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PENTACYCLIC TRITERPENES FROM *COMBRETUM NIGRICANS*

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Key Word Index—*Combretum nigricans*; Combretaceae; bark; arjungenin; combregenin; arjunglucoside; combreglucoside; pentacyclic triterpenes.

Abstract—From the bark of *Combretum nigricans*, four pentacyclic triterpenes have been isolated and their structures elucidated from spectral data as the known arjungenin and arjunglucoside, a new pentacyclic triterpene, combregenin (2α,3β,6β,19α,23-pentahydroxyolean-12-en-28-oic acid) and a new saponin, combreglucoside (β-D-glucopyranosyl 2α,3β,6β,19α,23-pentahydroxyolean-12-en-28-oate).

INTRODUCTION

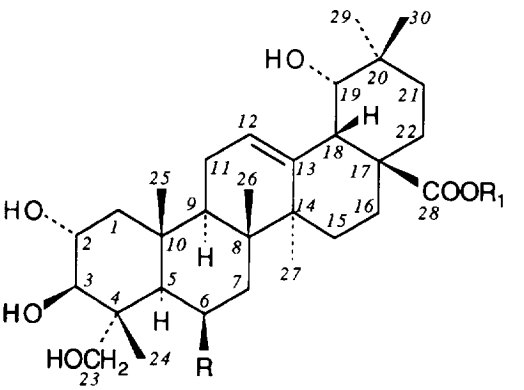
Combretum nigricans is a small tree widespread in the savanna of the Central African Republic [1]. The plant is used in folk medicine for the treatment of gastrointestinal diseases and also as a fish poison [2]. In a continuation of our phytochemical study on this genus [3], the stem bark components of *C. nigricans* have been examined. Previous studies on this species only reported the characterization of its gum, which is used as an adulterant and misrepresented as gum arabic [4]. This paper reports on the isolation, from the stem bark, of two new triterpenes, combregenin (**1**) and its glucoside, combre-glucoside (**2**), along with the known arjungenin (**3**) and arjunglucoside (**4**).

RESULTS AND DISCUSSION

The stem bark of *C. nigricans* was extracted successively with methylene chloride and methanol. The methanol extract yielded two pentacyclic triterpenic acids, **1** and **3**, and their glucosides, **2** and **4**.

Compound **1** gave rise to IR absorption bands for a carboxylic acid and a vinyl bond at ν_{max} 1696 and 1640 cm^{-1}, respectively. The CI mass spectrum showed $[M + H]^+$ at *m/z* 521, and the FAB HR mass spectrum $[M + Na]^+$ at *m/z* 543.3290, corresponding to the molecular formula $C_{30}H_{48}O_7$. The ^{13}C NMR spectrum displayed signals for 30 carbon atoms: one carbonyl (δ_C182.4), two ethylenic carbon atoms (–C=C<), four oxymethines, one oxymethylene, six methyls, seven methylenes, three methines and six quaternary carbon atoms (Table 1). The six methyls were tertiary as shown by the ^1H NMR spectrum and the olefinic proton gave a signal at δ_H5.37.

In the ^1H COSY spectrum, the proton at δ_H3.08 (H-18) was coupled with H-12 (δ_H5.37, broad singlet), H-16 (δ_H 2.25, *W* coupling), H-19 (δ_H3.29) and the methylene at C-11. The signal for H-18 was a broad singlet, indicating its β-disposition relative to the D-ring. Hydroxymethine H-19 was assigned the β configuration relative to the E-ring, as it gave a ^3J coupling constant value of 4 Hz with H-18, indicating they were in a *cis* relative configuration. The presence of the double bond at C-12 was confirmed by the chemical shifts of C-12 (δ_C124.9) and C-13 (δ_C143.9), characteristic of a Δ^{12}-oleonene [5].



	R	R_1
1	OH	H
2	OH	Glucose
3	H	H
4	H	Glucose

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Table 1. ^{1}H NMR data for compound **1** (CD_3OD) and its derivatives **2** (pyridine-d_5) and **5** ($CDCl_3$)

	1		**2**		**5**	
C	δ_H *m*	*J* (Hz)	δ_H *m*	*J* (Hz)	δ_H *m*	*J* (Hz)
1ax	0.87 *br t*	12	1.39 *br t*	13	1.06 *m*	
eq	1.90 *m*		2.30 *dd*	13, 4	1.96 *m*	
2ax	3.76 *ddd*	12, 10, 5	4.40 *m*		5.19 *ddd*	10, 10, 4
3ax	3.31 *d*	10	3.93 *m*		4.97 *d*	10
5ax	1.25 *br s*		1.93 *br s*		1.36 *br s*	
6eq	4.40 *br s*		5.01 *br s*		4.31 *br s*	
7eq	1.50 *m*		2.00 *m*		1.65 *m*	
ax	1.75 *br t*	12	2.00 *m*		1.35 *br t*	13
9	1.86 *m*		2.16 *m*		1.78 *m*	
11	2.05 *m*		2.34 *m*		1.95 *m*	
	2.05 *m*		2.15 *m*		2.09 *m*	
12	5.37 *br s*		5.55 *m*		5.46 *br t*	3
15ax	1.76 *br t*	10, 4	2.45 *br t*	13	1.35 *m*	
eq	1.05 *m*		1.25 *br d*	13	1.00 *m*	
16ax	2.25 *t d*	11, 4	2.78 *br t*	13	2.24 *br t*	13
eq	1.65 *m*		2.10 *m*		1.59 *br d*	13
18	3.08 *br s*		3.46 *br s*		3.04 *br s*	
19eq	3.29 *d*	4	3.55 *d*	4	3.31 *d*	4
21ax	1.63 *m*		1.97 *m*		1.69 *m*	
eq	1.01 *m*		1.00 *br t*	12	1.05 *br t*	10
22a	1.80 *m*		2.00 *m*		1.67 *m*	
b	1.56 *m*		1.90 *m*		1.67 *m*	
23a	3.59 *d*	11	4.35 *d*	11	3.68 *d*	12
b	3.46 *d*	11	4.03 *d*	11	3.90 *d*	12
24	1.08 *s*		1.69 *s*		1.25 *s*	
25	1.38 *s*		1.75 *s*		1.42 *s*	
26	1.07 *s*		1.70 *s*		0.95 *s*	
27	1.29 *s*		1.51 *s*		1.17 *s*	
29	0.95 *s*		1.10 *s*		0.93 *s*	
30	0.98 *s*		0.92 *s*		0.91 *s*	
1′	—		6.25 *d*	8	5.54 *d*	8
2′	—		4.14 *t*	9	5.15 *dd*	8, 8
3′	—		4.20 *t*	9	5.25 *dd*	8, 8
4′	—		4.29 *t*	9	5.09 *dd*	10, 8
5′	—		4.20 *m*		3.76 *ddd*	10, 4, 2
6′a	—		4.35 *m*		4.25 *dd*	12, 4
6′b	—		4.35 *m*		4.02 *dd*	12, 2
Ac	—		—		1.95 *s*	
Ac	—		—		1.97 *s*	
Ac	—		—		1.99 *s*	
Ac	—		—		1.99 *s*	
Ac	—		—		2.00 *s*	
Ac	—		—		2.02 *s*	
Ac	—		—		2.02 *s*	

The hydroxymethylene C-23 at δ_C65.9 gave long-range couplings with the protons of the methyl-24 (δ_H1.08). Hydroxymethines at δ_H3.76 and 3.31 were assigned at −2 and −3, respectively; the value of their vicinal coupling constant (10 Hz) indicated a *trans*-diaxial disposition, and the values of their ^{13}C chemical shifts were in agreement with those of 2α,2β,23-trihydroxylated oleanes [5]. C-3 gave long-range correlations with protons at C-23 and C-24.

The remaining hydroxymethine carbon (δ_C68.7) was linked with the proton at δ_H4.40 and located at C-6 as shown by the ^{1}H–^{1}H COSY correlations between this proton and both H-5 and methylene-7. The H-6 signal was a broad singlet, indicating that this proton was α-equatorial. Combregenin was thus assigned the structure **1** (2α,3β,6β,19α,23-pentahydroxyolean-12-en-28-oic acid).

Compound **2** was obtained as an amorphous solid. The positive FAB-HR mass spectrum showed the pseudomolecular ion $[M + Na]^+$ at *m/z* 705.3827, corresponding to the molecular formula $C_{36}H_{58}O_{12}$. The highest ions observed at *m/z* 538 and 521 in the CI

mass spectrum were those of the pseudomolecular ($[M' + NH_4]^+$ and $[M' + H]^+$) ions of **1**.

The ^{13}C NMR spectrum of **2** displayed signals for 36 carbon atoms. Close inspection of the spectrum disclosed the same signals as those of **1**, together with six more signals between δ_C60 and 100, suggesting that **2** was a glycoside derivative of **1** (Table 1).

The aglycone moiety was confirmed by analysis of the ^1H–^1H, ^1H–^{13}C and long-range ^1H–^{13}C COSY spectra which allowed shift assignments (Tables 1 and 2). The ^{13}C chemical shifts of the glycoside part suggested that it was glucose moiety.

Compound **2** was acetylated with acetic anhydride/pyridine to yield the hepta-acetyl derivative **5**, as shown by NMR analyses (1D and 2D ^1H and ^{12}C spectra). After acetylation, the carbinol protons were well resolved in the ^1H NMR spectrum, allowing the determination of their coupling constant values. All the vicinal coupling constants of the pyranose moiety had 7–10 Hz values (Table 2), indicating a β-glucopyranose structure. The *trans*-diaxial disposition of H-2 and H-3 of the aglycone was confirmed from their coupling constant value (10 Hz), as well as the *cis* relative disposition of H-18 and H-19 (^3J = 4 Hz). The proton of the hydroxymethine C-6 was α-equatorial, as it gave only small coupling constants with both the axial methine H-5 and the methylene protons at C-7. The NOESY spectrum of **5** showed cross-peaks between H-5 and both H-3 and H-6, indicating their α-disposition. The relative stereochemistry was confirmed from the observation of correlations of H-18 with H-12 and Me-30, of H-19 with H-12 and Me-29 and Me-30, and of Me-25 with Me-26. Compound **2** was thus β-D-glucopyranosyl 2α,3β,6β,19α,23-pentahydroxyolean-12-en-28-oate.

Compound **3** had the molecular formula $C_{30}H_{48}O_6$ (CI mass spectrum) and was identified, from the analysis of its NMR spectra, as arjungenin a compound, previously isolated from *Terminalia bellerica* (Combretaceae) [6]. Spectral analysis of the glycoside **4** identified it as arjunglucoside, previously characterized in several species of Combretaceae (*T. bellerica* [6] and *T. arjuna* [7]), Rosaceae (*Rubus* species [8]) and Apocynaceae (*Trachelospermum asiaticum* [9]).

Table 2. ^{13}C NMR data for compounds **1** and **3** (CD_3OD), **2** (pyridine-d_5), **4** (DMSO-d_6) and **5*** ($CDCl_3$)

C	1	2	3	4	5
1	49.7	49.5	46.9	46.7	45.3
2	69.6	68.9	69.2	67.6	69.8
3	78.2	78.9	78.5	77.8	74.9
4	44.7	44.3	43.6	42.7	42.4
5	49.2	49.1	48.3	46.4	48.5
6	68.7	67.6	18.9	17.8	67.9
7	41.2	41.1	33.5	28.5	40.9
8	39.9	39.5	40.3	39.3	38.8
9	49.4	48.8	48.4	47.5	48.0
10	38.6	38.1	38.8	37.7	37.5
11	24.7	24.2	24.5	23.5	23.6
12	124.9	123.8	124.5	122.6	124.9
13	143.9	143.5	144.0	143.4	141.3
14	43.0	42.6	42.2	41.4	41.8
15	29.5	28.9	29.0	32.0	27.9
16	28.5	28.0	28.2	28.0	27.3
17	46.6	46.3	46.1	45.5	45.5
18	45.1	44.4	44.6	43.4	43.3
19	82.4	80.9	82.1	80.2	81.5
20	35.9	35.4	35.5	35.0	34.6
21	29.3	28.9	28.9	27.2	27.8
22	34.0	32.8	32.9	32.0	31.9
23	65.9	66.3	67.0	64.1	65.2
24	15.1	15.7	13.5	13.8	15.2
25	18.8	18.7	17.3	16.9	18.1
26	18.4	18.4	17.5	16.9	17.9
27	25.1	24.7	25.0	24.5	24.5
28	182.4	177.1	181.9	176.1	175.8
29	28.7	28.6	28.5	28.3	27.7
30	25.2	24.7	25.0	24.7	24.3
1′	—	95.7	—	94.3	91.8
2′	—	73.8	—	72.5	69.9
3′	—	78.4	—	76.7	72.8
4′	—	70.9	—	69.6	67.9
5′	—	78.4	—	75.8	72.5
6′	—	61.9	—	60.8	61.4

*Acetyl groups of **5** had signals at δ21.0, 20.8, 20.7, 20.6, 20.6, 20.5 and 20.5 (Me) and 170.8, 170.5, 170.4, 170.3, 170.1, 169.1 and 168.8 (CO).

EXPERIMENTAL

General. NMR: 300 MHz (^1H) and 75 MHz (^{13}C), or 500 MHz (^1H) and 125 MHz (^{13}C), the chemical shifts were measured either from 1D or from 2D COSY spectra for complex entangled systems, with TMS as int. standard; FAB-MS: Nermag N10-10 mass spectrometer; CI-MS; Nermag R30-10 mass spectrometer.

Plant material. The stem bark of *C. nigricans* Lepr. ex Guill. et Perrot was collected near Damara (Central African Republic) in May 1994. A voucher specimen is deposited at the National Herbarium of the Forest Ministry of the Central African Republic.

Extraction and isolation. The air-dried and pulverized stem bark of *C. nigricans* (911 g), was extracted at room temp. with CH_2Cl_2 and then with MeOH. The latter was concd to dryness under red. pres. to yield a dark red residue (118 g). A part (70 g) was fractionated by reversed-phase chromatography (RP2) eluted with a H_2O–MeOH gradient starting with H_2O, and afforded 4 frs: F1 (7:3, 48.4 g), F2 (1:1, 6.8 g), F3 (1:3, 2.5 g) and F4 (0:1, 5.4 g). Fr. F3 on CC over silica gel (CH_2Cl_2–MeOH, 4:1) yielded (**4**) (0.18 g) and (**2**) (0.37 g). Fr. F4 on CC over silica gel eluted with CH_2Cl_2–MeOH (9:1) gave crude arjungenin and combregenin. Further purification of each crude compound by CC (silica gel CH_2Cl_2–MeOH 23:2) yielded pure (**3**) (0.30 g) and (**1**) 0.53 g).

Combregenin (2α,3β,6β,19α,23-*pentahydroxyolean*-12-*en*-28-*oic acid*) (**1**). $C_{30}H_{48}O_7$; crystals, mp 275° (MeOH); IR (KBr), ν_{max} (cm^{-1}): 3441, 2934, 1696, 1640, 1466, 1400, 1045, 650. $[\alpha]_D^{21}$ 0 (MeOH, *c*1); CIMS (NH_3)

m/z: 538 $[M + NH_4]^+$, 521 $[M + H]^+$; EIMS m/z (rel. int.): 520 (0.5), 502 (1), 458 (9), 264 (28), 246 (32), 231 (21), 201 (56), 187, (47), 173 (54), 159 (45), 147 (36), 133 (54), 119 (89), 105 (87), 91 (100); HR FAB-MS m/z: 543.3290 $[M + Na]^+$, calc. for $C_{30}H_{48}O_7Na$: 543.3298.

Combreglucoside (β-D-*glucopyranosyl* 2α,3β,6β,19α,23-*pentahydroxyolean*-12-*en*-28-*oate*) (**2**). $C_{36}H_{58}O_{12}$; crystals, mp 260° (MeOH); IR (KBr), ν_{max} (cm^{-1}): 3394, 2934, 1730sh, 1644, 1595, 1381, 1137, 1098, 591; $[\alpha]_D^{21}$ 0 (pyridine; *c* 1); CIMS (NH_3) m/z: 538 $[M - Glc + NH_4]^+$, 521 $[M - Glc + H]^+$; FAB-MS (thioglycerol) m/z (rel. int.): 705 $[M + Na]^+$ (27), 503 (12), 429 (19), 391 (9), 360 (8), 321 (17), 269 (26), 252 (75), 237 (84), 215 (100); HR FAB-MS m/z: 705.3827 $[M + Na]^+$, calc. for $C_{36}H_{58}O_{12}Na$: 705.3828.

Combreglucoside hepta-acetate (*hepta*-O-*acetyl*-β-D-*glucopyranosyl* 2α,3β,6β,19α,23-*pentahydroxyolean*-12-*en*-28-*oate*) (**5**). $C_{50}H_{72}O_{19}$; compound **2** (10 mg) in dry pyridine (1 ml) was kept overnight with Ac_2O (0.5 ml). The reaction mixt. was poured into H_2O and extracted with CH_2Cl_2. The organic phase was dried with Na_2SO_4 evapd and chromatographed (silica gel, CH_2Cl_2–MeOH, 49:1 to yield **5**. CIMS (NH_3) m/z: 994 $[M + NH_4]^+$.

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