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ALKALOIDS OF *PTEROTABERNA INCONSPICUA*

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Key Work Index—*Pterotaberna inconspicua*; Apocynaceae; seeds; root bark; indole alkaloids.

Abstract—Sixteen alkaloids were isolated from the seeds and root-bark of *Pterotaberna inconspicua*, collected in Zaïre. They were voacangine 3-carbonitrile, voacangine, voacristine, 3,6-oxido-voacangine, vobasine, 10-hydroxycoronaridine, 10-hydroxyheyneanine, methuenine, apparicine, tubotaiwine, 16-epi-isositsirikine, 16-epimethuenine, 3,14-dihydroellipticine, an isomer of corynantheol, 16-epimethuenine *N*-oxide and tetrahydroellipticine.

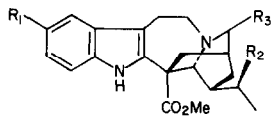
INTRODUCTION

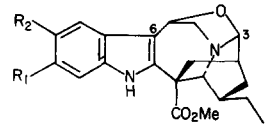
Pterotaberna inconspicua Stapf belongs to a monotypic genus from central west Africa [1]. Although fairly abundant in Zaïre, its alkaloidal content has never been examined to the best of our knowledge. It is the purpose of this communication to report on the indole alkaloids found in *P. inconspicua*. Extractions were conducted in the usual fashion on the seeds and root-bark, which respectively contained 10.3 and 13.6 g/kg of crude alkaloid mixture (AM). They were separated and purified by a combination of crystallization, CC and thick layer chromatography.

RESULTS AND DISCUSSION

The seeds were defatted in petrol and the alkaloids separated by an acid–base extraction; after this treatment another extraction was performed with ethyl acetate. The petrol extract (8.4 g/kg) showed one spot on TLC which was identified as voacangine **1**. The ethyl acetate extract (1.9 g/kg) was more complex but CC allowed the isolation and identification of seven components. They were by order of increasing polarity: voacangine 3-carbonitrile **2** (0.35% AM), voacangine **1** (83% AM; 8.7 g/kg), voacristine **3** (0.4% AM), 3, 6-oxido-voacangine **4** (0.5% AM), vobasine **5** (1.5% AM), 10-hydroxycoronaridine **6** (0.3% AM) and 10-hydroxyheyneanine **7** (0.2% AM). Derivatives **1**, **3**, **5**, **6** and **7** were identified by direct comparison with reference samples. Among these, **6** and **7**, still unknown at the beginning of this work have recently been isolated from *Tabernanthe pubescens* (**6**, **7**)[2] and *Ervatamia heyneana* (**6**)[3]. Compound **4** possessed spectral properties reminiscent of those of alkaloid D of *Muntafara sessilifolia* **8**[4]; among these, the mass spectrum showed a M^+ at *m/z* 382 with unusual fragmentations at *m/z* 368 ($M^{+\cdot} - 14$) and 364 ($M^{+\cdot} - 16$). This pattern, typical of the 3, 6-oxido iboga skeleton, has previously been observed by us and others[3–5]. Striking differences between **4** and **8** were noted in the UV spectra (maxima at 225, 285 and 300 (sh) for **4** vs 225, 270, 295 nm for **8**) and also in the aromatic part of the ^1H NMR spectrum. These data fitted a 3, 6-oxidovoacangine hypothesis which was confirmed by partial synthesis of **4** by I_2–$NaHCO_3$ mediated oxidation of voacangine.

The last unknown compound **2** displayed in its mass spectrum an odd M^+ at *m/z* 393, 25 amu higher than that of the corresponding ion of voacangine **1**. The presence of a nitrile was deduced from the IR spectrum (2220 cm^{-1}) and it was located at C-3 by synthesis based on related work in the coronaridine series by one us (T.M.)[6]. Thus, dichlorodicyanobenzoquinone oxidation of voacangine, under Yonemitsu's conditions[7], gave a single compound in all respects identical to **2**; this unusual oxidation and cyanide transfer will be the object of a forthcoming publication[8]. Finally, compound **2** was reduced to **1** ($NaBH_4$, MeOH), as would be expected for an α-amino nitrile.



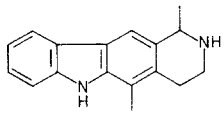


1 R_1 = OMe, R_2 = R_3 = H
2 R_1 = OMe, R_2 = H, R_3 = CN
3 R_1 = OMe, R_2 = OH, R_3 = H
6 R_1 = OH, R_2 = R_3 = H
7 R_1 = R_2 = OH, R_3 = H

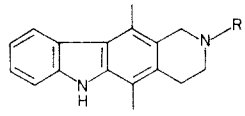
4 R_1 = H, R_2 = OMe
8 R_1 = OMe, R_2 = H

Ten alkaloids were separated from the root-bark alkaloid mixture. They are, in order of increasing polarity: vobasine **5** (1.1% AM), methuenine **9** (34% AM), apparicine **10** (0.03% AM), (+)-tubotaiwine **11** (0.02% AM), 16-epi-isositsirikine **12** (0.02% AM), 16-epimethuenine **13** (28% AM), 3, 14-dihydroellipticine **14** (0.03% AM) [9], an isomer of corynantheol **15** (0.01% AM), 16-epimethuenine *N*-oxide **16** (0.01% AM) and tetrahydroellipticine **17** (0.05% AM)[10]. Alkaloids **5**, **10** and **11** were identified by direct comparison with authentic samples. 16-Epi-isositsirikine **12** was identified with the least polar reduction product of geissoschizine according to ref. [12]. Alkaloids **14** and **17** were identified by comparison of their spectral properties with lit. data [9, 10]; compound **17**

which showed in its mass spectrum a misleading peak at $M^{+\cdot} - 15$ was, at one time, erroneously thought to be janetine [11]. Due to the low solubility of **17**, it was methylated according to a modified Eschweiler–Clarke reaction, yielding **18**, which showed in its ^1H NMR spectrum three 3-proton singlets ruling out the tetrahydroolivacine (janetine) structure.

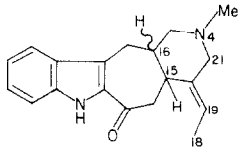


Janetine



17 R = H Tetrahydroellipticine
18 R = Me

Methuenine **9** was authenticated by direct comparison with a sample kindly provided by Dr P. Potier [13]. Alkaloids **9** and **13** presented very similar spectral properties but subtle differences in the NMR spectral were observed for the signals of the ethylidene side-chain (deshielded, δ 0.05) in **13** and also in the δ 2.9–3.6 area which showed more protons in **13** than in **9**. On the basis of the NMR spectra which presented the usual long-range couplings between H-18, H-19, H-21 and H-15, it was concluded that **9** and **13** were not double bond isomers. The only other point of differentiation between the two compounds being the ring-junction, we attributed **13** to 16-epi methuenine (*trans*-descarbomethoxy-dehydroervatamine) structure, for it is unlikely that epimerization had occurred at C-15, a biogenetically invariant center. Epimerization at C-16 is a natural consequence of the origin of **9** and **13** by decarbomethoxylation of dehydroervatamine and the configuration at C-16 may reflect the respective stabilities of the *cis* and *trans* C/D ring junctions. These two alkaloids are accompanied by the *N*-oxide of one of them **16**, whose structure was demonstrated by its reduction (ϕ_3P/HOAc) to **13** and its synthesis (MCPBA) from **13**; it has not been so far possible to determine the configuration at N-4.



9 H(16) : β
13 H(16) : α

Compound **15** showed mass spectral fragmentations identical with those of corynantheol (*m/z*: 296, 295, 265, 251, 225, 223) but was not identical with a sample prepared from corynantheine. Its CD spectrum was in favor of a 3αH isomer but the scarcity of material did not allow any further investigation.

The seeds of *P. inconspicua* contained mostly *iboga* alkaloids amongst which voacangine predominated. To the best of our knowledge, it is the first time that an indole alkaloid bearing the α-aminonitrile function has been isolated. Such a functionality has previously been encountered in a piperidine alkaloid from *Girgensohnia oppositiflora* [14]. At the present time, it is difficult to formulate hypotheses on the biogenesis of these compounds. In the root-bark, the major alkaloids are of the methuenine type (62% AM), in full agreement with Pichon's localization of this genus in between the *Ervatamia* and *Hazunta* [15]. The occurrence of ellipticine derivatives which usually are found in the *Ochrosia* is more intriguing. However, such alkaloids have recently been discovered in *Hazunta* species [13] and also in a *Strychnos* from Africa [16].

EXPERIMENTAL

General. Plant material was collected by one of us (C.D.) in Zaire and identified by H. Breyne. A herbarium specimen is deposited in the Brussels National Gardens under No. HB 3433. Mps are uncorr. Rotations were determined in $CHCl_3$ and unless otherwise stated, NMR were measured in $CDCl_3$ solns at 60 MHz, chemical shifts are given in δ values with TMS as the int. standard. Chromatographic columns were packed with Merck H 60 Si gel. Prep. TLC plates were Merck 60F-254. Colour reactions (CR) were obtained by spraying plates with a soln of $Ce(IV)(NH_4)_2SO_4$.

Extraction of seeds. Ground seeds (190 g), alkalized with NH_4OH were lixiviated with 2 l. petrol. Evapn gave 2.4 g of a gum shown by NMR to be a mixture of voacangine **1** and triolein. Acid–base extraction yielded 1.6 g of **1** which crystallized from MeOH (mp 135°). The dried seeds were again treated with NH_4OH and lixiviated overnight with EtOAc. After evpn, the extract was taken-up in 1% H_2SO_4, alkalized with NH_4OH and extracted with $CHCl_3$. Drying (Na_2SO_4) and evapn yielded 0.358 g of a mixture which was separated by CC using $CHCl_3$ and a gradient of MeOH in $CHCl_3$; fractions were 25 ml and were pooled according to composition (TLC). Fractions 1–9 yielded **1** (which crystallized from MeOH); mother liquors were purified by prep. TLC to give voacangine-3 carbonitrile **2** (7 mg). Fractions 10–12 were purified by TLC to give voacristine **3** (8 mg) and 3–6 gave oxidovoacangine **4** (10 mg). Vobasine **5** was found in fractions 13–17 (crystallized as a HCl in Me_2CO, 11 mg) as well as in fractions 18–36 (25 mg, TLC). Fractions 18–36 gave 10-hydroxy-coronaridine **6** (5 mg); the polar fractions eluted with $CHCl_3$–MeOH (49:1) contained 10-hydroxyheyneanine **7** (3 mg).

Extractions of root-bark. The bark (1 kg) was finely ground and extracted as previously described by means of EtOAc. It was thus obtained 13.6 g of crude alkaloid mixture, which was purified by medium pres. LC; 20 ml fractions were collected and examined by TLC. Fractions 31–51 yielded 151 mg of vobasine **5**; methuenine **9** was found in fractions 61–80 (4.6 g) and crystallized from Me_2CO (1.23 g crystals mp 192–204°). Fractions 81–85 were purified by prep. TLC and gave (−)apparicine **10** (48 mg, $[\alpha]_D = -120°$) and (+)tubotaiwine **11** (24 mg; $[\alpha]_D + 400°$ $c = 3$). Fractions 90–96 were 16-epi-isositsirikine **12** (23 mg). Fractions 100–220 (3.9 g) contained mostly 16-epi-methunenine **13** which crystallized in MeOH to give an analytical sample (mp 147°). Fractions 221–342 (170 mg) were purified by prep. TLC, which gave an additional 70 mg of **13** and 50 mg of 3, 14-dihydroellipticine **14**. The isomer of corynantheol **15** (21 mg) was separated from fractions 351–360 along with 16-epi-methuenine *N*(4)-oxide **16** (13 mg). MeOH elution of the column gave a fraction from which tetrahydroellipticine **17** (91 mg) was separated.

Novel alkaloids. Voacangine-3-carbonitrile **2**, $C_{23}H_{27}N_3O_3$(CR blue), $[\alpha]_D = -68°$ ($c = 1.1$); UV λ_{max} (MeOH) nm: 225, 285, 300; IR($CHCl_3$) cm^{-1}: 3380, 2220, 1730; MS *m/z*: 393 ($M^{+\cdot}$, 65%), 366, 334, 307, 244, 161, 160

(100%); ^{1}H NMR ($CDCl_3$) δ: 7.95 (*s*, 1H, N-H), 7.10 (*m*, 1H, H-12), 6.80 (*m*, 2H), 3.85 (*s*, 3H), 3.75 (*s*, 3H), 3.70 (*s*, 1H), 0.95 (*t*, $J = 7$ Hz, 3H). 3, 6-*Oxidovoacangine* **4**, $C_{22}H_{26}N_2O_4$ (CR grey), UV λ_{max} (MeOH) nm: 225, 285, 300; IR($CHCl_3$) cm^{-1}: 3380, 1730, 1715; MS *m/z*: 382 ($M^{+\cdot}$), 368 (100%), 366, 323, 307, 283, 268, 244, 184, 173, 160, 136, 124, 122; ^{1}H NMR($CDCl_3$) δ: 7.65 (*s*, 1H), 7.10, (*m*, 1H), 6.80 (*m*, 2H), 4.20 (*m*, 1H), 3.85 (*s*, 3H), 3.70 (*s*, 3H), 0.90 (*m*, 3H).

16-*Epimethuenine* **13** [13] $C_{19}H_{22}N_2O$ (CR yellow), mp 251° (hydrochloride); $[\alpha]_D = -140°$ ($c = 1$); UV λ_{max} (MeOH) nm: 240, 315; IR($CHCl_3$) cm^{-1}: 3330, 2780, 1625, 1535, 1330; MS *m/z*: 294, 266, 265, 136, 122; ^{1}H NMR ($CDCl_3$) δ: 9.10 (*s*, 1H), 7.60 (*d*, $J = 7$ Hz, 1H), 5.45 (*q*, $J = 7$ Hz, 1H), 2.40 (*s*, 3H), 1.62 (*dd*, $J = 7$ Hz, 2 Hz, 3H). *Isomer of corynantheol* **15**, amorphous, $C_{19}H_{24}N_2O$ (CR yellow), $[\alpha]_D = -15°$ ($c = 0.2$); UV λ_{max} (MeOH) nm: 225, 282, 290; IR($CHCl_3$) cm^{-1}: 3500, 3300, 2830, 2800, 1460, 1210; MS *m/z*: 296, 295, 251, 225, 223 (100%), 197, 156; ^{1}H NMR ($CDCl_3$) δ: 9.50 (*s*), 7.50–6.90 (*m*, 4H), 5.90–4.80 (*m*, 3H), 4.10 (*m*, 2H).

16-*Epimethuenine* N-*oxide* **16**. Amorphous, $C_{19}H_{22}N_2O_2$ (CR yellow), UV λ_{max} (MeOH) nm: 238, 313; IR($CHCl_3$) cm^{-1}: 3200 (broad), 1630, 1330; MS *m/z*: 310 ($M^{+\cdot}$, 100%), 294, 293, 251, 208; ^{1}H NMR ($CDCl_3$) δ: 10.7 (*s*, 1H), 5.70 (*q*, $J = 7$ Hz, 1H), 4.35 (*d*, $J = 15$ Hz, 1H), 3.25 (*s*, 3H), 1.65 (*d*, $J = 7$ Hz, 3H).

Partial synthesis of 3,6-*oxido-voacangine* **1**→**4**. Voacangine **1** (100 mg, 0.27 mmol) was dissolved in 5 ml of THF; after cooling at 0°, a soln of 100 mg $NaHCO_3$ in 5 ml H_2O was added followed by a soln of 100 mg of I_2 (0.4 mol) in 5 ml of THF. The mixture was stirred for 30 min, and diluted by 15 ml of H_2O and 20 ml of CH_2Cl_2. Usual treatment of the organic layer left 75 mg of a gum which was purified by TLC. The yields were 17 mg of **4**, 24 mg of 6-hydroxy-3-oxovoacangine and 30 mg of starting material.

Synthesis of voacangine 3-*carbonitrile from voacangine* **1**→**2**. Voacangine (140 ml, 0.38 mmol) was dissolved in 8 ml THF containing 5 drops of H_2O and DDQ (190 mg, 0.83 mmol, 2.2 equiv.) was added. After the addition an intense green color developed which gradually faded; after 40 min, the reaction mixture was poured into satd Na_2CO_3 soln and extracted in the usual fashion. Prep. TLC of the resulting gum (127 mg) yielded 80 mg (53%) of pure nitrile in all respects identical with the natural product **2**.

Methylation of tetrahydroellipticine **17**→**18**. Tetrahydroellipticine **17** (20 mg) was dissolved in a mixture of 40% aq. HCHO (1 ml), HOAc (0.2 ml) and $NaBH_3CN$ (60 mg) was gradually added over 30 min. Usual work-up yielded 26 mg of an oil which was purified by prep. TLC; an analytical sample was crystallized from Et_2O, mp 165°; UV λ_{max} (MeOH): 243, 252, 263, 285, 315, 327, 342 nm; MS *m/z*: 264, 263 (100%), 249, 221, 140; ^{1}H NMR ($CDCl_3$): 2.70 (*s*, 3H), 2.55 (*s*, 3H), 2.35 (*s*, 3H).

Reduction of 16-*epimethuenine* N(4)-*oxide* **16**→**13**. Compound **16** (10 mg) was dissolved in 2 ml of HOAc to which 50 mg of PPh_3 were added. The reaction was stirred at 80° for 24 hr, diluted with H_2O, alkalized with NH_4OH and extracted with $CHCl_3$. After the usual work-up, compound **13** was purified by prep. TLC (yield 6 mg).

Oxidation of 16-*epimethuenine* **13**→**16**. Starting material (25 mg) was dissolved in 5 ml of CH_2Cl_2 and 20 mg of MCPBA added in three portions over 1 hr. After TLC showed disappearance of starting material, the reaction mixture was diluted with 20 ml of CH_2Cl_2 and washed ×3 with satd Na_2CO_3 soln. TLC indicated the presence of two compounds, the least polar one being identical to **16** after TLC separation (yield, 12 mg 48%).

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