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Cycloartane and Friedelane Triterpenoids from the Leaves of *Caloncoba glauca* and Their Evaluation for Inhibition of 11β-Hydroxysteroid Dehydrogenases

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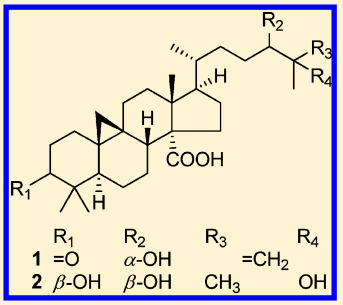
Supporting Information

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 3β,21β-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 11β-hydroxysteroid dehydrogenase (11β-HSD1 and 11β-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) 11β-HSD1.

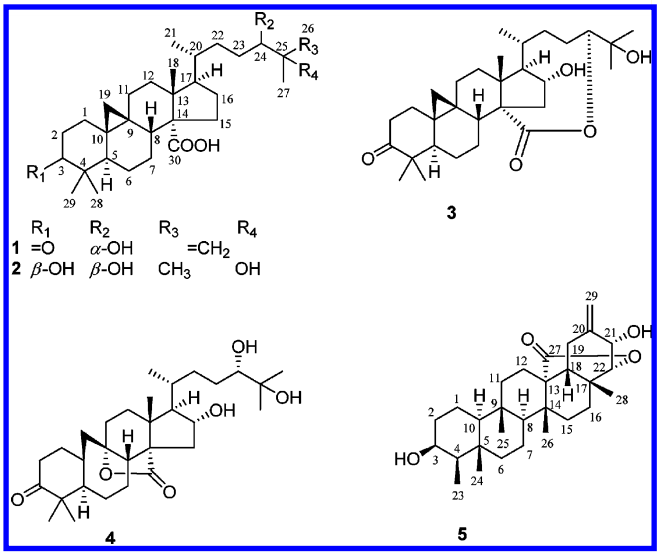


Caloncoba glauca (P. Beauv.) Gilg. (Flacourtiaceae) is a tree common in the tropical rain forests of Western Cameroon.[1,2] Information collected directly from traditional healers has indicated that the leaves are used as a purgative, while the fruits, leaves, and stem bark are used to treat inflammation 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.[1,3] 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 11β-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 3β,21β-dihydroxy-30-nor-(D:A)-friedo-olean-20(29)-en-27-oic acid (**6**)[3] and acetyltrichadenic acid B (**7**).[4]

Compound **1** was obtained as a white powder. The molecular formula was determined as $C_{30}H_{46}O_4$ by positive-ion HRESIMS, which showed a pseudomolecular ion peak at m/z 493.3305 $[M + Na]^+$, in conjunction with the NMR data. The IR spectrum showed absorptions for hydroxy (3425 cm^{-1}) and carbonyl (1708 and 1693 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) showed singlets for four tertiary methyl groups at δ_H 0.99, 1.09, 1.10, and 1.67, a secondary methyl at



δ_H 0.89 (3H, d, J = 6.2 Hz), a terminal methylene at δ_H 4.77 (1H, brs) and 4.86 (1H, brs), and characteristic cyclopropane methylene proton signals at δ_H 0.51 (1H, d, J = 4.2 Hz) and 0.86 (1H, overlapped), indicating a cycloartane-type skeleton.[5] The signals at δ_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.[6] The ^1H NMR spectrum also showed a signal of an oxymethine proton at δ_H 3.92 (1H, t, J = 6.4 Hz).

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Table 1. ^{1}H NMR Data of Compounds 1−4 (δ in ppm, J in Hz)

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

[a]Run in $CDCl_3$. Other spectra run in $CDCl_3$−MeOD. [b]Overlapped signals within a column.

Table 2. ^{13}C NMR Data of Compounds 1−4 (δ in ppm)

position	1	2	3[a]	4[a]
1	34.1, CH_2	32.7, CH_2	33.2, CH_2	28.9, CH_2
2	37.9, CH_2	30.4, CH_2	37.3, CH_2	36.8, CH_2
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_2	21.4, CH_2	20.8, CH_2	25.3, CH_2
7	27.4, CH_2	27.8, CH_2	26.5, CH_2	23.9, CH_2
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_2	28.6, CH_2	28.2, CH_2	33.9, CH_2
12	34.3, CH_2	34.5, CH_2	33.4, CH_2	34.6, CH_2
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_2	32.4, CH_2	42.1, CH_2	35.2, CH_2
16	30.1, CH_2	28.8, CH_2	78.4, CH	76.7, CH
17	52.9, CH	53.3, CH	57.6, CH	60.9, CH
18	18.4, CH_3	18.5, CH_3	18.6, CH_3	15.5, CH_3
19	30.5, CH_2	31.4, CH_2	30.8, CH_2	39.9, CH_2
20	36.2, CH	36.2, CH	32.9, CH	32.8, CH
21	18.8, CH_3	18.6, CH_3	18.5, CH_3	18.2, CH_3
22	32.5, CH_2	33.9, CH_2	31.3, CH_2	31.2, CH_2
23	31.9, CH_2	30.1, CH_2	25.7, CH_2	25.4, CH_2
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_2	25.5, CH_3	28.3, CH_3	25.9, CH_3
27	17.5, CH_3	24.5, CH_3	27.8, CH_3	23.5, CH_3
28	22.4, CH_3	25.8, CH_3	22.1, CH_3	25.5, CH_3
29	21.3, CH_3	14.4, CH_3	20.9, CH_3	22.2, CH_3
30	179.7, C	180.0, C	173.2, C	182.7, C

[a]Run in $CDCl_3$. Other spectra run in $CDCl_3$−MeOD.

The ^{13}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 δ_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 ^{13}C NMR spectrum also showed signals at δ_C 148.1 (C-25) and 111.3 (C-26), assignable to two olefinic carbons. The ^{1}H−^{1}H COSY spectrum (Figure 1a) together with the HSQC data revealed several proton spin systems, among which two −CH_2−CH_2− units, a −CH−CH_2−CH_2−CH− unit, and a −CH(−OH)−CH_2−CH_2−CH−CH_3 unit were evident. In the HMBC spectrum (Figure 1a), correlations between H-11ax (δ_H 2.27)/H-11eq (δ_H 1.32) and C-19 (δ_C 30.5), C-9 (δ_C 21.1), and C-10 (δ_C 27.9) gave evidence of a cyclopropane ring (C-9, C-10, and C-19).[7] This spectrum also exhibited long-range correlations from H_3-28 (δ_H 0.99) and H_3-29 (δ_H 1.09) to C-3 (δ_C 219.4), C-4 (δ_C 50.7), and C-5 (δ_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 (δ_H 1.78) and H_2-15 (δ_H 2.11 and 1.21) to C-14 (δ_C 63.2) and C-30 (δ_C 179.7). Further HMBC correlations of the deshielded methyl proton H_3-27 (δ_H 1.67) and the H_2-26 terminal methylene protons (δ_H 4.77, 4.86) with the oxymethine carbon C-24 (δ_C 76.7), as well as between the oxymethine proton H-24 (δ_H 3.92) and C-27 (δ_C 17.5), C-26 (δ_C 111.3) C-25 (δ_C 148.1), and C-23 (δ_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_3-18 and H-8 and H-20, on one hand, and H_3-28 and H-5, H-5 and H-7ax, H-7ax and H-17, and H-17 and H_3-21 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_3-18 with H-23β and that of H-23β with H-24. Therefore, compound **1** was characterized as 24(*S*)-hydroxy-3-

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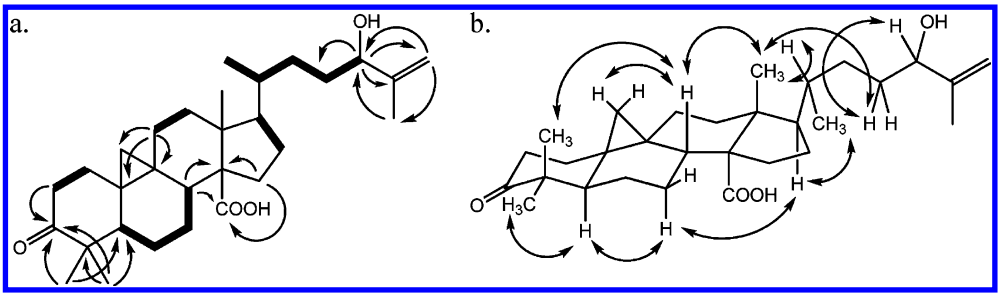

Figure 1. (a) Selected HMBC (H→C) and ^{1}H–^{1}H COSY (—) correlations of **1**. (b) Selected ROESY correlations of **1**.

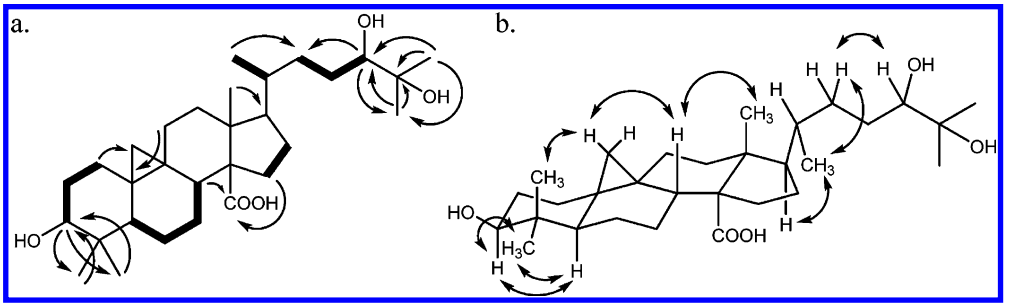

Figure 2. (a) Selected HMBC (H→C) and ^{1}H–^{1}H COSY (—) correlations of **2**. (b) Selected ROESY correlations of **2**.

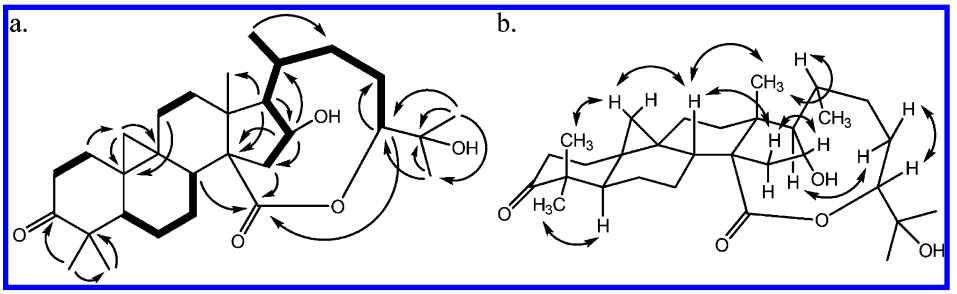

Figure 3. (a) Selected HMBC (H→C) and ^{1}H–^{1}H COSY (—) correlations of **3**. (b) Selected ROESY correlations of **3**.

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 $C_{30}H_{50}O_5$ on the basis of positive-ion HRESIMS (m/z 513.3565 $[M + Na]^+$). Its IR spectrum showed absorption bands at ν_{max} 3427 cm^{-1} (hydroxy) and 1686 cm^{-1} (carboxy), but did not show any carbonyl group absorption as in **1**. The ^{1}H NMR spectrum (Table 1) exhibited typical signals of a cycloartane triterpene including a characteristic pair of doublets at δ_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 δ_H 1.04 (J = 5.5 Hz, Me-21) and five tertiary methyl singlets at δ_H 0.93 (Me-29), 1.07 (Me-28), 1.24 (Me-18), and 1.28 (Me-26 and Me-27). The ^{13}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, δ_C 73.6 and C-3, δ_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 H_3-26 and 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 (δ_H 3.36) and C-28 and C-29 and between H_3-28 and C-3 as well as between 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 H_3-28 (Figure 2b) enabled the β-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 H_3-21 and H-22α, and H-22α and H-24. All these data suggest that compound **2** (caloncobic acid B) is a stereoisomer of protolyofoligenic acid,[8,9] and it was thus characterized as 3β,24(R),25-trihydroxycycloartan-30-oic acid.

The molecular formula of compound **3** was established as $C_{30}H_{46}O_5$ by HRESIMS analysis (m/z 995.6600 $[2 M + Na]^+$) in conjunction with the NMR data. The IR spectrum exhibited absorption bands for hydroxy (3474, 3417 cm^{-1}) and carbonyl (1709 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 δ_C 216.4 (C-3) suggested that these two compounds are both based on a cycloartane skeleton. Further analysis of the ^{1}H NMR spectrum indicated two relatively deshielded methyl proton signals at δ_H 1.21 (H_3-26) and 1.40 (H_3-27) and revealed their attachment to the hydroxylated C-25 carbon (δ_C 72.4); this was supported by the HMBC correlations from H_3-26 and H_3-27 to C-25. The ^{1}H NMR spectrum also showed

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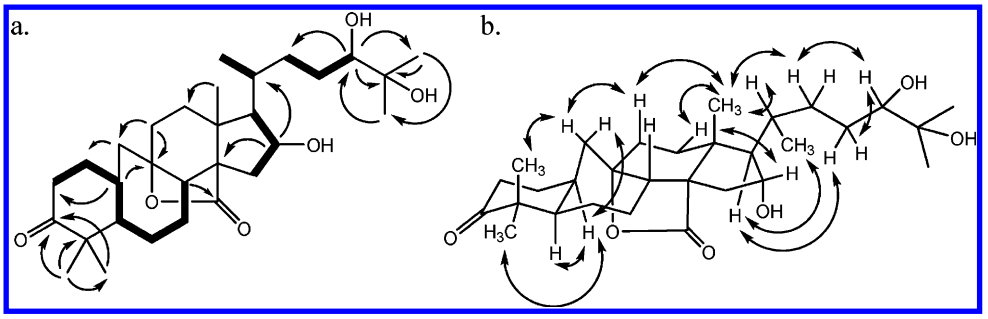

Figure 4. (a) Selected HMBC (H→C) and ^{1}H–^{1}H COSY (—) correlations of **4**. (b) Selected ROESY correlations of **4**.

signals of two oxymethine protons at δ_H 4.10 (H-16, brt, J = 8.1 Hz) and 4.77 (H-24, dd, J = 10.1, 2.4 Hz). The ^{13}C NMR spectrum exhibited a signal at δ_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_2-15 and C-30. Pertinent correlations in the latter spectrum were also observed between H_3-26 and the carbon resonances at δ_C 81.2 (C-24) and 27.8 (C-27) and between H_3-27 and C-24 and C-26 (δ_C 28.3). Further long-range ^{1}H–^{13}C couplings were observed between the H-16 proton (δ_H 4.10) and C-20 (δ_C 32.9), C-15 (δ_C 42.1), and C-14 (δ_C 60.9), between H-17 and C-14, C-16 (δ_C 78.4) and C-18, and between H_2-15 (δ_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 β-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 α-configuration of the hydroxy group at C-16 was confirmed by the ROESY correlations of H_3-18 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-23β; H-23α and H-17; and H-17 and H_3-21. Compound **3** (caloncobalactone A) was thus determined as 16α,25-dihydroxy-3,30-dioxocycloartan-30,24(*R*)-lactone.

Compound **4** was obtained as a white powder. Its molecular formula was deduced as $C_{30}H_{48}O_6$ on the basis of positive-ion HRESIMS (m/z 527.3343 $[M + Na]^+$) 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^{-1}) and carbonyl (1738, 1703 cm^{-1}) groups. The ^{13}C NMR spectrum (Table 2) revealed the presence of two carbonyl groups at δ_C 182.7 (C-30) and 216.2 (C-3) and four oxygenated carbons at δ_C 72.8 (C-25), 76.7 (C-16), 78.4 (C-24), and 88.4 (C-9). In the ^{1}H NMR spectrum (Table 1), five quaternary methyl signals at δ_H 0.93 (H_3-18), 1.06 (H_3-29), 1.12 (H_3-27), 1.13 (H_3-28), and 1.16 (H_3-26) and a secondary methyl group signal at δ_H 0.88 (d, J = 6.4 Hz, H_3-21) were observed. This spectrum also showed two oxymethine protons at δ_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_2-11 (δ_H 1.71, 1.69) and C-9 and C-19. A long-range correlation between H_2-11 and C-10 evident for compounds **1**–**3** was missing, as well the ^{1}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.[10] 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_2-15 and C-30 and between H-10/H_2-12 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 (δ_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_3-26 and C-24, C-25, and C-27, and between H_3-27 and C-24, C-25, and C-26. The ROESY correlations (Figure 4b) of H-19β/H_3-29 and H-11ax; H_3-18/H-11ax, H-8, H-20, H-12eq, and H-22β; H-12eq/H-16; and H-24/H-22β and H-23β, on one hand, and H-23α/H-17; H_3-21/H-17; and H-10/H-19α, H_3-28, and H-5 on the other hand showed that Me-29, Me-18, H-8, H-16, and H-24 are all β-oriented, whereas Me-28, Me-21, H-17, H-10, and H-5 are α-oriented. Consequently, the configuration of C-24 was determined to be *S*. Compound **4** (caloncobalactone B) was thus elucidated as 16β,24(*S*),25-trihydroxy-3,30-dioxo-9,10-*seco*-cycloartan-30,9α-lactone.

Compound **5** was obtained as a white powder. Its molecular formula was deduced as $C_{29}H_{44}O_4$ on the basis of the pseudomolecular ion peak at m/z 479.3133 $[M + Na]^+$ in the HRESIMS. This molecular formula accounted for eight degrees of unsaturation. The IR spectrum exhibited absorption bands corresponding to hydroxy (3555, 3433 cm^{-1}), carbonyl (1710 cm^{-1}), and exocyclic methylene (3075*w*, 1639 cm^{-1}) functionalities. Its ^{13}C NMR data were closely related to those of 3β,21β-dihydroxy-30-(D:A)-friedo-olean-20(29)-en-27-oic acid (**6**),[3] 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 δ_C 12.5 was suggestive of a friedelane-type triterpene skeleton with a hydroxy group at C-3.[1,3] The presence of a carbonyl group was confirmed by the signal at δ_C 175.2 (C-27). Further oxygenated carbon atoms were observed at δ_C 75.5 (C-21) and 87.4 (C-22). Its ^{1}H NMR spectrum revealed the presence of an exocyclic methylene group at δ_H 5.23 and 5.25 (each 1H, brs, H_2-29) and three oxymethine protons at δ_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 δ_H 0.91 (H_3-26), 0.95 (H_3-25), 1.26 (H_3-24), and 1.54 (H_3-28). 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 δ_H 1.10. The HMBC

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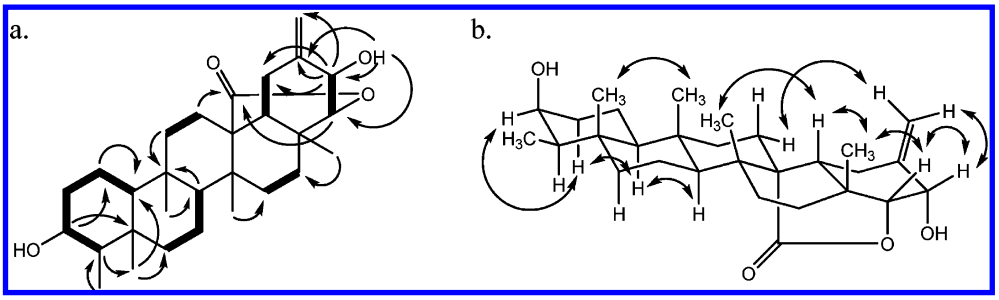

Figure 5. (a) Selected HMBC (H→C) and ^{1}H–^{1}H COSY (—) correlations of **5**. (b) Selected ROESY correlations of **5**.

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 (δ_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}H NMR data as well as the analysis of the ROESY spectrum (Figure 5b). In the ^{1}H NMR spectrum, the H-3 proton was observed as a broad singlet ($W_{1/2}$ = 8.2 Hz), which indicated its α-equatorial orientation.[3] The β-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 β-oriented.[11] Their orientation was confirmed by the ROESY correlations between H-21 and H-22; H-22 and H_3-28; H_3-28 and H-18; and H-18 and H_3-26. Therefore, compound **5** (glaucalactone) was characterized as 3β,21α-dihydroxy-27-oxo-30-nor-(D:A)-friedo-olean-20(29)-en-27,22α-lactone.

The enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) can influence factors affecting metabolic syndrome such as insulin resistance and dyslipidemia.[12] The inhibitory effects of compounds **1**–**7** on mouse and human 11β-HSD1 and 11β-HSD2 were evaluated. All assays were carried out in duplicate with glycyrrhizinic acid and carbenoxolone as positive controls. Compounds **1** and **2** showed inhibitory activities against mouse and human 11β-HSD1 (EC_{50} 132 and 13 nM and EC_{50} 105 and 72 nM, respectively) (Table 3). Compounds **3**–**7** were inactive (inhibition <50% at 1 μM).

Table 3. Inhibitory Activities of Compounds 1 and 2 against 11β-Hydroxysteroid Dehydrogenase Isozymes

compound	mouse 11β-HSD1 (IC_{50})	mouse 11β-HSD2 (IC_{50})	human 11β-HSD1 (IC_{50})	human 11β-HSD2 (IC_{50})
1	132 nM	>100 μM	105 nM	>100 μM
2	13 nM	>100 μM	72 nM	0.4 μM
glycyrrhizinic acid[a]	3 nM		6 nM	0.4 nM
carbenoxolone[a]		206 nM		

[a]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}H and ^{13}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 (δ) 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 μm, Amersham Pharmacia Biotech AB, Sweden). MPLC was performed on a Büchi Sepacore System (Büchi Labortechnik AG, Switzerland), and columns were packed with Chromatorex C_{18} (40–75 μm, Fuji Silysia Chemical Ltd., Japan) and MCI gel CHP-20P (75–150 μm, 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_2SO_4 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 × 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_2CO (100:0 to 70:30) to yield **7** (4 mg). Fraction B (13 g) was chromatographed on a MCI gel column (MeOH–H_2O, 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_2CO, 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 $CHCl_3$–MeOH (1:1), then on silica gel (petroleum ether–Me_2CO, 80:20), to yield **3** (4.6 mg). Successive chromatography of subfraction BII3 (2 g) over a reversed-phase C_{18} and over normal-phase silica gel eluted with MeOH–H_2O (50:50 to 100:0) and petroleum ether–Me_2CO, respectively, yielded **4** (41.5 mg) and **2** (5 mg). Fraction C (14 g) was chromatographed by MCI gel MPLC (MeOH–H_2O, 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 $CHCl_3$–Me_2CO (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 ($CHCl_3$–Me_2CO, 100:0 to 70:30) and passage over Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) to give **1** (13 mg).

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*Caloncobic acid A (**1**):* white powder (MeOH); mp 198−200 °C; $[\alpha]^{23}_{D}$ −35.1 (*c* 0.067, $CHCl_3$−MeOH, 1:2); IR (KBr) ν_{max} 3425, 2958, 2927, 1708 cm^{-1}; ^1H NMR ($CDCl_3$−CD_3OD, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$−CD_3OD, 100 MHz), see Table 2; positive ESIMS *m/z* 493 $[M + Na]^+$, 964 $[2 M + H + Na]^+$; HRESIMS *m/z* 493.3305 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_4Na$, 493.3293).

*Caloncobic acid B (**2**):* white powder (Me_2CO); mp 242−244 °C; $[\alpha]^{23}_{D}$ −2.0 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3427, 2964, 2930, 1686, 1637, 1629 cm^{-1}; ^1H NMR ($CDCl_3$−CD_3OD, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$−CD_3OD, 125 MHz), see Table 2; positive ESIMS *m/z* 513 $[M + Na]^+$, 1004 $[2 M + H + Na]^+$; HRESIMS *m/z* 513.3565 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_5Na$, 513.3555).

*Caloncobalactone A (**3**):* white powder (Me_2CO); mp 348−350 °C; $[\alpha]^{23}_{D}$ +70.2 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3474, 3417, 2968, 2921, 1709, 1233, 1186 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; positive ESIMS *m/z* 995 $[2 M + Na]^+$; HRESIMS *m/z* 995.6600 $[2 M + Na]^+$ (calcd for $[C_{30}H_{46}O_5]_2Na$, 995.6588).

*Caloncobalactone B (**4**):* white powder (Me_2CO); mp 194−196 °C; $[\alpha]^{23}_{D}$ +17.0 (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3342, 2967, 2930, 2870, 1738, 1703, 1438, 1075, 934 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; positive ESIMS *m/z* 505 $[M + H]^+$, 527 $[M + Na]^+$, 1031 $[2 M + Na]^+$; HRESIMS *m/z* 527.3343 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_6Na$, 527.3348).

*Glaucalactone (**5**):* white powder (Me_2CO); mp 251−253 °C; $[\alpha]^{23}_{D}$ −18.9 (*c* 0.1, pyridine); IR (KBr) ν_{max} 3555, 3433, 2943, 2921, 1710, 1630, 1047 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ 7.35 (1H, d, *J* = 4.0 Hz, OH-21), 5.49 (1H, brs, OH-3), 5.25 (1H, brs, H-29α), 5.23 (1H, brs, H-29β), 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); ^{13}C NMR (C_5D_5N, 100 MHz) δ 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 $[M + H]^+$, 479 $[M + Na]^+$, 914 $[2 M + 2H]^+$; HRESIMS *m/z* 479.3133 $[M + Na]^+$ (calcd for $C_{29}H_{44}O_4Na$, 479.3137).

Bioassay. The inhibitory activities of the compounds on human and mouse 11β-HSD1 and 11β-HSD2 were determined using a scintillation proximity assay (SPA) with microsomes containing 11β-HSD1 or 11β-HSD2, as described in a previous study.[13] Briefly, the full-length cDNAs of human or murine 11β-HSD1 and 11β-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 μg/mL of G418. The microsomal fraction overexpressing 11β-HSD1 or 11β-HSD2 was prepared from the HEK-293 cells stably transfected with either 11β-HSD1 or 11β-HSD2 and used as the enzyme source for SPA. Microsomes containing human or mouse 11β-HSD1 were incubated with NADPH and [^{3}H]cortisone, and then the product [3H]cortisol was specifically captured by a monoclonal antibody coupled to protein A-coated SPA beads. 11β-HSD2 screening was performed by incubating 11β-HSD2 microsomes with [^{3}H]cortisol and NAD^+ and monitoring substrate disappearance. The experiments were repeated twice, and IC_{50} values were calculated using Prism Version 4 (GraphPad Software, San Diego, CA, USA) and expressed as average values. Glycyrrhizinic acid was set as the positive control for the human and mouse 11β-HSD1 and human 11β-HSD2 assay, whereas carbenoxolone was used as the positive control for the mouse 11β-HSD2 assay.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

HRESIMS and 1D and 2D NMR spectra of the new compounds (**1**−**5**); ESIMS and ^1H and ^{13}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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