Indoloquinazoline Alkaloids from *Araliopsis tabouensis*

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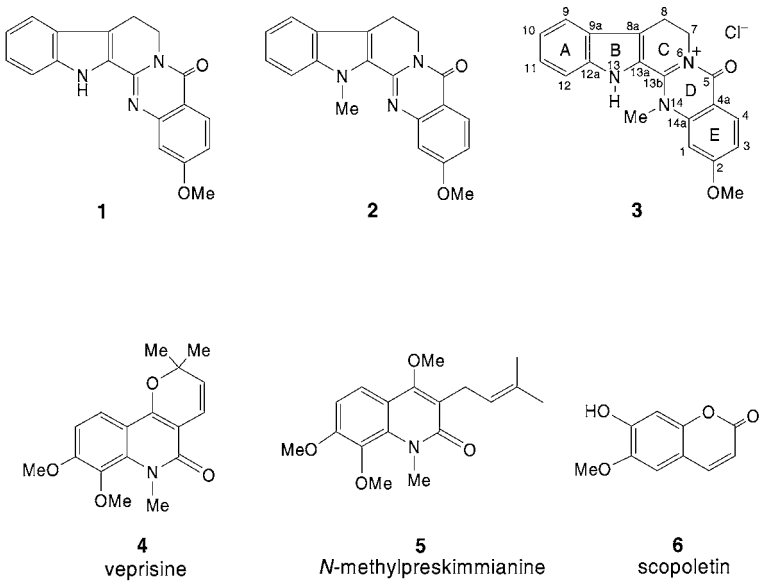
Three new indoloquinazolidine-type alkaloids, 8,13-dihydro-2-methoxyindolo[2′,3′:3,4]pyrido[2,1-*b*]quinazolin-5(7*H*)-one (**1**), 8,13-dihydro-2-methoxy-13-methylindolo[2′,3′:3,4]pyrido[2,1-*b*]quinazolin-5(7*H*)-one (**2**), and 5,8,13,14-tetrahydro-2-methoxy-14-methyl-5-oxo-7*H*-indolo[2′,3′:3,4]pyrido[2,1-*b*]quinazolim-6-iun chloride (**3**) were isolated from *Araliopsis tabouensis*, together with three known compounds. The structures of the new compounds were determined primarily from 1D- and 2D-NMR analysis. The antimalarial activities of compounds **1**–**5** were evaluated against *Plasmodium falciparum* D6 and W2 clones. The IC_{50} values in antimalarial bioassay for compounds **2**–**5** varied from 1.8 to 4.7 μg/ml.

Introduction. – *Araliopsis tabouensis* AUBREV. et PELLEGR. (Rutaceae) is a large evergreen tree that grows in the tropical forests of West and Central Africa. Its medicinal use is for the treatment of sexually transmitted diseases. An infusion of the exceedingly bitter-tasting bark is drunk as a cure for gonorrhea in the Ivory Coast [1]. In the previous chemical investigations of *A. tabouensis*, large quantities of the protolimonoid triterpene flindissol, quinoline, and indoloquinazoline type alkaloids were isolated and identified [2–4].

As part of our ongoing biological evaluation of West-African medicinal plants, we undertook a bioactivity-guided phytochemical investigation of the stem bark of *A. tabouensis*, which resulted in the isolation of indoloquinazoline and quinoline alkaloids. Their structures were assigned by spectroscopic methods (IR, HR-ESI-MS, 1D (^{1}H and ^{13}C), and 2D NMR (G-DQF-COSY, G-HMQC, G-HMBC, and ^{15}N,^{1}H G-HMBC)). The present study describes the structure elucidation of the new indoloquinazoline alkaloids **1**–**3**.

Results and Discussion. – Bioassay-guided fractionation of *A. tabouensis* resulted in the isolation of the three new indoloquinazoline alkaloids **1**–**3**, as well as of three known compounds, veprisine (**4**), *N*-methylpreskimmianine (**5**), and scopoletin (**6**). The known compounds were identified on the basis of comparison of their spectral data with the reported values [4][5].

Compound **1** was isolated as a yellow amorphous powder. The molecular formula of **1** was determined as $C_{19}H_{15}N_3O_2$ by HR-ESI-MS (positive mode), which exhibited ions at *m/z* 318.1247 ($[M+H]^+$), 340.1064 ($[M+Na]^+$), and 635.2321 ($[2M+H]^+$). Based



on further spectral data, the structure of **1**, a new natural product, was established as 2-methoxyrutaecarpine (= 8,13-dihydro-2-methoxyindolo[2′,3′:3,4]pyrido[2,1-*b*]quinazolin-5(7*H*)-one).

The ^{1}H-NMR spectrum of **1** exhibited a deshielded proton at δ 13.1 (*s*, H–N(13)), an MeO group at δ 3.77 (*s*), and two symmetrical *t* at δ 3.07 ($J = 6.3$ Hz) and 4.55 ($J = 6.3$ Hz). The chemical shifts of the latter protons are typical of the CH_2(7) and CH_2(8) protons of quinazolinecarboline (indoloquinazoline)-type alkaloids [6] [7]. This assumption was supported by the ^{13}C-NMR data, which indicated characteristic CH_2(7) and CH_2(8) signals at δ 41.3 and 19.9, respectively, based on the G-HMQC connectivities. In addition, the ^{1}H-NMR spectrum showed seven aromatic protons of an *ortho*-disubstituted (δ 7.27 (*t*, $J = 7.5$ Hz, H–C(10)); 7.44 (*t*, $J =$ 7.6 Hz, H–C(11)); 7.74 (*d*, $J = 7.8$ Hz, H–C(9)); 7.80 (*d*, $J = 8.0$ Hz, H–C(12)) and one trisubstituted aromatic ring (δ 7.09 (*d*, $J = 8.8$ Hz, H–C(3)); 7.15 (br. *s*, H–C(1)); 8.44 (*d*, $J = 8.8$ Hz, H–C(4)). The substitution pattern and the δ(H) and δ(C) values of the *ortho*-disubstituted aromatic ring (δ 120.7 (C(9)), 120.6 (C(10)), 125.5 (C(11)), and 113.1 (C(12)); ring A), and the signals attributed to rings C and D (above-mentioned CH_2 signals, and δ 118.5 (C(8a)), 128.6 (C(13a)), 150.7 (C(13b)), 115.6 (C(4a)), and 161.3 (C(5)), each quaternary C-atom) were in clear agreement with those reported for rutaecarpine [6–8], except for the differences associated with the presence of a MeO group (ring E). The combined use of G-DQF-COSY, G-HMQC, and G-HMBC experiments with **1** permitted the complete assignment of the indoloquinazoline skeleton and its substitution pattern. Thus, the deshielded ^{1}H-NMR signal at δ 13.11 showed correlations with two quaternary C-atoms at δ 140.0 and 128.6, which were attributed to C(12a) and C(13a), respectively. The aromatic proton observed at δ 8.44 (H–C(4)) indicated that it was adjacent to an electron-withdrawing substituent. This assumption was supported by long-range correlation in the G-HMBC spectrum between H–C(4) and C(5) at δ 161.3, assigned to the amide carbonyl group. H–C(4) also exhibited HMBC connectivity with another downfield-shifted C-atom at δ 164.8 (C(2)) indicating a substitution of the latter with an electron-donating group, either an OH or a MeO group. C(2) showed correlations with the MeO protons (δ 3.77) in the G-HMBC spectrum, hence allowing its unambiguous assignment and locating the MeO group at C(2). Moreover, the position of the MeO group was confirmed by the ^{1}H,^{15}N-NMR HMBC correlations between H–C(1) (δ 7.15, br. *s*) and N(14) (δ 223.1), which ruled out the possibility of MeO substitution.

The HR-ESI-MS of **2** displayed a molecular ion at *m/z* 332.1405 ($[M+H]^+$, $C_{20}H_{17}N_3O_2^+$), which was 14 mass units higher than that of **1**, confirming the presence of

an extra Me group. The signals arising from the indoloquinazoline skeleton of **2** and **1** were superimposible, with the exception of an additional signal at δ 4.45 (*s*, 3 H) in the ^{1}H-NMR spectrum, implying the presence of a MeN group. The assignment of the ^{1}H- and ^{13}C-NMR signals of **2** was secured by G-DQF-COSY, G-HMQC, and G-HMBC experiments. The location of the extra Me group was confirmed by a ^{1}H,^{15}N NMR HMBC experiment, which showed correlation between the MeN protons (δ 4.45) and N(13) (δ 131.0).

Based on these observations, the structure of **2** was elucidated as 2-methoxy-13-methylrutaecarpine (= 8,13-dihydro-2-methoxy-13-methylindolo[2′,3′:3,4]pyrido[2,1-*b*]quinazolin-5(7*H*)-one). Compound **3** was isolated as a yellow amorphous solid. The HR-ESI-MS of **3** displayed a molecular ion at *m/z* 332.1313 (M^+), supporting a molecular formula of $C_{20}H_{17}N_3O_2$. On the basis of the spectral evidence, the structure of **3** was established as 5,8,13,14-tetrahydro-2-methoxy-14-methyl-5-oxo-7*H*-indolo[2′,3′:3,4]pyrido[2,1- *b*]quinazolin-6-ium chloride.

The ^{1}H-NMR spectrum of **3** displayed the typical spin patterns of compounds **1** and **2**: seven aromatic-proton signals, of which two appeared as *t* at δ 7.28 (J = 7.5 Hz) and 7.46 (J = 7.5 Hz) attributed to H–C(10) and H–C(11), four as *d* at δ 7.73 (J = 8.5 Hz), 7.77 (J = 8.5 Hz), 7.79 (J = 9.0 Hz), and 6.22 (J = 1.0 Hz) assigned to H–C(12), H–C(9), H–C(4), and H–C(1), and one as *dd* at δ 6.31 (J = 9.5, 1.0 Hz) attributed to H–C(3); a MeO signal at δ 3.66 (*s*, 3 H); the symmetrical *t* at δ 3.38 (J = 6.3 Hz, 2 H) and 4.58 (J = 6.3 Hz, 2 H) correlating with δ(C) 47.2 (*t*) and 20.3 (*t*) in the G-HMQC spectrum, indicating the presence of the CH_2(7) and CH_2(8) groups. Three N-signals at δ 149.0, 128.9, and 63.2 correlating with proton signals at δ 3.38 (CH_2(8)), 13.3 (H–N(13)), and 2.71 (Me–N(14)), respectively, were observed in the ^{15}N,^{1}H NMR HMBC spectrum (*Fig.*). Detailed examination of 1D and 2D NMR spectra of **3** indicated that **3** differs from **2** and **1** in the position of the double bond in ring C/D (N(6)=C(13b) for **3**; N(14)=C(13b) for **2**), implying a quaternary N-atom, and the position of *N*-methyl group (N(14) for **3**; N(13) for **2**).

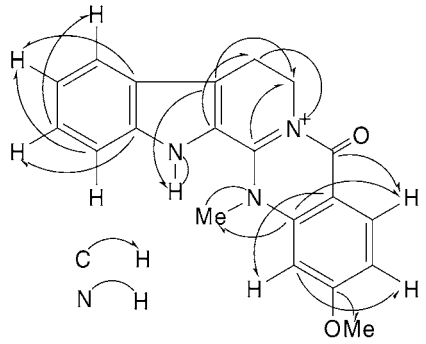


Figure. Selected ^{1}H,^{13}C- and ^{1}H,^{15}N HMBC of compound **3**

In regard to the rearrangement of rings C and D, five factors were taken into consideration: *i*) The N-signal attributed to N(6) appears at δ 149.0, *i.e.*, *ca.* 15.0 ppm upfield-shifted compared to the N(6) signal of compound **1** (δ 164.0). *ii*) H–C(1) is upfield-shifted (δ 6.22, (*d*, J = 1.0 Hz) for **3** and 7.15 (br. *s*) for **1**), *i.e.*, it is not deshielded due to the absence of the N(14)=C(13b) bond. *iii*) C(5) and C(13b) are downfield shifted (C(5): δ 175.1 for **3** and 161.3 for **1**, $\Delta\delta \approx 14$ ppm; C(13b): δ 161.3 for **3** and 146.6 for **1**, $\Delta\delta \approx 15$ ppm) because of the positive charge at N(6). *iv*) The ^{15}N,^{1}H-NMR HMBC shows correlations (see *Fig.*) between N(6) (δ 149.0) and the CH_2(7) and CH_2(8) protons (δ 3.38, 4.58), and between N(14) (δ 63.2) and the Me group at δ 2.71 and the aromatic H–C(1) at δ 6.22. *v*) The HR-ESI-MS spectra of **1** and **2** provided $[M+H]^+$ and $[M+Na]^+$ ions, while the HR-ESI-MS of **3** gave only a molecular ion M^+ due to the positive charge in the molecule.

Compounds **2**–**5** were active against *Plasmodium falciparum* D6 and W2 clones (*Table*). Although compounds **1**–**3** were from the same chemical class with minor differences, only compounds **2** and **3** exhibited significant antimalarial activity, while compound **1** unexpectedly lacked activity (see *Table*). Compounds **1**–**5** showed no cytotoxicity.

Table. *Antiprotozoal Activities* (IC_{50} in μg/ml) *of Compounds* **1**–**5**

	1	**2**	**3**	**4**	**5**	Artemisinin
P. falciparum (D6 Clone)	n.a.[a])	1.8	3.3	2.0	2.1	< 0.026
P. falciparum (W2 Clone)	n.a.[a])	> 4.7	> 4.7	> 4.7	1.8	< 0.026

[a]) n.a. = not active.

Experimental Part

1. *General.* TLC: precoated silica gel *250F* plates (*Baker*); eluents: $CHCl_3$/MeOH 90 : 10 and 85 : 15, $CHCl_3$/MeOH/H_2O 80 : 20 : 1, 80 : 20 : 2, and 70 : 30 : 3; visualization: *Dragendorff* reagent. Column chromatography (CC): silica gel (230–400 mesh), reversed phase *C-18* (40 mμ), *Merck*, or *Sephadex* (*LH-20*). UV: λ_{max} (log ε) in nm. 1D and 2D NMR Spectra: *Bruker® Avance-DRX 500-FT* spectrometer operating at 500 (^{1}H) and 125 (^{13}C) MHz; δ in ppm rel. to $SiMe_4$ for ^{1}H and ^{13}C, and rel. to liq. ammonia (by calibrating nitromethane to δ 380.2) for ^{15}N, coupling constants *J* in Hz; ^{13}C-multiplicities by a DEPT experiment. HR-ESI-FT-MS: *Bruker BioApex FT* mass spectrometer in ESI (electrospray ionization) positive mode.

2. *Plant Material.* The plant material was collected from Owi, Cross River State, Nigeria, on the 6th of December 2001 and identified by Mr. *Ozioko*, a taxonomist at the *Bioresources Development and Conservation Programme* (BDCP) at Nsukka. Voucher specimens are deposited at the BDCP Herbarium.

3. *Extraction and Isolation.* The stem bark (2 kg) of *A. tabouensis* was extracted at r.t. with MeOH: 340 g of extract. Thereof, a portion (60 g) was suspended in 1.0 N HCl (1 l) and then ammonia was added and the mixture partitioned with $CHCl_3$ to give a crude alkaloid extract. The alkaloid extract (8.0 g) was submitted to CC (silica gel, MeOH/$CHCl_3$ gradient), and the eluates were pooled (TLC) into 17 major fractions: *Fr. I–XVII*. *Fr. VII* (114 mg) was purified by gel filtration (*Sephadex LH-20*, MeOH), followed by centrifugal chromatography (*Chromatotron* system, model *8924*, 4-mm plate, flow rate 8–10 ml/min, $CHCl_3$MeOH mixtures): 200 mg of **3** (EC-AT-01). *Fr. XIII* and *XIV* (59.7 mg and 49.8 mg, resp.) precipitated: 109.5 mg of **1** (EC-AT-02). *Fr. VIII* (65.6 mg) was chromatographed (*Chromatotron*, $CHCl_3$/MeOH mixtures): 15.7 mg of **2** (EC-AT-06). Further chromatography (SiO_2, *Sephadex LH-20*, *Chromatotron*) resulted in the isolation of the known compounds **4**–**6** (24.6, 44.5, and 12.4 mg, resp.).

4. *Antimalarial Assay.* The *in vitro* antimalarial activity was determined against two strains of *Plasmodium falciparum*, D6 (chloroquine-sensitive) and W2 (chloroquine-resistant). The assay is based on the determination of parasite LDH activity using *Malstat*™ reagent [9]. The two clones are subcultered daily with fresh medium and blood cells, gassed with a mixture of 90% N_2, 5% O_2, and 5% CO_2, and incubated at 37°. On the day of assay, a suspension of infected red blood cells (2% parasitemia and 2% hematocrit) is prepared from type-A human red blood cells (*Interstate Blood Bank*, Memphis, TN) in RPMI 1640 medium supplemented with 10% human serum (*Interstate Blood Bank*, Memphis, TN) and amikacin (60 mg/ml, *Sigma*, St. Louis, MO). To a 96-well flat-bottomed microplate, 200 μl of the cell suspension is added along with 10 μl of the samples, which have been diluted in the medium, in duplicate. The plate is placed into a modular incubation chamber (*Billups-Rothenberg*, CA) and flushed with the gas mixture. The chamber containing the plates is then placed in a 37° incubator for 48 h. After incubation, the cultures are mixed, and 20 μl from each well is transferred to another microplate containing the *Malstat*™ reagent, and this plate is incubated at r.t. for 30 min. Then 20 μl of NBT (nitroblue tetrazolium chloride)/PES (phenazine ethosulfate) 1 : 1 (*Sigma*, St. Louis, MO) is added, and the plate is incubated in the dark for 1 h. The reaction is then stopped by the addition of 100 μl of a 5% AcOH soln. to each well. The plate is read at 650 nm on an *EL-340-Biokinetic* reader (*Bio-Tek Instruments*, Vermont). IC_{50} is

calculated from dose curves of growth inhibition. Chloroquine and artemisin are included as control drugs in each assay.

5. *New Alkaloids. 2-Methoxyrutaecarpine* (**1**). Yellow amorphous powder. UV (MeOH): 214 (1.95), 242 (1.11), 324 (0.70), 376 (1.42) ^{1}H-NMR (500 MHz, C_5D_5N): 13.11 (*s*, H–N(13)); 8.44 (*d*, *J* = 8.8, H–C(4)); 7.80 (*d*, *J* = 8.0, H–C(12)); 7.74 (*d*, *J* = 7.8, H–C(9)); 7.44 (*t*, *J* = 7.6, H–C(11)); 7.27 (*t*, *J* = 7.5, H–C(10)); 7.15 (br. *s*, H–C(1)); 7.09 (*d*, *J* = 8.8, H–C(3)); 4.55 (*t*, *J* = 6.8, CH_2(7)); 3.77 (*s*, MeO); 3.07 (*t*, *J* = 6.8, CH_2(8)). ^{13}C-NMR (125 MHz, in C_5D_5N): 164.8 (*s*, C(2)); 161.3 (*s*, C(5)); 150.7 (*s*, C(14a)); 146.6 (*s*, C(13b)), 140.0 (*s*, C(12a)); 129.1 (*d*, C(4)); 128.6 (*s*, C(13a)); 126.3 (*s*, C(9a)); 125.5 (*d*, C(11)); 120.7 (*d*, C(9)); 120.6 (*d*, C(10); 118.5 (*s*, C(8a)); 115.6 (*s*, C(4a)); 115.7 (*d*, C(3)); 113.1 (*d*, C(12)); 108.5 (*d*, C(1)); 55.7 (*q*, MeO); 41.3 (*t*, C(7)); 19.9 (*t*, C(8)). HR-ESI-MS: 318.1247 ($[M+H]^+$, $C_{19}H_{15}N_3O_2^+$; calc. 318.1242); 340.1064 ($[M+Na]^+$), 635.2321 ($[2M+H]^+$).

2-Methoxy-13-methylrutaecarpine (**2**). Yellow amorphous powder. UV (MeOH): 212 (1.80), 326 (0.90), 372 (1.52). ^{1}H-NMR (500 MHz, CD_3OD): 8.02 (*d*, *J* = 9.0, H–C(4)); 7.84 (*d*, *J* = 8.2, H–C(12)); 7.81 (*d*, *J* = 2.6, H–C(1)); 7.69 (*d*, *J* = 8.4, H–C(9)); 7.67 (*dd*, *J* = 2.6, 9.0, H–C(3)); 7.53 (*t*, *J* = 7.5, H–C(11)); 7.30 (*t*, *J* = 7.5, H–C(10); 4.58 (*t*, *J* = 6.7, CH_2(7)); 4.45 (*s*, MeN); 4.00 (*s*, MeO); 3.39 (*t*, *J* = 6.7, CH_2(8)). ^{13}C-NMR (125 MHz, in CD_3OD): 161.8 (*s*, C(2)); 160.1 (*s*, C(5)); 150.6 (*s*, C(14a)); 144.1 (*s*, C(13b)); 135.7 (*s*, C(12a)); 132.3 (*s*, C(13a)); 130.9 (*d*, C(4)); 126.8 (*s*, C(9a)); 125.8 (*d*, C(11)); 123.9 (*d*, C(10)); 123.0 (*d*, C(9)); 122.3 (*s*, C(8a)); 121.9 (*d*, C(12)); 121.7 (*s*, C(4a)); 115.0 (*d*, C(3)); 111.0 (*d*, C(1)); 57.4 (*q*, MeO); 42.1 (*t*, C(7)); 20.7 (*t*, C(8)). HR-ESI-MS: 332.1405 ($[M+H]^+$, $C_{20}H_{17}N_3O_2^+$; calc. 332.1400).

*5,8,13,14-Tetrahydro-2-methoxy-14-methyl-5-oxo-7*H-*indolo[2′,3′:3,4]pyrido[2,1-*b*]quinazolin-6-ium Chloride* (**3**). Yellow amorphous powder. UV (MeOH): 212 (1.91), 284 (0.49), 362 (0.70), 376 (0.35). ^{1}H-NMR (500 MHz, C_5D_5N): 13.30 (*s*, H–N(13)); 7.79 (*d*, *J* = 8.8, H–C(4)); 7.77 (*d*, *J* = 8.0, H–C(9)); 7.73 (*d*, *J* = 7.5, H–C(12)); 7.46 (*t*, *J* = 7.5, H–C(11)); 7.28 (*t*, *J* = 7.5, H–C(10)); 6.31 (*dd*, *J* = 9.0, 1.0, H–C(3)); 6.22 (*d*, *J* = 1.0, H–C(1)); 4.18 (*t*, *J* = 6.3, CH_2(7)); 3.66 (*s*, MeO); 3.38 (*t*, *J* = 6.3, CH_2(8)). ^{13}C-NMR (125 MHz, C_5D_5N): 175.1 (*s*, C(5)); 164.6 (*s*, C(2)); 161.3 (*s*, C(13b)); 153.3 (*s*, C(14a)); 138.4 (*s*, C(12a)); 135.2 (*d*, C(4)); 126.9 (*s*, C(13a)); 124.9 (*d*, C(11)); 124.7 (*s*, C(9a)); 121.3 (*s*, C(8a)); 120.3 (*d*, C(9)); 119.6 (*d*, C(10)); 112.2 (*d*, C(12)); 109.1 (*s*, C(4a)); 101.8 (*d*, C(3)); 94.0 (*d*, C(1)); 55.7 (*q*, MeO); 47.2 (*t*, C(7)); 20.3 (*t*, C(8)). HR-ESI-MS: 332.1313 (M^+, $C_{20}H_{18}N_3O_2^+$; calc. 332.1399).

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