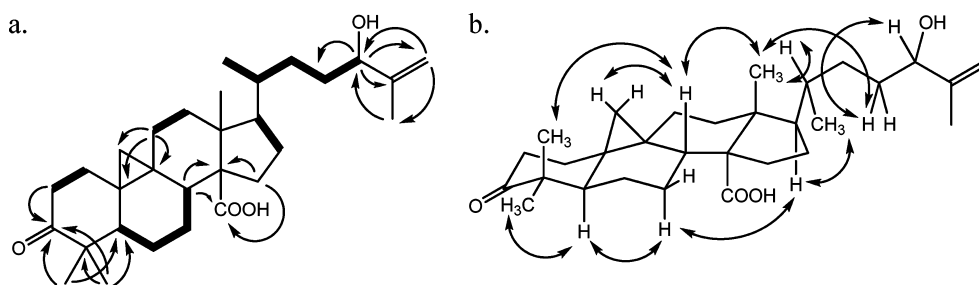
The <sup>13</sup>C NMR spectrum (Table 2) of **1** displayed 30 carbon resonances, which were assigned by DEPT and HSQC experiments as five methyls, 12 methylenes, five methines, and eight quaternary carbons. The signals at  $\delta_C$  76.7, 179.7, and 219.4 were attributed to an oxymethine carbon (C-24), a carboxylic acid carbon (C-30), and a carbonyl (C-3), respectively. The <sup>13</sup>C NMR spectrum also showed signals at  $\delta_C$  148.1 (C-25) and 111.3 (C-26), assignable to two olefinic carbons. The <sup>1</sup>H–<sup>1</sup>H COSY spectrum (Figure 1a) together

Table 2. <sup>13</sup>C NMR Data of Compounds 1–4 ( $\delta$  in ppm)

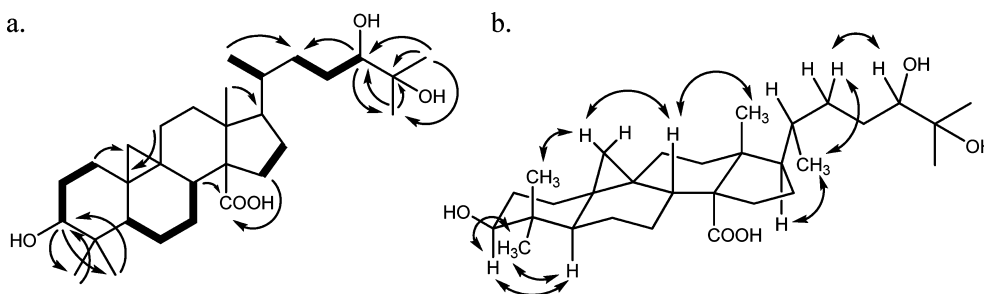
position	1	2	3 <sup>a</sup>	4 <sup>a</sup>
1	34.1, CH <sub>2</sub>	32.7, CH <sub>2</sub>	33.2, CH <sub>2</sub>	28.9, CH <sub>2</sub>
2	37.9, CH <sub>2</sub>	30.4, CH <sub>2</sub>	37.3, CH <sub>2</sub>	36.8, CH <sub>2</sub>
3	219.4, C	79.0, CH	216.4, C	216.2, C
4	50.7, C	41.0, C	49.9, C	48.5, C
5	48.6, CH	47.3, CH	47.5, CH	48.2, CH
6	21.6, CH <sub>2</sub>	21.4, CH <sub>2</sub>	20.8, CH <sub>2</sub>	25.3, CH <sub>2</sub>
7	27.4, CH <sub>2</sub>	27.8, CH <sub>2</sub>	26.5, CH <sub>2</sub>	23.9, CH <sub>2</sub>
8	46.3, CH	47.0, CH	46.2, CH	51.3, CH
9	21.1, C	20.0, C	19.3, C	88.4, C
10	27.9, C	28.1, C	27.2, C	28.7, CH
11	29.0, CH <sub>2</sub>	28.6, CH <sub>2</sub>	28.2, CH <sub>2</sub>	33.9, CH <sub>2</sub>
12	34.3, CH <sub>2</sub>	34.5, CH <sub>2</sub>	33.4, CH <sub>2</sub>	34.6, CH <sub>2</sub>
13	48.1, C	48.2, C	49.2, C	46.7, C
14	63.2, C	63.4, C	60.9, C	63.6, C
15	32.2, CH <sub>2</sub>	32.4, CH <sub>2</sub>	42.1, CH <sub>2</sub>	35.2, CH <sub>2</sub>
16	30.1, CH <sub>2</sub>	28.8, CH <sub>2</sub>	78.4, CH	76.7, CH
17	52.9, CH	53.3, CH	57.6, CH	60.9, CH
18	18.4, CH <sub>3</sub>	18.5, CH <sub>3</sub>	18.6, CH <sub>3</sub>	15.5, CH <sub>3</sub>
19	30.5, CH <sub>2</sub>	31.4, CH <sub>2</sub>	30.8, CH <sub>2</sub>	39.9, CH <sub>2</sub>
20	36.2, CH	36.2, CH	32.9, CH	32.8, CH
21	18.8, CH <sub>3</sub>	18.6, CH <sub>3</sub>	18.5, CH <sub>3</sub>	18.2, CH <sub>3</sub>
22	32.5, CH <sub>2</sub>	33.9, CH <sub>2</sub>	31.3, CH <sub>2</sub>	31.2, CH <sub>2</sub>
23	31.9, CH <sub>2</sub>	30.1, CH <sub>2</sub>	25.7, CH <sub>2</sub>	25.4, CH <sub>2</sub>
24	76.7, CH	79.4, CH	81.2, CH	78.4, CH
25	148.1, C	73.6, C	72.4, C	72.8, C
26	111.3, CH <sub>2</sub>	25.5, CH <sub>3</sub>	28.3, CH <sub>3</sub>	25.9, CH <sub>3</sub>
27	17.5, CH <sub>3</sub>	24.5, CH <sub>3</sub>	27.8, CH <sub>3</sub>	23.5, CH <sub>3</sub>
28	22.4, CH <sub>3</sub>	25.8, CH <sub>3</sub>	22.1, CH <sub>3</sub>	25.5, CH <sub>3</sub>
29	21.3, CH <sub>3</sub>	14.4, CH <sub>3</sub>	20.9, CH <sub>3</sub>	22.2, CH <sub>3</sub>
30	179.7, C	180.0, C	173.2, C	182.7, C

<sup>a</sup>Run in CDCl<sub>3</sub>. Other spectra run in CDCl<sub>3</sub>–MeOD.

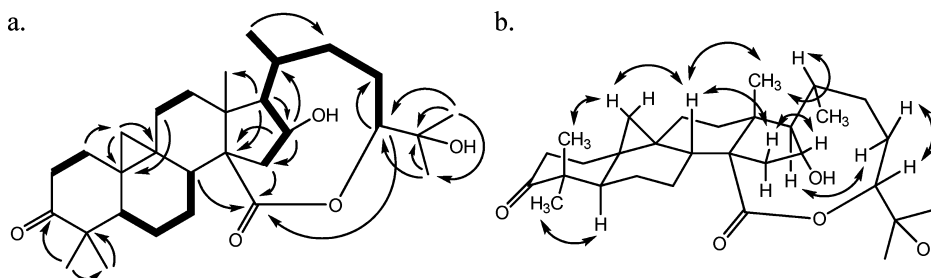
with the HSQC data revealed several proton spin systems, among which two –CH<sub>2</sub>–CH<sub>2</sub>– units, a –CH–CH<sub>2</sub>–CH<sub>2</sub>–CH– unit, and a –CH(–OH)–CH<sub>2</sub>–CH<sub>2</sub>–CH–CH<sub>3</sub> unit were evident. In the HMBC spectrum (Figure 1a), correlations between H-11ax ( $\delta_H$  2.27)/H-11eq ( $\delta_H$  1.32) and C-19 ( $\delta_C$  30.5), C-9 ( $\delta_C$  21.1), and C-10 ( $\delta_C$  27.9) gave evidence of a cyclopropane ring (C-9, C-10, and C-19).<sup>7</sup> This spectrum also exhibited long-range correlations from H<sub>3</sub>-28 ( $\delta_H$  0.99) and H<sub>3</sub>-29 ( $\delta_H$  1.09) to C-3 ( $\delta_C$  219.4), C-4 ( $\delta_C$  50.7), and C-5 ( $\delta_C$  48.6), indicating the location of the carbonyl group at C-3. A carboxyl group was placed at C-14 on the basis of the observed HMBC correlations from H-8 ( $\delta_H$  1.78) and H<sub>2</sub>-15 ( $\delta_H$  2.11 and 1.21) to C-14 ( $\delta_C$  63.2) and C-30 ( $\delta_C$  179.7). Further HMBC correlations of the deshielded methyl proton H<sub>3</sub>-27 ( $\delta_H$  1.67) and the H<sub>2</sub>-26 terminal methylene protons ( $\delta_H$  4.77, 4.86) with the oxymethine carbon C-24 ( $\delta_C$  76.7), as well as between the oxymethine proton H-24 ( $\delta_H$  3.92) and C-27 ( $\delta_C$  17.5), C-26 ( $\delta_C$  111.3) C-25 ( $\delta_C$  148.1), and C-23 ( $\delta_C$  31.9) indicated that the hydroxy group is located at C-24. The relative configuration of **1** was determined by careful analysis of the ROESY spectrum (Figure 1b). The cross-peaks observed in this spectrum between H<sub>3</sub>-18 and H-8 and H-20, on one hand, and H<sub>3</sub>-28 and H-5, H-5 and H-7ax, H-7ax and H-17, and H-17 and H<sub>3</sub>-21 on the other hand were in accordance with the presence of a cycloartane ring between C-9 and C-10. The configuration of C-24 was derived as *S* from the ROESY correlation of H<sub>3</sub>-18 with H-23 $\beta$  and that of H-23 $\beta$  with H-24. Therefore, compound **1** was characterized as 24(*S*)-hydroxy-3-



**Figure 1.** (a) Selected HMBC (H→C) and  $^1\text{H}$ - $^1\text{H}$  COSY (—) correlations of **1**. (b) Selected ROESY correlations of **1**.



**Figure 2.** (a) Selected HMBC (H→C) and  $^1\text{H}$ - $^1\text{H}$  COSY (—) correlations of **2**. (b) Selected ROESY correlations of **2**.



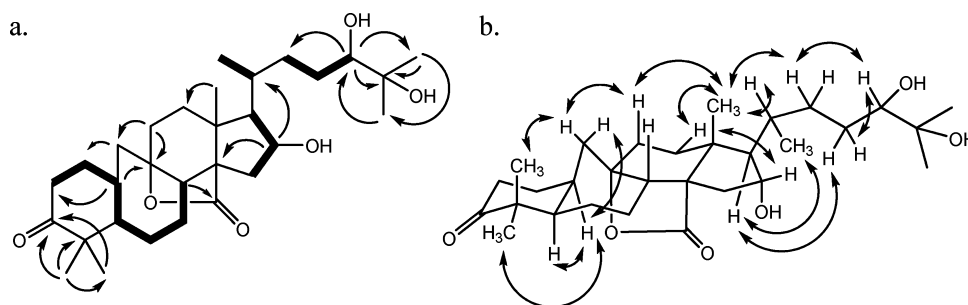
**Figure 3.** (a) Selected HMBC (H→C) and  $^1\text{H}$ - $^1\text{H}$  COSY (—) correlations of **3**. (b) Selected ROESY correlations of **3**.

oxocycloart-25-en-30-oic acid and has been assigned the trivial name caloncobic acid A.

Compound **2** was obtained as a white powder. Its molecular formula was determined to be  $\text{C}_{30}\text{H}_{50}\text{O}_5$  on the basis of positive-ion HRESIMS ( $m/z$  513.3565 [ $M + \text{Na}$ ] $^+$ ). Its IR spectrum showed absorption bands at  $\nu_{\text{max}}$  3427  $\text{cm}^{-1}$  (hydroxy) and 1686  $\text{cm}^{-1}$  (carboxy), but did not show any carbonyl group absorption as in **1**. The  $^1\text{H}$  NMR spectrum (Table 1) exhibited typical signals of a cycloartane triterpene including a characteristic pair of doublets at  $\delta_{\text{H}}$  0.76 (1H,  $J = 3.9$  Hz) and 0.40 (1H,  $J = 4.0$  Hz), corresponding to the C-19 methylene protons of the cyclopropane ring and a methyl doublet at  $\delta_{\text{H}}$  1.04 ( $J = 5.5$  Hz, Me-21) and five tertiary methyl singlets at  $\delta_{\text{H}}$  0.93 (Me-29), 1.07 (Me-28), 1.24 (Me-18), and 1.28 (Me-26 and Me-27). The  $^{13}\text{C}$  NMR data of compound **2** (Table 2) were closely related to those of **1** except that **2** did not exhibit resonances for a keto group and double bond as in **1** and showed two additional hydroxylated carbons (C-25,  $\delta_{\text{C}}$  73.6 and C-3,  $\delta_{\text{C}}$  79.0). The relatively low-field value of the proton chemical shift of Me-26 and Me-27 suggested that these two methyl groups are attached to the hydroxylated C-25 carbon atom. The locations of these two methyl groups were confirmed by the HMBC correlations (Figure 2a) observed from  $\text{H}_3$ -26 and  $\text{H}_3$ -27 to C-25 and C-24. The second additional hydroxylated carbon was placed at the C-3 position, based on the HMBC correlations between H-3 ( $\delta_{\text{H}}$  3.36) and

C-28 and C-29 and between  $\text{H}_3$ -28 and C-3 as well as between  $\text{H}_3$ -29 and C-3. The relative configuration of compound **2** was determined by analysis of its ROESY spectrum. The observed correlations in this spectrum between H-5, H-3, and  $\text{H}_3$ -28 (Figure 2b) enabled the  $\beta$ -orientation of the C-3 hydroxy group to be determined. The configuration of C-24 was determined as *R* based on the ROESY correlations between  $\text{H}_3$ -21 and H-22 $\alpha$ , and H-22 $\alpha$  and H-24. All these data suggest that compound **2** (caloncobic acid B) is a stereoisomer of protolyfoligenic acid,<sup>8,9</sup> and it was thus characterized as 3 $\beta$ ,24(*R*),25-trihydroxycycloartan-30-oic acid.

The molecular formula of compound **3** was established as  $\text{C}_{30}\text{H}_{46}\text{O}_5$  by HRESIMS analysis ( $m/z$  995.6600 [ $2M + \text{Na}$ ] $^+$ ) in conjunction with the NMR data. The IR spectrum exhibited absorption bands for hydroxy (3474, 3417  $\text{cm}^{-1}$ ) and carbonyl (1709  $\text{cm}^{-1}$ ) groups. The chemical shifts of C-18, C-19, C-21, C-28, and C-29 (Table 2) and those of the protons of the C-19 methylene group were almost identical with those of caloncobic acid A (**1**) (Table 1). In addition, the presence of a carbonyl signal at  $\delta_{\text{C}}$  216.4 (C-3) suggested that these two compounds are both based on a cycloartane skeleton. Further analysis of the  $^1\text{H}$  NMR spectrum indicated two relatively deshielded methyl proton signals at  $\delta_{\text{H}}$  1.21 ( $\text{H}_3$ -26) and 1.40 ( $\text{H}_3$ -27) and revealed their attachment to the hydroxylated C-25 carbon ( $\delta_{\text{C}}$  72.4); this was supported by the HMBC correlations from  $\text{H}_3$ -26 and  $\text{H}_3$ -27 to C-25. The  $^1\text{H}$  NMR spectrum also showed



**Figure 4.** (a) Selected HMBC (H→C) and <sup>1</sup>H-<sup>1</sup>H COSY (—) correlations of 4. (b) Selected ROESY correlations of 4.

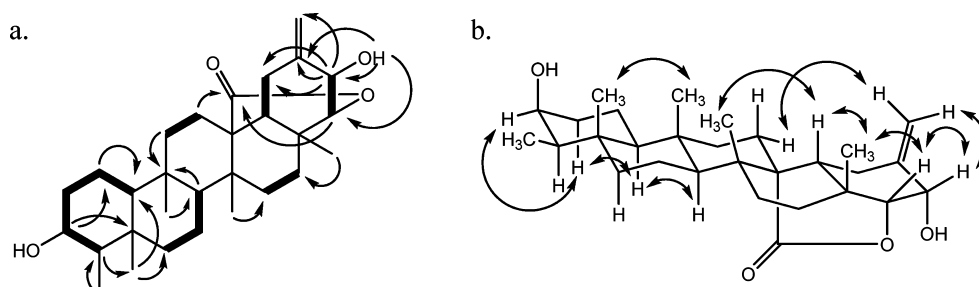
signals of two oxymethine protons at  $\delta_{\text{H}}$  4.10 (H-16, brt,  $J = 8.1$  Hz) and 4.77 (H-24, dd,  $J = 10.1, 2.4$  Hz). The <sup>13</sup>C NMR spectrum exhibited a signal at  $\delta_{\text{C}}$  173.2 ascribable to the carbonyl carbon of a lactone group located at C-30 on the basis of the observed correlations in the HMBC spectrum (Figure 3a) between H-8/H<sub>2</sub>-15 and C-30. Pertinent correlations in the latter spectrum were also observed between H<sub>3</sub>-26 and the carbon resonances at  $\delta_{\text{C}}$  81.2 (C-24) and 27.8 (C-27) and between H<sub>3</sub>-27 and C-24 and C-26 ( $\delta_{\text{C}}$  28.3). Further long-range <sup>1</sup>H-<sup>13</sup>C couplings were observed between the H-16 proton ( $\delta_{\text{H}}$  4.10) and C-20 ( $\delta_{\text{C}}$  32.9), C-15 ( $\delta_{\text{C}}$  42.1), and C-14 ( $\delta_{\text{C}}$  60.9), between H-17 and C-14, C-16 ( $\delta_{\text{C}}$  78.4) and C-18, and between H<sub>2</sub>-15 ( $\delta_{\text{H}}$  2.01 and 2.15) and C-16, indicating the location of a second hydroxy group to be at C-16. A cross-peak in the same spectrum between H-24 and C-30, together with the deshielded value of C-24 as compared to compound 1, indicated lactonization of the C-30 carboxylic acid group to C-24. The  $\beta$ -equatorial orientation of H-16 was determined from its broad triplet resonance due to the equatorial-equatorial couplings with both H-15eq and H-17eq and the equatorial-axial coupling with H-15ax. The  $\alpha$ -configuration of the hydroxy group at C-16 was confirmed by the ROESY correlations of H<sub>3</sub>-18 with H-20 and H-8; H-8 with H-15eq; and H-15eq with H-16. The configuration of C-24 was determined to be *R* based on the observed ROESY correlations between H-24 and H-23 $\beta$ ; H-23 $\alpha$  and H-17; and H-17 and H<sub>3</sub>-21. Compound 3 (caloncobalactone A) was thus determined as 16 $\alpha$ ,25-dihydroxy-3,30-dioxocycloartan-30,24(*R*)-lactone.

Compound 4 was obtained as a white powder. Its molecular formula was deduced as C<sub>30</sub>H<sub>48</sub>O<sub>6</sub> on the basis of positive-ion HRESIMS ( $m/z$  527.3343 [ $M + \text{Na}$ ]<sup>+</sup>) and was supported by its NMR data, indicating its triterpenoid nature. This molecular formula accounted for seven degrees of unsaturation. The IR spectrum exhibited absorption bands of hydroxy (3422 cm<sup>-1</sup>) and carbonyl (1738, 1703 cm<sup>-1</sup>) groups. The <sup>13</sup>C NMR spectrum (Table 2) revealed the presence of two carbonyl groups at  $\delta_{\text{C}}$  182.7 (C-30) and 216.2 (C-3) and four oxygenated carbons at  $\delta_{\text{C}}$  72.8 (C-25), 76.7 (C-16), 78.4 (C-24), and 88.4 (C-9). In the <sup>1</sup>H NMR spectrum (Table 1), five quaternary methyl signals at  $\delta_{\text{H}}$  0.93 (H<sub>3</sub>-18), 1.06 (H<sub>3</sub>-29), 1.12 (H<sub>3</sub>-27), 1.13 (H<sub>3</sub>-28), and 1.16 (H<sub>3</sub>-26) and a secondary methyl group signal at  $\delta_{\text{H}}$  0.88 (d,  $J = 6.4$  Hz, H<sub>3</sub>-21) were observed. This spectrum also showed two oxymethine protons at  $\delta_{\text{H}}$  3.28 (brd,  $J = 10.3$ , H-24) and 4.01 (m, H-16). In the HMBC spectrum (Figure 4a), correlations were observed between H<sub>2</sub>-11 ( $\delta_{\text{H}}$  1.71, 1.69) and C-9 and C-19. A long-range correlation between H<sub>2</sub>-11 and C-10 evident for compounds 1–3 was missing, as well the <sup>1</sup>H NMR upfield cyclopropane methylene signals of the cycloartane skeleton. This suggested that the C-9/C-10 bond is cleaved and that compound 4 is a

9,10-*seco*-cycloartane triterpenoid.<sup>10</sup> This skeleton, consisting of four rings, associated with the presence of two carbonyl groups and seven degrees of unsaturation, gave evidence of the presence of an additional ring, which, in conjunction with the NMR data, was assignable to a lactone group. Pertinent correlations observed in the HMBC spectrum between H-8/H<sub>2</sub>-15 and C-30 and between H-10/H<sub>2</sub>-12 and C-9 for the locations of a carbonyl at C-30 and the oxygenated quaternary carbon at C-9, respectively, together with the relatively deshielded value of the C-9 chemical shift ( $\delta_{\text{C}}$  88.4), implied C-30/C-9 lactonization. Further correlations were observed between H-16 and C-20 and C-14, as well as between H-24 and C-25 and C-26, between H<sub>3</sub>-26 and C-24, C-25, and C-27, and between H<sub>3</sub>-27 and C-24, C-25, and C-26. The ROESY correlations (Figure 4b) of H-19 $\beta$ /H<sub>3</sub>-29 and H-11ax; H<sub>3</sub>-18/H-11ax, H-8, H-20, H-12eq, and H-22 $\beta$ ; H-12eq/H-16; and H-24/H-22 $\beta$  and H-23 $\beta$ , on one hand, and H-23 $\alpha$ /H-17; H<sub>3</sub>-21/H-17; and H-10/H-19 $\alpha$ , H<sub>3</sub>-28, and H-5 on the other hand showed that Me-29, Me-18, H-8, H-16, and H-24 are all  $\beta$ -oriented, whereas Me-28, Me-21, H-17, H-10, and H-5 are  $\alpha$ -oriented. Consequently, the configuration of C-24 was determined to be *S*. Compound 4 (caloncobalactone B) was thus elucidated as 16 $\beta$ ,24(*S*),25-trihydroxy-3,30-dioxo-9,10-*seco*-cycloartan-30,9 $\alpha$ -lactone.

Compound 5 was obtained as a white powder. Its molecular formula was deduced as C<sub>29</sub>H<sub>44</sub>O<sub>4</sub> on the basis of the pseudomolecular ion peak at  $m/z$  479.3133 [ $M + \text{Na}$ ]<sup>+</sup> in the HRESIMS. This molecular formula accounted for eight degrees of unsaturation. The IR spectrum exhibited absorption bands corresponding to hydroxy (3555, 3433 cm<sup>-1</sup>), carbonyl (1710 cm<sup>-1</sup>), and exocyclic methylene (3075w, 1639 cm<sup>-1</sup>) functionalities. Its <sup>13</sup>C NMR data were closely related to those of 3 $\beta$ ,21 $\beta$ -dihydroxy-30-(D:A)-friedeo-olean-20(29)-en-27-oic acid (6),<sup>3</sup> a known compound also obtained in the present study, and revealed 29 signals assigned by HSQC and DEPT spectra to seven quaternary carbons, 10 methylenes, seven methines, and five methyl carbons. The carbon signal at  $\delta_{\text{C}}$  12.5 was suggestive of a friedelane-type triterpene skeleton with a hydroxy group at C-3.<sup>1,3</sup> The presence of a carbonyl group was confirmed by the signal at  $\delta_{\text{C}}$  175.2 (C-27). Further oxygenated carbon atoms were observed at  $\delta_{\text{C}}$  75.5 (C-21) and 87.4 (C-22). Its <sup>1</sup>H NMR spectrum revealed the presence of an exocyclic methylene group at  $\delta_{\text{H}}$  5.23 and 5.25 (each 1H, brs, H<sub>2</sub>-29) and three oxymethine protons at  $\delta_{\text{H}}$  3.90 (brs, H-3), 4.39 (m, H-22), and 4.68 (t,  $J = 3.3$  Hz, H-21). This spectrum confirmed the presence of four tertiary methyl groups at  $\delta_{\text{H}}$  0.91 (H<sub>3</sub>-26), 0.95 (H<sub>3</sub>-25), 1.26 (H<sub>3</sub>-24), and 1.54 (H<sub>3</sub>-28). The Me-23 proton signal did not appear as a doublet as expected for a friedelane triterpene, which may be due to overlapping with the H-4 signal at  $\delta_{\text{H}}$  1.10. The HMBC





**Figure 5.** (a) Selected HMBC (H→C) and  $^1\text{H}$ – $^1\text{H}$  COSY (—) correlations of 5. (b) Selected ROESY correlations of 5.

correlations (Figure 5a) observed between H-3 and C-1 and C-5 confirmed the attachment of one of the hydroxy groups at C-3. The second hydroxy group was placed at C-21, based on the long-range correlations between H-21 and C-20, C-29, C-22, C-17, and C-19, as well as between OH-21 and C-20, C-21, and C-22. The position of the oxymethine proton ( $\delta_{\text{H}}$  4.39) at C-22 was confirmed by the cross-peak observed in the COSY spectrum (Figure 5a) between H-21 and H-22. Further correlations observed in the HMBC spectrum between H-12, H-22 and C-27, in addition to the relatively low-field resonance of the oxygenated C-22 methine, supported a C-22,C-27 lactone ring. The relative configuration of compound 5 was determined on the basis of the  $^1\text{H}$  NMR data as well as the analysis of the ROESY spectrum (Figure 5b). In the  $^1\text{H}$  NMR spectrum, the H-3 proton was observed as a broad singlet ( $W_{1/2} = 8.2$  Hz), which indicated its  $\alpha$ -equatorial orientation.<sup>3</sup> The  $\beta$ -orientation of the hydroxy group at C-3 was confirmed by the observed ROESY cross-peaks between H-3 and H-2ax; H-2ax and H-10; and H-10 and H-8. The small coupling constant ( $J = 3.3$  Hz) between the oxymethine protons H-21 and H-22 indicated that they are cofacial and  $\beta$ -oriented.<sup>11</sup> Their orientation was confirmed by the ROESY correlations between H-21 and H-22; H-22 and H<sub>3</sub>-28; H<sub>3</sub>-28 and H-18; and H-18 and H<sub>3</sub>-26. Therefore, compound 5 (glaucalactone) was characterized as  $3\beta,21\alpha$ -dihydroxy-27-oxo-30-nor-(D:A)-friedo-olean-20(29)-en-27,22 $\alpha$ -lactone.

The enzyme  $11\beta$ -hydroxysteroid dehydrogenase type 1 ( $11\beta$ -HSD1) can influence factors affecting metabolic syndrome such as insulin resistance and dyslipidemia.<sup>12</sup> The inhibitory effects of compounds 1–7 on mouse and human  $11\beta$ -HSD1 and  $11\beta$ -HSD2 were evaluated. All assays were carried out in duplicate with glycyrrhizic acid and carbenoxolone as positive controls. Compounds 1 and 2 showed inhibitory activities against mouse and human  $11\beta$ -HSD1 ( $\text{EC}_{50}$  132 and 13 nM and  $\text{EC}_{50}$  105 and 72 nM, respectively) (Table 3). Compounds 3–7 were inactive (inhibition <50% at 1  $\mu\text{M}$ ).

**Table 3. Inhibitory Activities of Compounds 1 and 2 against  $11\beta$ -Hydroxysteroid Dehydrogenase Isozymes**

compound	mouse $11\beta$ -HSD1 ( $\text{IC}_{50}$ )	mouse $11\beta$ -HSD2 ( $\text{IC}_{50}$ )	human $11\beta$ -HSD1 ( $\text{IC}_{50}$ )	human $11\beta$ -HSD2 ( $\text{IC}_{50}$ )
1	132 nM	>100 $\mu\text{M}$	105 nM	>100 $\mu\text{M}$
2	13 nM	>100 $\mu\text{M}$	72 nM	0.4 $\mu\text{M}$
glycyrrhizic acid <sup>a</sup>	3 nM		6 nM	0.4 nM
carbenoxolone <sup>a</sup>		206 nM		

<sup>a</sup>Positive control.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** Melting points were measured on an X-4 micromelting point apparatus and are uncorrected. Optical rotations were obtained with a JASCO P-1020 digital polarimeter. A Tensor 27 spectrophotometer was used for scanning IR spectra with KBr pellets. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on Bruker AV-400 or DRX-500 NMR spectrometers, while 2D-NMR spectra were recorded on Bruker DRX-500 or AV-600 instruments. Chemical shifts ( $\delta$ ) are expressed in ppm with reference to TMS, and coupling constants ( $J$ ) are given in Hz. ESIMS and HRESIMS were carried out on an API Qstar time-of-flight spectrometer. Column chromatography was performed on silica gel G (100–200 and 300–400 mesh, Qingdao Haiyang Chemical Co., Ltd., People's Republic of China) and Sephadex LH-20 (40–70  $\mu\text{m}$ , Amersham Pharmacia Biotech AB, Sweden). MPLC was performed on a Büchi Sepacore System (Büchi Laborotechnik AG, Switzerland), and columns were packed with Chromatorex C<sub>18</sub> (40–75  $\mu\text{m}$ , Fuji Silysia Chemical Ltd., Japan) and MCI gel CHP-20P (75–150  $\mu\text{m}$ , Mitsubishi Chemical Co., Japan). TLC was carried out on precoated silica gel plates (Qingdao Haiyang Chemical Co.), and spots were visualized by heating the plates after they were dipped into a 10% ethanolic H<sub>2</sub>SO<sub>4</sub> solution. Solvents were distilled prior to use.

**Plant Material.** The leaves of *C. glauca* were collected in Bangang-Wabane Village, Cameroon, in May 2009. The plant was authenticated by Mr. François Nana, a botanist of the National Herbarium of Cameroon (Yaoundé, Cameroon), where a voucher specimen has been deposited (accession number: 55064/HNC).

**Extraction and Isolation.** Air-dried and powdered leaves of *C. glauca* (2 kg) were extracted with MeOH (3  $\times$  3 L) at room temperature for 72 h. After filtration, the solvent was removed under vacuum to give a crude extract (200 g). This extract was defatted using *n*-hexane, and the insoluble portion (150 g) was fractionated over a silica gel column. Elution with gradients of petroleum ether–EtOAc (90:10, 70:30, 50:50, 20:80) and EtOAc–MeOH (100:0, 90:10, 70:30, 50:50, 0:100) gave five main fractions, A–E. Fraction A (7 g) was separated on a silica gel column using a gradient of petroleum ether–Me<sub>2</sub>CO (100:0 to 70:30) to yield 7 (4 mg). Fraction B (13 g) was chromatographed on a MCI gel column (MeOH–H<sub>2</sub>O, 60:40 to 100:0) to give three subfractions: BI, BII, and BIII. Subfraction BII (6 g) was subjected to passage over a silica gel column (petroleum ether–Me<sub>2</sub>CO, 90:10 to 0:100) and afforded subfractions BII1–BII4. Subfraction BII2 (1 g) was purified on a Sephadex LH-20 column by elution with CHCl<sub>3</sub>–MeOH (1:1), then on silica gel (petroleum ether–Me<sub>2</sub>CO, 80:20), to yield 3 (4.6 mg). Successive chromatography of subfraction BII3 (2 g) over a reversed-phase C<sub>18</sub> and over normal-phase silica gel eluted with MeOH–H<sub>2</sub>O (50:50 to 100:0) and petroleum ether–Me<sub>2</sub>CO, respectively, yielded 4 (41.5 mg) and 2 (5 mg). Fraction C (14 g) was chromatographed by MCI gel MPLC (MeOH–H<sub>2</sub>O, 60:40 to 100:0), and subfractions CI to CV were obtained. Repeated purification of subfraction CII (4 g) on a silica gel column eluted with CHCl<sub>3</sub>–Me<sub>2</sub>CO (100:0 to 50:50) afforded 5 (17 mg) and 6 (200 mg). Subfraction CIII (3 g) was subjected successively to silica gel column chromatography (CHCl<sub>3</sub>–Me<sub>2</sub>CO, 100:0 to 70:30) and passage over Sephadex LH-20 (CHCl<sub>3</sub>–MeOH, 1:1) to give 1 (13 mg).

**Caloncobic acid A (1):** white powder (MeOH); mp 198–200 °C;  $[\alpha]_D^{25}$  –35.1 (c 0.067, CHCl<sub>3</sub>–MeOH, 1:2); IR (KBr)  $\nu_{\max}$  3425, 2958, 2927, 1708 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>–CD<sub>3</sub>OD, 400 MHz), see Table 1; <sup>13</sup>C NMR (CDCl<sub>3</sub>–CD<sub>3</sub>OD, 100 MHz), see Table 2; positive ESIMS *m/z* 493 [M + Na]<sup>+</sup>, 964 [2 M + H + Na]<sup>+</sup>; HRESIMS *m/z* 493.3305 [M + Na]<sup>+</sup> (calcd for C<sub>30</sub>H<sub>46</sub>O<sub>4</sub>Na, 493.3293).

**Caloncobic acid B (2):** white powder (Me<sub>2</sub>CO); mp 242–244 °C;  $[\alpha]_D^{25}$  –2.0 (c 0.1, MeOH); IR (KBr)  $\nu_{\max}$  3427, 2964, 2930, 1686, 1637, 1629 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>–CD<sub>3</sub>OD, 500 MHz), see Table 1; <sup>13</sup>C NMR (CDCl<sub>3</sub>–CD<sub>3</sub>OD, 125 MHz), see Table 2; positive ESIMS *m/z* 513 [M + Na]<sup>+</sup>, 1004 [2 M + H + Na]<sup>+</sup>; HRESIMS *m/z* 513.3565 [M + Na]<sup>+</sup> (calcd for C<sub>30</sub>H<sub>50</sub>O<sub>5</sub>Na, 513.3555).

**Caloncobalactone A (3):** white powder (Me<sub>2</sub>CO); mp 348–350 °C;  $[\alpha]_D^{25}$  +70.2 (c 0.1, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3474, 3417, 2968, 2921, 1709, 1233, 1186 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz), see Table 1; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz), see Table 2; positive ESIMS *m/z* 995 [2 M + Na]<sup>+</sup>; HRESIMS *m/z* 995.6600 [2 M + Na]<sup>+</sup> (calcd for [C<sub>30</sub>H<sub>46</sub>O<sub>5</sub>]<sub>2</sub>Na, 995.6588).

**Caloncobalactone B (4):** white powder (Me<sub>2</sub>CO); mp 194–196 °C;  $[\alpha]_D^{25}$  +17.0 (c 0.1, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3342, 2967, 2930, 2870, 1738, 1703, 1438, 1075, 934 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz), see Table 1; <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz), see Table 2; positive ESIMS *m/z* 505 [M + H]<sup>+</sup>, 527 [M + Na]<sup>+</sup>, 1031 [2 M + Na]<sup>+</sup>; HRESIMS *m/z* 527.3343 [M + Na]<sup>+</sup> (calcd for C<sub>30</sub>H<sub>48</sub>O<sub>6</sub>Na, 527.3348).

**Glauclactone (5):** white powder (Me<sub>2</sub>CO); mp 251–253 °C;  $[\alpha]_D^{25}$  –18.9 (c 0.1, pyridine); IR (KBr)  $\nu_{\max}$  3555, 3433, 2943, 2921, 1710, 1630, 1047 cm<sup>-1</sup>; <sup>1</sup>H NMR (C<sub>2</sub>D<sub>5</sub>N, 400 MHz)  $\delta$  7.35 (1H, d, *J* = 4.0 Hz, OH-21), 5.49 (1H, brs, OH-3), 5.25 (1H, brs, H-29 $\alpha$ ), 5.23 (1H, brs, H-29 $\beta$ ), 4.68 (1H, t, *J* = 3.3 Hz, H-21), 4.39 (1H, m, H-22), 3.90 (1H, brs, H-3), 2.94 (1H, m, H-19eq), 2.60 (1H, td, *J* = 13.1, 4.9 Hz, H-11ax), 2.44 (1H, brd, *J* = 13.3 Hz, H-19ax), 2.11 (1H, m, H-2eq), 2.03 (1H, m, H-12eq), 1.91 (1H, dd, *J* = 12.4, 3.2 Hz, H-1ax), 1.87 (1H, brs, H-18), 1.77 (2H, m, H-11eq, H-16eq), 1.72 (1H, dt, *J* = 12.8, 2.9 Hz, H-6eq), 1.66 (1H, dd, *J* = 11.1, 3.1 Hz, H-8), 1.61 (1H, dt, *J* = 13.2, 3.6, H-2ax), 1.55 (1H, m, H-16ax), 1.54 (3H, s, H-28), 1.53 (1H, m, H-1eq), 1.51 (1H, m, H-15eq), 1.42 (1H, dd, *J* = 11.6, 7.9 Hz, H-15ax), 1.37 (1H, m, H-7eq), 1.32 (2H, m, H-7ax, H-12ax), 1.26 (3H, s, H-24), 1.17 (1H, brd, *J* = 11.4 Hz, H-10), 1.10 (4H, overlap, H-4, H-23), 0.95 (3H, s, H-25), 0.92 (1H, m, H-6ax), 0.91 (3H, s, H-26); <sup>13</sup>C NMR (C<sub>2</sub>D<sub>5</sub>N, 100 MHz)  $\delta$  175.2 (C-27), 144.5 (C-20), 118.7 (C-29), 87.4 (C-22), 75.5 (C-21), 71.4 (C-3), 61.1 (C-10), 50.0 (C-8), 49.9 (C-13), 49.6 (C-4), 41.6 (C-6), 41.5 (C-18), 40.2 (C-14), 38.1 (C-5), 37.3 (C-11), 37.2 (C-16), 36.5 (C-2, C-9), 34.4 (C-15), 33.0 (C-17), 27.9 (C-19), 25.2 (C-12), 24.6 (C-28), 19.1 (C-25), 17.9 (C-7), 16.9 (C-26), 16.6 (C-1), 16.7 (C-24), 12.5 (C-23); positive ESIMS *m/z* 457 [M + H]<sup>+</sup>, 479 [M + Na]<sup>+</sup>, 914 [2 M + 2H]<sup>+</sup>; HRESIMS *m/z* 479.3133 [M + Na]<sup>+</sup> (calcd for C<sub>29</sub>H<sub>44</sub>O<sub>4</sub>Na, 479.3137).

**Bioassay.** The inhibitory activities of the compounds on human and mouse 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 were determined using a scintillation proximity assay (SPA) with microsomes containing 11 $\beta$ -HSD1 or 11 $\beta$ -HSD2, as described in a previous study.<sup>13</sup> Briefly, the full-length cDNAs of human or murine 11 $\beta$ -HSD1 and 11 $\beta$ -HSD2 were isolated from the cDNA libraries provided by the NIH Mammalian Gene Collection and cloned into a pcDNA3 expression vector. HEK-293 cells were transfected with the pcDNA3-derived expression plasmid and selected after cultivation in the presence of 750  $\mu$ g/mL of G418. The microsomal fraction overexpressing 11 $\beta$ -HSD1 or 11 $\beta$ -HSD2 was prepared from the HEK-293 cells stably transfected with either 11 $\beta$ -HSD1 or 11 $\beta$ -HSD2 and used as the enzyme source for SPA. Microsomes containing human or mouse 11 $\beta$ -HSD1 were incubated with NADPH and [<sup>3</sup>H]cortisone, and then the product [<sup>3</sup>H]cortisol was specifically captured by a monoclonal antibody coupled to protein A-coated SPA beads. 11 $\beta$ -HSD2 screening was performed by incubating 11 $\beta$ -HSD2 microsomes with [<sup>3</sup>H]cortisol and NAD<sup>+</sup> and monitoring substrate disappearance. The experiments were repeated twice, and IC<sub>50</sub> values were calculated using Prism Version 4 (GraphPad Software, San Diego, CA, USA) and expressed as average values. Glycyrrhizic acid was set as the positive control for

the human and mouse 11 $\beta$ -HSD1 and human 11 $\beta$ -HSD2 assay, whereas carbenoxolone was used as the positive control for the mouse 11 $\beta$ -HSD2 assay.

## ■ ASSOCIATED CONTENT

### Supporting Information

HRESIMS and 1D and 2D NMR spectra of the new compounds (1–5); ESIMS and <sup>1</sup>H and <sup>13</sup>C NMR spectra of compounds 6 and 7. This material is 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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