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ALKALOIDS FROM LEAVES OF *PTEROTABERNA INCONSPICUA* AND THE KISANTU HYBRID PROBLEM

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Abstract—In the leaves of *Pterotaberna inconspicua*, two alkaloids with the vincadifformine skeleton have been found. One is a monomer and corresponds to 5-oxo vincadifformine, the other, kisantine, is a dimeric species (M^+ 810) composed of two highly oxidized tabersonine units. These results suggest a parenthood between the Kisantu hybrid and *P. inconspicua*.

INTRODUCTION

The Kisantu hybrid is a spontaneous hybrid originating from a sowing of *Daturicarpa elliptica*, it was described and discussed from a botanal standpoint in 1957, by Hürlimann, who suggested its likely parenthood with *Tabernanthe iboga* [1]. Examination of the fruit of the first and second generation of the hybrid's offspring allowed us to propose *Pterotaberna inconspicua* as a more probable parent of the Kisantu hybrid [2]. This opinion was strengthened by comparing the alkaloid contents of the seeds and leaves of the hybrid, of *D. elliptica* and of *T. iboga* and of the seed of *P. inconspicua* [2]. In a previous investigation, the alkaloids of the root and stem bark of *P. inconspicua* were studied but it was found that the alkaloids of the leaves were present in too small a quantity to justify in-depth studies [3]. This was contradicted by an independent work [4] and it was thus decided to resume the earlier investigations in connection with the hybrid problem.

RESULTS AND DISCUSSION

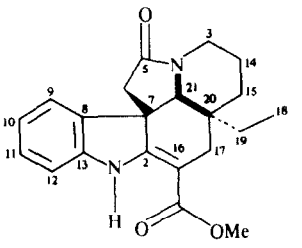
Plant material was collected anew at the place of the early collections and the same low yield of alkaloids was obtained (1.2 g/kg). The bulk of the material proved to be highly unstable and its tentative purification led to progressive and inevitable destruction. From it, small quantities of a stable monomer **1** and of a short lived dimeric species **2**, were isolated.

Monomer **1** colours blue by TLC upon Ce(IV) spraying and displays the three maxima UV spectrum of the β-anilino acrylate esters (225, 300 and 330 nm). Its IR spectrum shows bands at 3350, 1675, 1635 and 1605 cm^{-1} for N–H, C=O and C=C vibrations. The mass spectrum of **1** is dominated by the *m/z* 214 ion of the tabersonine type of alkaloids [5]. The $[M]^+$ is detected at *m/z* 352 ($C_{21}H_{24}N_2O_3$), i.e. 14 mass units higher than in vincadifformine. The 300 MHz ^1H NMR spectrum of **1** is fully assignable by a 2D-COSY experiment. It shows a deshielded NH, four aromatic protons, a methoxy group, an ethyl chain, six coupled protons and two AB doublets of doublets. All these characteristics are best explained by the 5-oxo vincadifformine structure **1**, with the AB systems featuring methylenes 6 and 17. Further support for this analysis is obtained by observation of noticeable deshielding for H-21 ($\delta = 3.69$ ppm) and for one of the H-3 ($\delta = 4.29$ ppm). It is also worth pointing out cross peaks due to long range couplings between H-21 and H-6α, H-21 and H-17α, H-17β and one of the H-19; they all obey the W-rule. A similar formula has been proposed for ervinidinine from *Vinca erecta* [6].

Alkaloid **2** is an unstable species and probably a dimer according to its ^1H and ^{13}C NMR spectra. Its UV spectrum is of the anilinoacrylic type and this is confirmed by the observation in the ^1H NMR spectrum of two exchangeable resonances at δ 9.05 and 8.76 ppm. Recording of the mass spectrum of **2** under EI conditions gives no significant peak above 400 mass units. Using Fast Atom Bombardment (FAB) (sample dissolved in glycerol), however, an intense quasi $[M]^+$ is observed at *m/z* 811; this corresponds to a M_r of 810. The sample was submitted to a high resolution (20000) analysis using an alternating FAB probe; the measured mass value (811.3503) corresponds to a $C_{44}H_{51}N_4O_{11}$ (calc. 811.3554).

The ^1H NMR spectrum of **2** shows two aromatic protons as singlets at δ 6.37 and 5.53 ppm, two sets of two coupled olefinic protons, four MeO groups and a single ethyl chain. Analysis of this spectrum, by a normal COSY experiment and by a delayed COSY to emphasize long range couplings, indicates that the ethyl side chain is on quaternary carbon atom and that another quaternary carbon is bound to a CH_2–CH_2–OH unit. One of the olefinic systems couples to two protons appearing at 3.4 ppm and thus are part of a CH=CH–CH_2 unit, the two other olefinic protons couple to a single allylic CH at 4.16 ppm (*d*, $J = 3.2$ Hz).

The ^{13}C NMR spectrum of **2** confirms the presence of two anilinoacrylate systems with four quaternary carbons in the neighborhood of 168 ppm (C-2 and C=O) and two other around 90 ppm (C-16). It also shows an unusually large number of resonances above 130 ppm,



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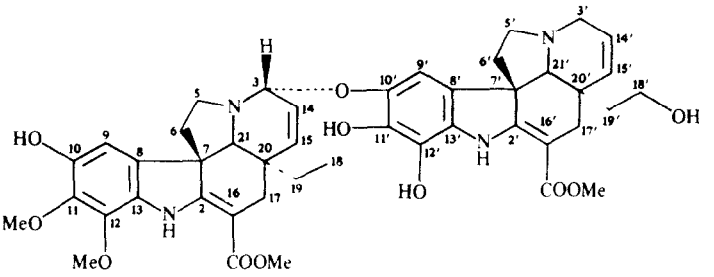
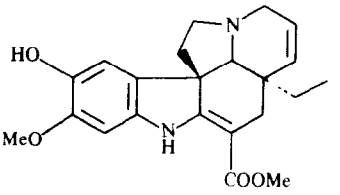


2



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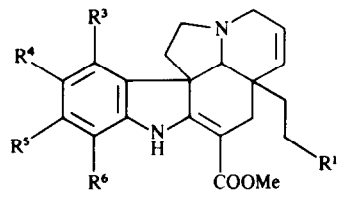
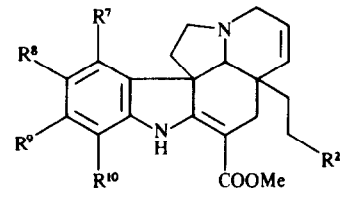
which indicates highly oxygenated aromatic rings At a field lower than the resonance of $CDCl_3$, only seven protonated carbons are found, they correspond to two aromatic, four olefinic and one carbinolamine type carbons ($\delta = 84.8$ ppm)

As a working hypothesis, a structure composed of two tabersonine units is proposed, giving $C_{42}O_4N_4$ as a partial formula As a consequence, two carbon and seven oxygen atoms remain to be located The presence of only two aromatic protons (instead of eight) may suggest that six oxygen atoms substitute the A rings of both tabersonines, the above mentioned occurrence of a CH_2–CH_2OH unit will take care of the seventh oxygen atom The two extra carbon atoms are part of aromatic methoxy groups as shown by ^1H and ^{13}C NMR spectra Their abnormal chemical shifts (60 3 and 60 7 ppm) are typical of encumbered ArOMe groups as those found in the 10-hydroxy-11,12-dimethoxy tabersonine part of pandicine [7] The inclusion of these proposals in the working hypothesis, leads to a formula $C_{44}H_{52}N_4O_{11}$ Two hydrogen atoms should be removed to link the two moieties and in order to obtain the correct formula The presence of a single allylic hydrogen atom on one of the moieties suggests that C-3 is a locus of substitution This carbon is probably also substituted by an oxygen atom as shown by ^{13}C NMR ($\delta_{CH} = 85.2$ ppm) and by ^1H NMR ($\delta = 4.16$ ppm instead of 3 4 ppm in tabersonine) Five of the OH functions of the molecule are obvious candidates for this substitution but the chemical shift of the sole oxygenated methylene ($\delta = 59.3$ ppm) favours a free primary alcohol rather than an ether All this evidence leads to partial structure **3**

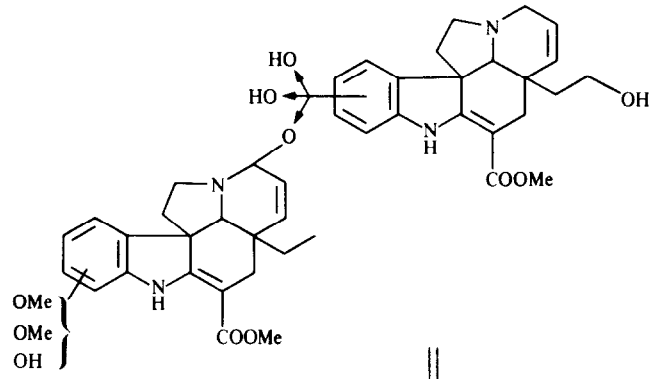
The gross substitution of the aromatic nuclei can be deduced from mass spectral fragmentations which are well established in the tabersonine series The retro-Diels–Alder cleavages of the 'lower part' of the molecule gives rise to a m/z 290 ion, which means that the corresponding aromatic ring is substituted by two OMe and one OH ($290 = 214 + 2 \times 30 + 16$), no significant peak at m/z 262 (3 OH) or 278 (2 OH + 1 OCH_3) could be detected Fragmentation of the upper part of the molecule yields a m/z 138 ion, indicating that the hydroxyethyl chain belongs to this moiety These results best fit formula **4** in which the location of the substituents has to be determined

Location of the substituents in the 'lower' aromatic ring is made possible by comparison of the ^{13}C spectra of **2** and of the tabersonine part of pandicine [7] In pandicine, the 10-hydroxy-11,12-dimethoxy-tabersonine moiety is characterized by an aromatic CH (C-9) at 104 2 ppm in the ^{13}C spectrum and at 6 34 ppm in the ^1H spectrum, the corresponding figures of 105 3 and 6 37 ppm favour an identical substitution pattern for **2** Assuming a 3βH configuration, which is justified by the low





3 R^1 or R^2 = H or OH
R^3 or R^{10} = 2H, 3OH, 2OMe + one bond



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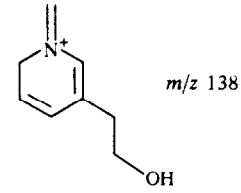
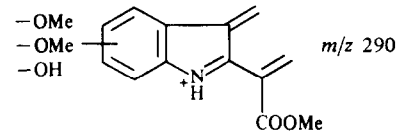


m/z 138



m/z 290

value of J_{3-14} (3.8 Hz), there remain 12 structural possibilities Among these, only those with a 9-hydrogen atom ought to be considered to account for a long range coupling between the N(1′)H and the remaining aromatic proton ($\delta = 5\ 53$ ppm) The abnormal shielding of this latter resonance when compared to the other H-9 (6.37 ppm, $\Delta\delta = 0\ 74$ ppm) is best explained by the proximity of the second tabersonine moiety. These interpretations are all incorporated in final structure **2** which fully justifies the spectral interpretations given in the Experimental We propose the name of kisantine for alkaloid **2**.

The identification of **2** in *P inconspicua* and of 10-hydroxy-11-methoxy tabersonine **5** in the Kisantu hybrid is in favour of our previous hypothesis that the two species were related [2] since the occurrence of an oxygenated indole ring is too rare The conflicting reports on the occurrence of alkaloids in the leaves of *P. inconspicua* [3, 4], may be simply explained by the presence of twigs in the sample investigated by the Antwerp group [4].

EXPERIMENTAL

General ^{1}H NMR spectra were obtained at 300 MHz ^{13}C NMR spectra at 75 MHz and at 22 15 MHz

Extraction of alkaloids Dried powdered leaves of *P inconspicua* (200 g) were wetted with 120 ml 50% NH_4OH and lixiviated with 2 l of EtOAc The lixiviate was extd with 2% H_2SO_4 and the aq phase made alkaline with NH_4OH and extd with $CHCl_3$ The $CHCl_3$ layers were dried (Na_2SO_4) and evapd *in vacuo* to yield 240 mg of crude alkaloid mixt Silica gel CC of this material yielded 10 mg of **1** ($R_f = 0\ 8$ in $CHCl_3$–MeOH, 3 2) and 65 mg of **2** ($R_f = 0\ 6$)

'Ervinidimine' **1** (CR blue, turns to purple with a yellow centre on standing), $[\alpha]_D = -152°$ (MeOH, *c* 0 25), mp 250°, UV λ_{max} nm 225, 300, 330, IR ν_{max} cm^{-1} 3450, 1675, 1645, 1605, 1240, 1215, 1100, MS *m/z* 352 $[M]^+$ (50%), 320(10), 293 (3), 214 (100), 154, 111, ^{1}H NMR (300 MHz, $CDCl_3$) 8 95 (*s*, NH), 7 2 (*m*, 2H), 6 92 (*t*, $J = 8$ Hz), 6 84 (*d*, $J = 8$ Hz), 4 29 (*dt*, $J = 13, 4$, H-3), 3 78 (*s*, 3H, OMe), 3 69 (*d*, $J = 1\ 5$ Hz, H-21), 2 82 (*m*, H-3), 2 8 (*d*, $J = 18$ Hz, H-6), 2 58 (*dd*, $J = 18, 1$ Hz, H-6), 2.39 (*dd*, $J = 16$,

1 5 Hz, H-17), 2 21 (*d*, $J = 16$ Hz, H-17), 1 9 (*m*, 1H, H-14), 1 6 (*m*, 3H, H-14 + 2H-15), 1 06 (*dq*, $J = 13$ 5, 7 Hz, H-19), 0 79 (*dq*, $J = 13$ 5, 7 Hz, H-19), 0 64 (*t*, $J = 7$ Hz, Me-18)

Dimer **2** (CR purple), $[\alpha]_D = -21$ (MeOH, *c* 0 33), UV λ_{max} nm 240, 310, 335, IR ν_{max} ($CHCl_3$) cm^{-1} 3390, 1680, 1610, 1470, 1440, 1260, MS (FAB) *m/z* 811 $[M+1]^+$ (100%), 403 (25), 371 (20), 329 (20), 304 (70), 290 (50), 268 (35), 208 (40), 185 (45), 151 (65), 138 (45), 112 (85), ^{1}H NMR ($CDCl_3$, 300 MHz) 9 05 (s, 1H), 8 76 (s, 1H), 6 37 (s, 1H), 5 8 (*m*, 1H, H-14′), 5 7 (*m*, 1H, H-15′), 5 53 (s, 1H), 5 05 (*dd*, $J = 7$ 6, 3 2 Hz, H-14), 4 8 (*d*, $J = 7$ 6 Hz, H-15), 4 16 (*d*, $J = 3$ 2 Hz, H-3), 3 84 (s, 3H), 3 785 (s, 3H), 3 78 (s, 3H), 3 77 (s, 3H), 0 70 (*t*, $J = 7$ Hz, Me-18), ^{13}C NMR ($CDCl_3$, 22 MHz) 168 5 ($\underline{C}O_2Me$, 165 9 ($\underline{C}O_2Me$), 164 7 (C-2), 160 9 (C-2′), 144 9 (C-12), 143 5 (C-12′), 139 7 (C-11), 136 9 (C-11′), 133 3 (C-15′), 133 3 (C-8), 130 7 (C-8′), 128 8 (C-13), 124 9 (C-15), 124 5 (C-14), 119 3 (C-14′), 105 3 (C-9), 93 1 (C-9′), 91 7 (C-16), 90 6 (C-16′), 85 3 (C-3), 69 5 (C-21′), 65 2 (C-21), 60 3 (ArOMe), 60 2 (ArOMe), 58 6 (C-18″), 55 3 (C-7), 54 6 (C-7′), 51 0 (C-5′), 50 8 ($CO_2\underline{Me}$), 50 6 ($CO_2\underline{Me}$), 50 0 (C-3′), 45 7 (C-5), 44 8 (C-6′), 41 7 (C-6), 39 3 (C-20), 33 7 (C-19′), 29 5 (C-19), 26 4 (C-17), 22 1 (C-17′), 7 5 (C-18) (assignments of carbons with similar values may be interchanged)

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