Alkaloids from the Stem Bark of *Turraeanthus africanus* (Meliaceae)

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Fractionation of the methanol extract of the stem bark of *Turraeanthus africanus* led to the isolation of two new alkaloids designated turraeanthin A and B, together with two known alkaloids. The structures of the new alkaloids were elucidated by means of spectroscopic analysis and characterized as 10-*O*-demethyl-17-*O*-methyl isoarnottianamide and 11-demethoxyl-12-methoxyl oxynitidine respectively.

Key words *Turraeanthus africanus*; Meliaceae; turraeanthin A; turraeanthin B; alkaloid

The genus *Turraeanthus* (Meliaceae) occurs in tropical and subtropical regions and comprises about four species in Cameroon.[1)] These species have been used in Cameroonian traditional medicine for the treatment of cardiovascular disease, stomach ache, rheumatism pains, and asthma.[2)] Phytochemical studies on the seeds of *Turraeanthus africanus* (WELW. ex. C.D.C.) PELLEGR. revealed the presence of labdane diterpenoids and a limonoid.[3)] In this paper, we describe the isolation and structural elucidation of two new alkaloids from *T. africanus*.

Results and Discussion

The dried and ground stem bark of *T. africanus* was extracted with methanol. This extract was concentrated to dryness under a vacuum. Extensive column chromatography of the residue on silica gel yielded pure 10-*O*-demethyl-17-*O*-methyl isoarnottianamide (**1**) and 11-demethoxyl-12-methoxyl oxynitidine (**2**), together with two known compounds that were characterized based on their spectral data, and identified as decarine,[4,5)] and oxynitidine.[6)]

Alkaloid **1**, obtained as brown crystals, mp 237—239 °C, reacted positively to $FeCl_3$ reagent suggesting the presence of a phenolic hydroxyl group. Its high resolution electrospray-TOF mass spectrum (HR-ES-IMS) showed a pseudomolecular ion peak $(M+H)^+$ at *m*/*z* 382.1283 (Calcd for $C_{21}H_{20}O_6N$, 382.1285). The odd mass at *m*/*z* 381 indicated that it contained a nitrogen atom in the molecule. Its IR spectrum showed absorption bands for free hydroxyl at 3450 cm^{-1}, olefin (1631, 1517 cm^{-1}), carbonyl (1663 cm^{-1}), and ether (1285, 1142 cm^{-1}) functionalities. The UV spectrum of compound **1** exhibited absorption maxima at 237, 291, and 332 nm. The ^{1}H-NMR signals at δ 2.90 (3H) and δ 8.25 (1H) were typical of an $N(CH_3)CHO$ group in compound **1**.[7)] This was confirmed in the ^{13}C-NMR spectrum by signals at δ 32.6 and 163.3. Alkaloid **1** has also characteristic signals due to four aromatic protons appearing as, singlets at δ 7.01, 7.40, 6.60, and 6.51, and a pair of doublet at δ 7.80 (1H, J=8.7 Hz) and 7.25 (1H, J=8.7 Hz). On the other hand, the singlets of the aromatic rings should be located in *para* positions, and the two doublets indicated that the two aromatic protons were

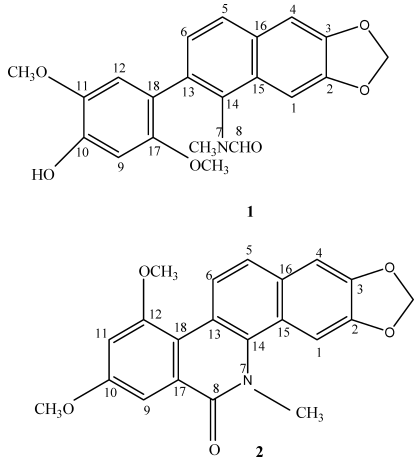

Fig. 1. Structures of Compounds **1** and **2**

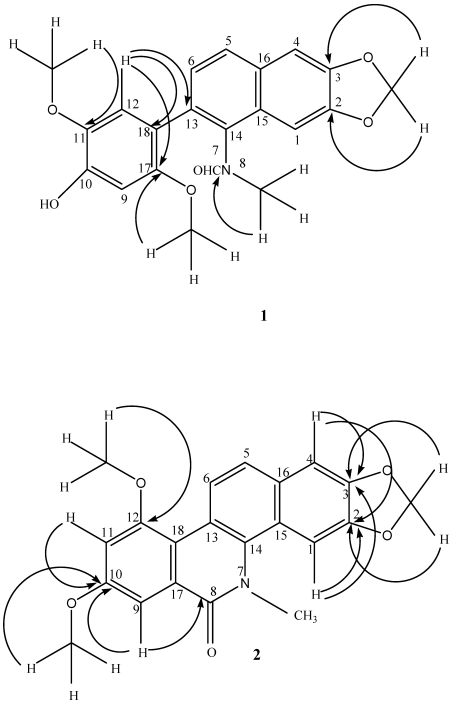

Fig. 2. Pertinent HMBC Correlations Observed in Compounds **1** and **2**

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in *ortho* positions. All these data suggest that alkaloid **1** is derived from a secobenzo[*c*]phenanthridine alkaloid.[7,8] Futhermore, the ^{1}H-NMR spectrum showed two 3H singlets at δ 3.75 and 3.63 corresponding to two methoxyl groups, and one 2H singlet at δ 6.15 ppm due to a methylenedioxy group. The positions of the hydroxyl, two methoxyl, and methylenedioxy groups on the secobenzo[*c*]phenanthridine derivative skeleton remain to be established unambiguously. The methylenedioxy group was located at positions 2 and 3. This was confirmed by the HMBC spectrum of **1** which showed, through ^2J and ^3J interactions, that a ^{1}H signal at δ 7.01 (H-1) was correlated with the ^{13}C signal at δ 148.3 (C-3, C-2), and the ^{1}H signal at δ 7.40 (H-4) exhibited correlations with the ^{13}C signal at δ 148.3 (C-3, C-2). The position of one of the methoxyl groups was established based on the NOESY spectrum of **1**, which showed spatial correlations between methoxyl protons at δ 3.63 ppm with NMe at 2.90 ppm. Thus the OMe at δ 3.63 occupied position 17. The second methoxyl substituent is located at position 11 and the hydroxyl at position 10. The *ortho* position of these two moieties was determined based on the ^{13}C-NMR spectrum which exhibited diagnostic signals at δ 149.1 and 148.7 due to *ortho* aromatic carbons bearing an oxygen atom.[9] Position 10 for the hydroxyl group was deduced on the basis of a phase-sensitive NOESY experiment, which showed interactions between the methoxyl protons at δ 3.75 and H-12 (δ 6.51). Furthermore, the HMBC spectrum of **1** showed long-range coupling of H-12 (δ 6.51) with C-18 (δ 116.2), C-17 (δ 141.4) and C-13 (δ 134.3). Based on this information, alkaloid **1**, turraeanthin A, was characterized as 10-*O*-demethyl-17-*O*-methyl isoarnottianamide.

Alkaloid **2**, mp 270—272 °C, was isolated as brown crystals of which the elemental composition was determined to be $C_{21}H_{17}NO_5$, corresponding to 14 degrees of unsaturation. The broad-band decoupled ^{13}C-NMR spectrum of alkaloid **2** showed 21 carbon signals that were determined using Jmod and HSQC techniques to be three methyls, one methylene, six methines, and quaternary carbons including a carbon (δ 164.1 ppm) of a carbonyl of amide, four oxygenated sp^2 carbons, and six sp^2 carbons. The IR spectrum of **2** indicated the presence of aromatic rings at 1609 and 1500 cm^{-1} and amide group at 1655 cm^{-1}. In the ^{1}H-NMR spectrum analyzed by ^{1}H–^{1}H COSY, a typical AB spin system of two one-proton doublets at δ 8.35 (d, J=8.5 Hz) and δ 7.70 (d, J=8.5 Hz) ppm was characteristic of a benzophenanthridine skeleton[10] and assignable to H-5 and H-6, respectively. The presence of two methoxyl groups and one NMe group was inferred from three 3H singlets at δ 3.88, 4.00 and 4.10 ppm in the ^{1}H-NMR spectrum of **2**. This was confirmed by the ^{13}C-NMR spectrum showing signals at δ 55.8, 56.1, and 41.1 ppm, respectively. The ^{1}H-NMR spectrum of alkaloid **2** exhibited a singlet of two protons at δ 6.18 ppm due to a methylenedioxy group, two singlets of one proton each at δ 7.33 and 8.25 ppm corresponding to two aromatic protons in the *para* position, and two doublets of one proton each at δ 7.94 (d, J=1.9 Hz) and 7.92 (d, J=1.9 Hz) ppm due to two aromatic protons in *meta* position. The positions of the methoxyl and methylenedioxy groups were given by HMBC correlations which showed long-range coupling of H-9 (δ 7.94) with C-8 (δ 164.1) and C-10 (δ 154.1). H-11 (δ 7.92) gave an HMBC correlation with C-10 (δ 154.1), C-12 (δ 151.2), and C-9 (δ 104.2). H-1 (δ 7.33) gave an HMBC correlation with C-2 (δ 147.8) and C-3 (δ 150.1). H-4 (δ 8.25) showed long range coupling with C-3 and C-2. This led to the conclusion that the two methoxyls are located at positions 10 and 12, and the methylenedioxy at positions 2 and 3. From all of the above spectroscopic observations, turreaeanthin B (**2**) was assigned to be 11-demethoxyl-12-methoxyl oxynitidine.

Table 1. ^{1}H and ^{13}C Assignments for Turraeanthin A (**1**) in DMSO-d_6 and Turraeanthin B (**2**) in Pyridine-d_5

Position	^{13}C (**1**)	Multiplicity	^{1}H [m, J (Hz)] (**1**)	^{13}C (**2**)	Multiplicity	^{1}H [m, J (Hz)] (**2**)
1	98.5	d	7.01 (s)	105.1	d	7.33 (s)
2	148.3	s	—	147.8	s	—
3	148.3	s	—	150.1	s	—
4	104.1	d	7.40 (s)	109.4	d	8.25 (s)
5	126.7	d	7.80 (d, 8.7)	119.4	d	8.35 (d, 8.5)
6	127.7	d	7.25 (d, 8.7)	123.5	d	7.70 (d, 8.5)
7	—	—	—	—	—	—
8	163.3	d	8.25 (s)	164.1	s	—
9	114.9	d	6.60 (s)	104.2	d	7.94 (d, 1.9)
10	147.5	s	—	154.1	s	—
11	149.1	s	—	103.2	d	7.92 (d, 1.9)
12	100.8	d	6.51 (s)	151.2	s	—
13	134.3	s	—	117.1	s	—
14	135.3	s	—	137.5	s	—
15	128.0	s	—	121.0	s	—
16	130.4	s	—	130.0	s	—
17	141.4	s	—	132.5	s	—
18	116.2	s	—	105.2	s	—
10-OMe	—	—	—	56.1	q	4.00 (s)
11-OMe	55.3	q	3.75 (s)	—	—	—
12-OMe	—	—	—	55.8	q	3.88 (s)
14-NMe	32.6	q	2.90 (s)	—	—	—
17-OMe	56.3	q	3.63 (s)	—	—	—
–NMe	—	—	—	41.1	q	4.10 (s)
–OCH_2O–	101.5	t	6.15 (s)	102.2	t	6.18 (s)

Experimental

General Experimental Procedures IR spectra were recorded on a Shimadzu 408 spectrophotometer in KBr disks. UV spectra were obtained on a Beckman model 25 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker spectrometer equipped with a 5 mm ^{1}H and ^{13}C probe operating at 300.135 and 75.469 MHz, respectively, with TMS as internal standard. Jmod and two-dimensional NMR spectra were measured with the usual pulse sequence and data processing was performed with standard software. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Silica gel, 60 GF 254, was used for TLC.

Plant Material Stem bark of *T. africanus* was collected in August 2003 at Bonepoupa, Cameroon. The sample was identified by Dr. L. Zapfack, Botanic Department, University of Yaounde I, where a voucher specimen is deposited.

Extraction and Isolation The air-dried powdered stem bark of *T. africanus* (8 kg) was extracted with MeOH at room temperature. The extract was concentrated under a vacuum to afford a viscous brown oil (150 g). The crude extract was subjected to column chromatography over Si gel 60 (70—230 mesh, ASTM, Merck) eluted with an *n*-hexane–EtOAc mixture with increasing polarity. A total of 200 fractions of *ca.* 200 ml each were collected and combined on the basis of TLC analysis leading to 12 series (A—L). The pure compounds were obtained either by direct crystallization or after futher purification on column chromatography. Series D (8.70 g) eluted with *n*-hexane–EtOAc (7 : 3), was column chromatographed over Si gel using an *n*-hexane–EtOAc mixture of increasing polarity. A total of 300 fractions of *ca.* 50 ml each were collected. The combined fractions 184—189, eluted with *n*-hexane–EtOAc (17 : 3) gave oxynitidine **4** (10 mg). Fractions 260—265, eluted with *n*-hexane–EtOAc (4 : 1) afforded turraeanthin B **2** (20 mg). Crystallization of combined fractions 87—95 of series E eluted with *n*-hexane–EtOAc (3 : 2), gave decarine **3** (150 mg). Combined fractions 96—105 of series F, eluted with *n*-hexane–EtOAc (1 : 1) yielded turraeanthin A (**1**) (50 mg).

Turraeanthin A (**1**): Brown crystals, mp 237—239 °C; acetone; IR ν_{max} (KBr) cm^{-1}: 3450, 1663, 1631, 1517, 1285 and 1142; UV λ_{max} nm (MeOH) (log ε): 237 (4.73), 291 (4.00), 332 (3.85); ^{1}H-NMR (DMSO-d_6), see Table 1; ^{13}C-NMR (DMSO-d_6), see Table 1; HR-ES-IMS *m/z* 382.1283 $[M+H]^+$ (Calcd for $C_{21}H_{20}NO_6$, 382.1285).

Turraeanthin B (**2**): Brown crystals, mp 270—272 °C; MeOH; IR ν_{max} (KBr) cm^{-1}: 1665, 1609 and 1500; ^{1}H-NMR (pyridine-d_5), see Table 1; ^{13}C-NMR (pyridine-d_5), see Table 1; HR-ES-IMS *m/z* 364.1177 $[M+H]^+$ (Calcd for $C_{21}H_{18}NO_5$, 364.1180).

Decarine[4,5] and oxynitidine[6] were identified by direct comparison of their spectral data (^{1}H-, ^{13}C-NMR and MS) with those published in the literature.

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