FULL PAPER

Combretins A and B, New Cycloartane-Type Triterpenes from *Combretum fragrans*

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The phytochemical studies on the leaves of the traditionally used medicinal plant *Combretum fragrans* F. Hoffm (Combretaceae) from Cameroon have led to the isolation of combretins A and B (**1** and **2**, resp.), two new cycloartane-type triterpenes from the AcOEt-soluble subfraction along with *β*-sitosterol (**3**), oleanolic acid (**4**), ursolic acid (**5**), and pratensein (**6**). The compounds **4** – **6** are reported for the first time from this species. The structures of the new triterpenes were elucidated by spectroscopic techniques including ^{1}H- and ^{13}C-NMR (DEPT), and 2D-NMR experiments.

Keywords: *Combretum fragrans*, Combretaceae, Combretins A and B, Cycloartanes, Triterpenes.

Introduction

Combretum fragrans F. Hoffm (Combretaceae) is a shrub or a small tree which grows up to 10 – 12 m height. It is common in deciduous woodland and wooded grassland associated with seasonally waterlogged clay soils, and sometimes also found on shallow, stony soils [1]. The bark is gray and reticulately fissured, and the branches peeling to give dark reddish color. Leaves are opposite or 3-(4)-verticillate, and the lamina is broadly ovate-elliptic. The flowers are greenish yellow to white and fragrant, particularly at night. The fruit is subcircular to elliptic in outline, yellow-brown to brown. In various parts of Africa, the plant is used for the treatment of leprosy, cough, syphilis, snakebite, diarrhea, new and chronic wounds, malaria, even septic wounds, and fungal infection of the scalp. It is also used for its aphrodisiac properties [2][3]. Roots, leaves, and stem bark extracts of this plant have been investigated and shown to possess antifungal [4 – 6], antibacterial [6][7], and antiproliferative [3] properties. Stem bark extract of C. *fragrans* have shown to exhibit significant *Clostridium chauvoei* neuraminidase enzyme inhibitory activity [8]. Previous phytochemical analyses have shown that extracts of stem barks, root, and leaves of *C. fragrans* contain flavonoids, tannins, and saponins [4][9]. *β*-Sitosterol and stigmasterol have previously been reported from the stem bark of *C. fragrans* [10].

In this article, we present the isolation and structure elucidation of combretins A and B (**1** and **2**, resp.), two new cycloartane-type triterpenes from the AcOEt-soluble subfraction of the MeOH extract of the leaves of *C. fragrans* along with *β*-sitosterol, oleanolic acid, ursolic acid, and an isoflavonoid pratensein, respectively. Aside from the sterol, the other compounds are reported for the first time from this species. Prior to our work, 23-deoxyjessic acid and 7*β*-hydroxy-23-deoxyjessic acid had been previously reported from *C. quadrangulare*, and further isolation of related compounds from *C. fragrans* is a fair indication that this class of triterpenes are of common occurrence in the genus *Combretum* [11 – 13].

Results and Discussion

The MeOH extract of the leaves of the plant was divided into fractions soluble in AcOEt, BuOH, and H_2O. Column chromatography of the AcOEt-soluble fraction provided compounds **1** – **6** as described in the *Experimental Part.*

Combretin A (**1**) was obtained as white amorphous solid ($[\alpha]_D^{28}$ = +82.14). It gave positive *Salkowski* and *Liebermann–Burchard* color reactions as well as brisk effervescence with dilute $NaHCO_3$ solution revealing a triterpene skeleton with a COOH moiety. The IR spectrum showed absorption bands of OH (3411 cm^{-1}), COOH C=O (1696 cm^{-1}), and C=C (1643 cm^{-1}). The molecular formula was determined as $C_{31}H_{50}O_4$ by HR-EI-MS showing an M^+ peak at m/z 486.3729 (calc. 486.3711). The presence of monounsaturated side chain

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was evident in the spectrum at *m/z* 359.2232 ($C_{22}H_{31}O_4$), formed by the loss of C_9H_{19} with two H-atoms transferred from the ring system [14]. It also revealed the presence of COOH moiety and OH groups in the ring system. The molecular formula was further substantiated by the broad-band-decoupled and DEPT ^{13}C-NMR spectra of **1** (*Table*) which showed 31 signals: six Me groups, eleven CH_2 groups including one olefinic C-atom at δ(C) 156.3, seven CH groups including two O-bearing C-atoms at δ(C) 78.0 and 74.9, seven quaternary C-atoms including one COOH C-atom at δ(C) 179.5, and an olefinic C-atom at δ(C) 105.6.

The ^{1}H-NMR spectrum of **1** (*Table*) displayed two upfield *doublet*s resonating as an *AX* system at δ(H) 0.60

Table. ^{1}H- and ^{13}C-NMR (600 and 150 MHz, resp., CD_3OD) data of compounds **1** and **2**. δ in ppm, *J* in Hz

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	1.28[a])	31.7	1.32[a])	33.3
	1.62[a])		1.64[a])	
2	1.58[a])	29.2	1.68[a])	30.2
	1.73[a])		1.98[a])	
3	4.02 (*dd*, *J* = 11.4, 4.8)	74.9	4.10 (br. *dd*)	86.3
4		54.4		55.5
5	1.93 (*dd*, *J* = 8.4, 4.2)	44.3	1.96 (br. *dd*)	47.5
6	0.92[a])	22.9	0.95[a])	24.3
	1.19[a])		1.22[a])	
7	2.08[a])	25.7	2.12 – 2.14 (*m*, 1 H)	27.6
	1.10[a])		1.66	
8	1.61[a])	48.8	1.65[a])	50.5
9		19.9		21.7
10		24.9		26.7
11	1.09[a])	25.5	1.18[a])	27.1
	1.62[a])		1.65[a])	
12	1.12[a])	34.8	–	34.4
	1.53[a])		–	
13		50.3		52.0
14		45.6		46.5
15	3.89 (*dd*, *J* = 9.6, 4.8)	78.0	3.26 (br. *dd*)	77.9
16	1.66[a])	39.0	1.69[a])	40.7
	1.83[a])		1.85[a])	
17	1.65[a])	50.1	1.67[a])	51.5
18	1.02 (*s*)	17.5	1.04 (*s*)	19.3
19	0.42 (*d*, *J* = 4.2)	29.9	0.46 (*d*, *J* = 4.2)	31.7
	0.60 (*d*, *J* = 4.2)		0.63 (*d*, *J* = 4.2)	
20	1.33[a])	35.7	1.35[a])	37.4
21	0.87 (*d*, *J* = 6)	17.4	0.89 (br. *d*)	19.2
22	1.68[a])	33.5	1.71[a])	35.2
	1.60[a])		1.63[a])	
23	2.11 (*td*, *J* = 9.6, 5.4)	30.9	2.10 – 2.11 (*m*, 1 H)	32.7
	1.88[a])		1.93[a])	
24		156.3		158.1
25	2.22 (*sept.*, *J* = 7.2)	33.5	2.22 (br. *sept.*)	35.3
26	1.00 (*d*, *J* = 4.8)	20.9	1.02 (br. *d*)	22.9
27	1.01 (*d*, *J* = 4.8)	21.1	1.03 (br. *d*)	22.7
28		179.5		181.3
29	1.07 (*s*)	8.6	1.16 (*s*)	11.1
30	0.94 (*s*)	10.7	0.96 (*s*)	12.6
31	4.70 (br. *d*)	105.6	4.72 (br. s)	107.4
	4.64 (br. *d*)		4.65 (br. *s*)	
1′			4.26 (*d*, *J* = 7.8)	106.6
2′			3.10 (br. *dd*)	75.5
3′			3.92 (br. *dd*)	79.7
4′			3.45 (br. *dd*)	71.5
5′			3.16 (br. *dd*)	67.2
			3.82 (br. *dd*)	

[a]) Overlapping, assignment determined by COSY, HMQC, and HMBC.

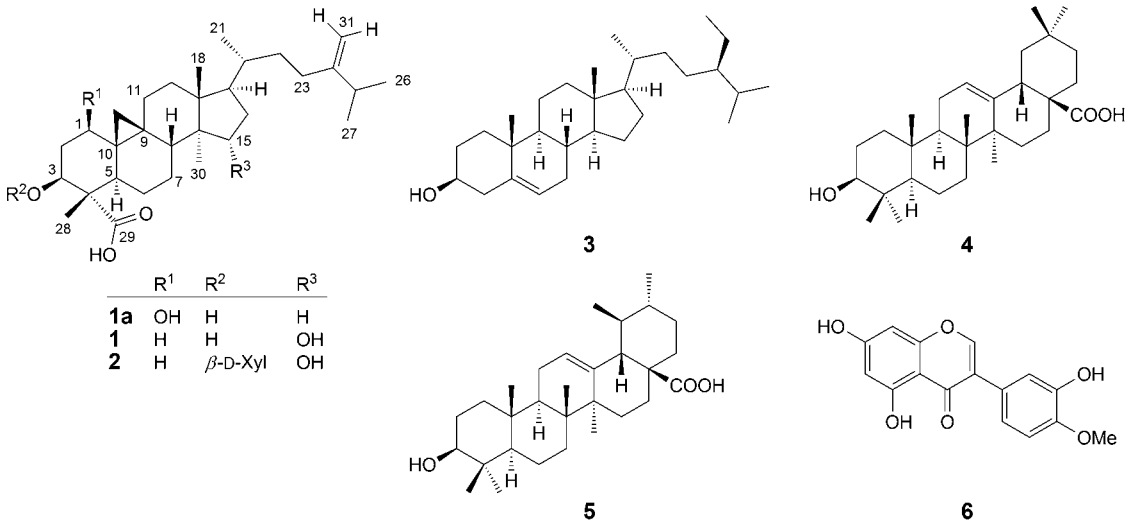

Fig. 1. Structures of the isolated compounds.

and 0.42 (both *d*, *J* = 4.2) assignable to cyclopropyl CH_2 H-atoms which are characteristic of 9*β*,19-cycloartane-type triterpenes [15 – 18]. It also displayed signals for three tertiary Me groups at *δ*(H) 1.07, 1.02, 0.94 and three secondary Me groups at *δ*(H) 1.00 and 1.01 (both *d*, *J* = 4.8), and 0.87 (*d*, *J* = 6). The signals at *δ*(H) 4.02 (*dd*, *J* = 6.6, 4.8) and 3.89 (*dd*, *J* = 7.8, 6.4) were due to H-atoms germinal to the OH groups. In addition, the two *singlet*s at *δ*(H) 4.70 and 4.64 were assigned to the terminal CH_2 H-atoms [12][13]. The structure of **1** (*Fig. 1*) was deduced by comparison of its ^{1}H- and ^{13}C-NMR data with those reported in the literature for 23-deoxyjessic acid (**1a**) [11]. The difference between the new derivative **1** and compound **1a** is the presence of a OH group at C (15) in **1**, instead of the OH group at C(1) in the case of derivative **1a**. The location of the OH group at C(15) was established according to observed HMBC cross peaks (*Fig. 2*) between H–C(15) and the signals corresponding to Me(30) group (*δ*(C) 10.7), and to C(8) (*δ*(C) 48.8). The configuration at C(3) and C(15) was determined on the basis of NOESY experiments. First, the observed correlation between H–C(3) (*δ*(H) 4.02) and *α*-axial oriented H–C(5) (*δ*(H) 1.93) established a *β*-equatorial configuration for the HO–C(3) group. Moreover, the correlation between H–C(15) (*δ*(H) 3.89) and the *β*-axial Me(18) (*δ*(H) 1.02) group suggests that H–C(15) and Me(18) are situated at the same side of the plane defined by the cyclopentane ring. Therefore, the OH group at C(15) should be *α*-equatorial oriented.

In summary, on the basis of cumulative evidence, the structure of combretin A (**1**) could be assigned as (3*β*,9*β*,15*β*)-3,15-dihydroxy-24-methylidene-9,19-cyclolanostan-28-oic acid (3*β*,15*α*-dihydroxycycloart-24(31)-en-29-oic acid).

Combretin B (**2**) was isolated as colorless amorphous solid ($[\alpha]_D^{28}$ = +97.69). It gave similar color reactions and chemical test as those of **1**. Its glycosidic nature was

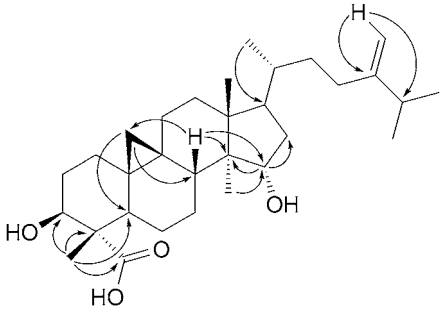

Fig. 2. Important HMBCs (H → C) of combetin A (**1**).

revealed by positive *Molisch*'s test. The molecular formula was ascertained as $C_{36}H_{58}O_8$ with the help of HR-FAB-MS (positive mode) showing *quasi*-molecular-ion peak $[M + H]^+$ at *m*/*z* 619.4228 (calc. $C_{36}H_{59}O_8$ 619.4211). The spectral data were similar to those of **1**, except the additional peaks for a pentapyranoside moiety in both ^{1}H- and ^{13}C-NMR spectra. The ^{13}C-NMR showed the anomeric C-atom at *δ*(C) 106.6, three O-bearing CH groups at *δ*(C) 79.7, 75.5, and 71.5, and one O-bearing CH_2 group at *δ*(C) 67.2. In the ^{1}H-NMR spectrum, the anomeric H-atom appeared comparatively downfield as a *doublet* at *δ*(H) 4.26. Its larger coupling constant (*J* = 7.8 Hz) confirmed the *β*-configuration at the anomeric C-atom of the sugar moiety. Other signals of the sugar residue are shown in the *Table*. On the basis of chemical shifts in both ^{1}H- and ^{13}C-NMR spectra, the sugar could be identified as D-xylose [18][19]. Comparison of the ^{13}C-NMR spectrum with that of compound **1** showed downfield shift of C(3) atom revealing the presence of *O*-*β*-D-xylopyranoside moiety at C(3). It was further confirmed through HMBC experiments that H–C(3) shown ^3J correlation with anomeric C(1′) and the anomeric H–C(1′) correlated with C(3). Further HMBCs were similar to those of

compound **1**. Thus, the structure of combretin B (**2**) could be assigned as (3*β*,9*β*,15*β*)-15-hydroxy-24-methylidene-3-(*β*-D-xylopyranosyloxy)-9,19-cyclolanostan-28-oic acid.

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Supporting Information

Supporting Information for this article is available on the WWW under http://dx.doi.org/10.1002/hlca.201600053.

Experimental Part

General

Thin-layer chromatography (TLC): silica gel F_{254} plates (SiO_2; *E. Mecrk*, Darmstadt, Germany). Column chromatography (CC): SiO_2 (250 – 400 mesh; *E. Merck*). UV Spectra: *Hitachi UV-3200* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Jasco 302-A* spectrophotometer; in KBr; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker 600* MHz instrument; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-, EI-, and HR-FAB-MS: *Joel JMS-HX-110* and *JMS-DA-500* mass spectrometers with glycerol as matrix; in *m*/*z*.

Plant Material

The leaves of *C. fragrans* were collected in February 2014 in Maroua, Far-North Province, Cameroon. The identification of the plant was performed by Mr. *Victor Nana* of the National Herbarium, Yaoundé, where a voucher specimen (30309/H.N.C.) has been deposited.

Extraction and Isolation

Dried leaves of *C. fragrans* (1.2 kg) were ground and extracted with MeOH (3 × 10 l) at r.t. After removing the solvent, the residue (126 g) was suspended in H_2O and extracted with hexane, AcOEt, and BuOH. The AcOEt-soluble fraction (48 g) was subjected to CC eluting with the mixture of hexane/AcOEt and AcOEt/MeOH in increasing order of polarity. Fractions from hexane/AcOEt 85:15 were combined and subjected to CC (SiO_2; 2 × 45 cm, 40 g), eluting with hexane/AcOEt gradient mixtures to afford *β*-sitosterol (**3**; 36 mg), oleanolic acid (**4**; 57 mg), and ursolic acid (**5**; 64 mg). Elution with hexane/AcOEt 7:3 provided a major fraction (3 g), which was further subjected to CC eluting with CH_2Cl_2 to yield combretin A (**1**; 13 mg) and pratensein (**6**; 43 mg), resp. Fractions from hexane/AcOEt 10:90 and pure AcOEt were combined and rechromatographed on SiO_2 and eluted with CH_2Cl_2/MeOH in increasing order of polarity to obtain combretin B (**2**; 11 mg).

Combretin A (= **(3*β*,9*β*,15*β*)-3,15-Dihydroxy-24-methylidene-9,19-cyclolanostan-28-oic Acid**; **1**). White amorphous solid. IR: 3411 (OH), 1696 (COOH C=O), 1643 (C=C). ^{1}H- and ^{13}C-NMR: *Table*. HR-EI-MS: 486.3729 (M^+, $C_{31}H_{50}O_4^+$; calc. 486.3711), 359.2232 ($[M - C_9H_{19}]^+$, $C_{22}H_{31}O_4^+$).

Combretin B (= **(3*β*,9*β*,15*β*)-15-Hydroxy-24-methylidene-3-(*β*-D-xylopyranosyloxy)-9,19-cyclolanostan-28-oic Acid**; **2**). Colorless amorphous solid. IR: 3418 (OH), 1709 (COOH C=O), 1644 (C=C). ^{1}H- and ^{13}C-NMR: *Table*. HR-FAB-MS: 619.4228 ($[M + H]^+$, $C_{36}H_{59}O_8^+$; calc. 619.4211).

The structures of the known compounds were elucidated through their spectroscopic data and subsequent comparison with literature values [10][17][18].

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