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MATADINE, A CYTOTOXIC ALKALOID FROM *STRYCHNOS GOSSWEILERI*

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Key Word Index—*Strychnos gossweileri*; Strychnaceae; root bark; indole alkaloid; matadine; 2D NMR spectra; cytotoxic activity.

Abstract—Matadine, a new alkaloid, has been isolated from the root bark of *Strychnos gossweileri*. Elucidation of its structure is mainly based on 1D and 2D NMR studies. Its cytotoxic activity has been tested *in vitro* on cancer cells and normal cells.

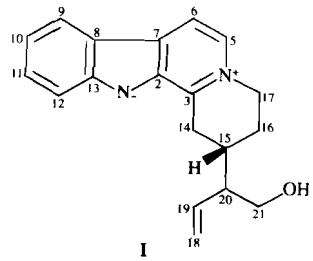
INTRODUCTION

Our previous studies on the constituents of *Strychnos gossweileri* have resulted in the isolation of numerous alkaloids [1–6], among which are two anhydronium bases (alstonine, strychnoxanthine). However, *in vitro* microtests indicated an antiproliferative activity on B16 melanoma at a concentration of 50 $\mu g\,ml^{-1}$ for a later fraction containing an uncharacterized alkaloid. We now report on the isolation and the structural elucidation of this remaining base which we have named matadine (**1**). The name matadine was chosen because *S. gossweileri* was collected in Matadi (Zaïre). We also describe the results of preliminary tests concerning its cytotoxicity, because, according to French scientists, some related alkaloids (serpentine, alstonine) exert a selective cytotoxicity on cancer cells [7, 8].

RESULTS AND DISCUSSION

Matadine (**1**) shows an intense blue fluorescence under UV light. The wavelengths of its three λ_{max} and the bathochromic shift in alkaline solution suggest the presence of a β-carbolinium chromophore. Its FAB mass spectrum showed a $[M+1]^+$ peak at *m/z* 293 corresponding to the elemental composition $C_{19}H_{21}N_2O$. Characteristic fragments of a β-carbolinium derivative were found at *m/z* 154, 167, 168 and 182 [5]. We also noted another important fragment at *m/z* 221 which is indicative of a loss of C_4H_8O from an antirhine or a corynantheol derivative [10]. The presence of a hydroxyl group, indicated by the peak at *m/z* 275 (loss of OH), was confirmed by acetylation (pyridine–Ac_2O) of **1** at room temperature to give the expected monoacetylated derivative (*m/z* 335 in the FAB spectrum). Furthermore, the IR spectrum showed the absence of any carbonyl band.

A more detailed understanding of the molecular structure of **1** was gained from its NMR spectra (Tables 1 and 2). In the 400 MHz ^1H NMR spectrum, the presence of a β-carbolinium structure was confirmed by the presence of six aromatic protons, four of which corresponded to an unsubstituted indole moiety, and two more deshielded doublets whose chemical shifts were in accordance with a pyridinium ring [5]. The spectrum also revealed the absence of any methyl group and the presence of only one double bond which was present in a vinyl side chain (δ5.67 and 5.39). We also noted the presence of four quite deshielded protons at δ4.6, 4.4, 3.85 and 3.77 respectively, two signals corresponding to a hydroxymethyl group (δ3.85 and 3.77) and others for a methylene in the α position of a quaternary nitrogen atom. Because of the unsaturation number, we must assume the presence of four rings.



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The ^{13}C NMR spectra (DEPT and total decoupling) confirm the above data and indicate the presence of five quaternary carbon atoms, seven methine and one methylene in the aromatic region, and two methine and four methylene in the aliphatic region. The comparison of the ^{13}C NMR spectra of matadine with those of antirhine (**2**) and corynantheol (**3**) shows that matadine is an antirhine derivative because of the noticeable shielding of several carbon resonances (in particular C-14, C-15 and C-16) and the position of C-20, as compared with corynantheol derivatives [10]. Assignations of protons and carbons were mainly determined by 2D NMR experiments (X–H Corr; COSY).

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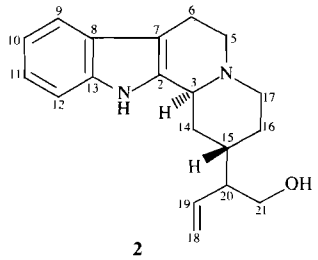

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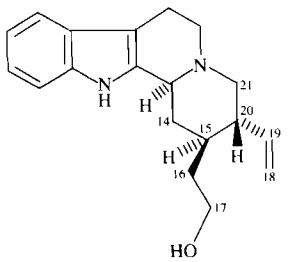

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Table 1. ^{13}C NMR spectrum of compounds **1**–**3**

C	**1** (CD_3OD, 100 MHz)	**2** [13] ($CDCl_3$, 100 MHz)	**3** [10] ($CDCl_3$–CD_3OD, 75 MHz)
2	142.3	132.4	134.5
3	145.4	53.9	60.0
5	134.1	49.2	52.9
6	116.8	17.4	21.4
7	132.6	105.6	107.0
8	121.6	126.6	127.0
9	124.2	116.9	117.8
10	123.3	120.2	121.0
11	133.0	118.1	118.9
12	114.1	110.4	110.8
13	136.0	135.7	136.1
14	30.8	30.7	34.1
15	31.7	30.4	37.0
16	26.3	27.3	35.9
17	56.9	46.0	61.0
18	119.9	116.5	116.9
19	137.9	137.8	139.2
20	52.4	51.0	46.8
21	64.1	62.6	59.9

The results of the COSY spectrum are shown in Table 2. In the aromatic region, in addition to the ubiquitous indolic protons, another isolated spin system can be established from the resonance (at δ7.88) of H-5 which is connected to H-6. In the aliphatic region, a larger structural fragment can be assembled. A convenient entry point in this system is afforded by the H-21A and H-21B protons resonating at δ3.85 and 3.77. These chemical shifts are indicative of a methylene bearing an hydroxyl group. The connectivities provide the means of assembling the multispin substructure related with

Table 2. Observed ^{1}H–^{1}H connectivities for compound **1** in the 2D COSY spectrum

H-5/H-6	H-14B/H-15	H-16B/H-17A
H-9/H-10	H-15/H-20	H-16B/H-17B
H-9/H-11	H-15/H-16A	H-18/H-19
H-10/H-11	H-15/H-16B	H-19/H-20
H-10/H-12	H-16A/H-16B	H-20/H-21A
H-11/H-12	H-16A/H-17A	H-20/H-21B
H-14A/H-14B	H-16A/H-17B	H-21A/H-21B
H-14A/H-15		

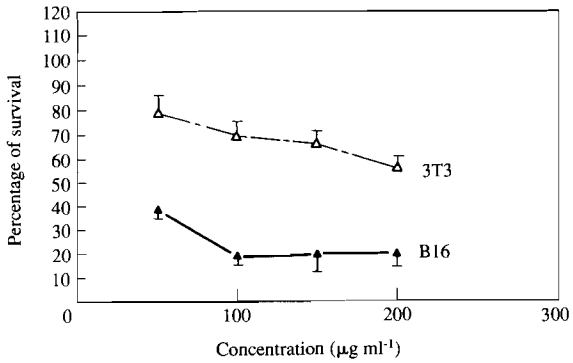

Fig. 1. Effects of matadine (50–200 μg ml^{-1}) on the percentage survival of non-cancer cells (—△— 3T3) or cancer cells (—▲— B16 melanoma) after 72 hr of treatment. Vertical lines represent standard error; MTT test; results obtained for controls are considered here as 100%.

the hydrogens bonded to C-21–C-20(–C-19–C-18)–C-15 (–C-14)–C-16–C-17–N-4 which further confirm the antirhine-type skeleton. On the basis of the above structural work, structure **1** is proposed for matadine. All the chemical shifts of the protons and carbon atoms are fully supportive of this structure as compared with data from known compounds [5, 10–12].

The proposed configuration of C-15 (*S*) agrees with the biogenetic hypothesis [14], where vallesiachotamine type alkaloids are characterized by a N-4/C-17 bond and H-15 in β-position. The stereochemistry of C-20 remains undetermined, because we require further material to prepare semi-synthetic derivatives.

Because of the selective toxicity of alstonine and other anhydronium bases on cancer cells, we decided to perform a preliminary *in vitro* microtest [9] in order to see if matadine possessed the same properties. The activity was tested from 50 to 200 μg ml^{-1} on cancer and normal cells. This preliminary test based upon the use of trypan blue and toluidine blue staining showed that matadine is more toxic for B16 mouse melanoma cells than for non-cancerous mouse 3T3 fibroblasts; a different level of activity exerted on 2002 human non-cancer cells and HeLa human tumour cells was doubtful. To confirm such a selective activity, we used a new quantitative colorimetric test which is more sensitive. This test is based on the transformation of the tetrazolium salt MTT into dark blue formazan by various dehydrogenase enzymes in active mitochondria, so that the reaction occurs only in living cells [15]. The results are shown in Figs 1–4. We

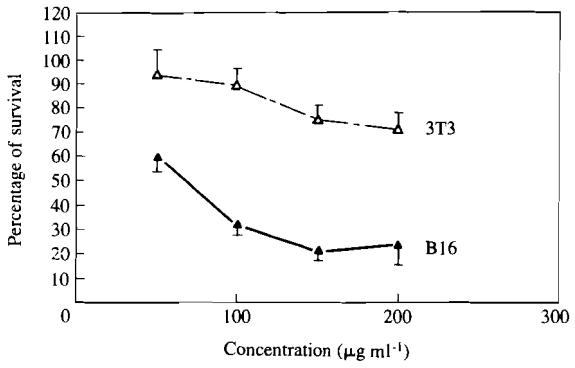

Fig. 2. Effects of serpentine (50–200 $\mu g\ ml^{-1}$) on the percentage survival of non-cancer cells (—△— 3T3) or cancer cells (—▲— B16 melanoma) after 72 hr of treatment. Vertical lines represent standard error; MTT test.

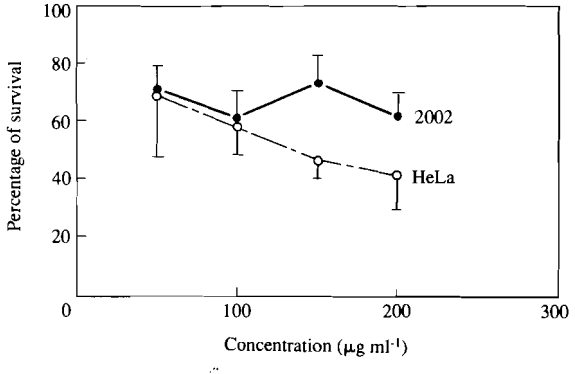

Fig. 3. Effects of matadine (50–200 $\mu g\ ml^{-1}$) on the percentage survival of non-cancer human cells (—●— 2002) or cancer cells (—○— HeLa) after 72 hr of treatment. Vertical lines represent standard error; MTT test.

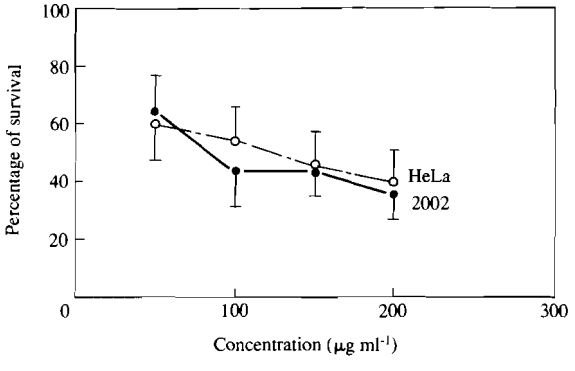

Fig. 4. Effects of serpentine (50–200 $\mu g\ ml^{-1}$) on the percentage survival of non-cancer cells (—●— 2002) or cancer cells (—○— HeLa) after 72 hr of treatment. Vertical lines represent standard error; MTT test.

used the well known serpentine as a reference for evaluating the activity of matadine.

Matadine, as well as serpentine, exerts a selective inhibiting activity on B16 melanoma cells at the same range of concentrations while it is less toxic than serpentine in human 2002 non-cancer cells. These promising results are very interesting because, though the active concentration is relatively high, as compared to that of non selective compounds such as strychnopentamine or emetine, we observe here a selective activity which might well be due, as it seems to be the case for serpentine or alstonine, to a higher affinity of the compound for destabilized single-stranded DNA as mainly present in cancer cells [7, 8].

EXPERIMENTAL

Plant material. Root bark of *Strychnos gossweileri* Exell collected in Zaïre, near Matadi by one of us (C.D.) and identified by Dr Breyne. Reference specimens (nr HB5690) are deposited at the Botanical Garden of Belgium at Meise.

Extraction and isolation. The powdered bark was macerated for 24 hr with EtOH–H_2O–HOAc (16:3:1) and then percolated with the same mixt. After concn under red. pres. and filtration, a 10% NaOH soln was added to pH 8. The soln was then extracted with $CHCl_3$. The $CHCl_3$ layer was dried over Na_2SO_4 and evapd to give fr. A. This fr. was partitioned between the two phases of a mixt. of $CHCl_3$–MeOH–H_2O (4:4:3). The upper aq. phase was evapd. The residue was fractionated firstly on a reversed-phase Lobar® column (LichroPrep RP-8) with the mixt. of Me_2CO and H_2O (1:9) and secondly on a medium pressure column (Superformance®) filled with Silica 60 using as solvent system $CHCl_3$ with increasing concentrations of MeOH. Frs containing matadine were finally purified on Fractogel TSK HW 40S with EtOH.

Cytotoxicity tests. Screening microtests (on B16 melanoma cells, 2002 human fibroblasts and HeLa human epithelial cells) were performed as previously described [9] on NUNC 96-well plates. Cultured Flow mouse 3T3 fibroblasts (9000) derived from normal embryonic mice were inoculated and treated 24 hr later for 72 hr. The culture medium was GIBCO DEM 90% supplemented with 10% foetal calf serum and 100 U ml^{-1} penicillin. Matadine was tested at concentrations of 50, 100, 150 and 200 $\mu g\ ml^{-1}$.

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazoliumbromide) test was applied after 72 hr of treatment under the same conditions as for the first test but using media devoid of penicillin: 10 μl of a 1.5 mg ml^{-1} aq. soln of MTT was added to the culture medium of each well of a 96-well plate and allowed to incubate at 37° for one or two hr, depending of the type of cell. The cultured medium was then removed and 50 μl of iso-PrOH containing 0.08 M HCl was added at room temp. and thoroughly mixed to dissolve the dark blue crystals. After a few minutes, plates were read on a Biotek Elisa 309 Reader at 540 and 650 nm. Eight wells were used for each condition.

Matadine. UV λ_{max}^{MeOH} nm (log ε): 215 (4.72), 253 (4.34), 307 (4.14); λ_{max}^{MeONa} nm (log ε): 220 (4.16), 283 (4.47), 328 (3.77); FT IR ν_{max}^{KBr} cm^{-1}: 3316, 3055, 2967, 2928, 2878, 2801, 1635, 1575, 1526, 1506, 1450, 1439, 1407, 1386, 1371, 1334, 1279, 1257, 1253, 1165, 1149, 1103, 1052, 1038, 994, 920, 880, 817, 774, 760, 741, 719, 644, 594, 546, 505, 438; FABMS *m/z* (rel. int.): 293 (100) $[M+1]^+$, 275 (2) [M − OH], 221 (47), 207 (9), 193 (6), 182 (18), 168 (6), 167 (5), 154 (5), 140 (3), 131 (34), 115 (5); ^1H NMR spectrum: (400 MHz D_2O): δ7.88 (1H, *d*, *J* = 6.5 Hz, H-5) 7.82 (1H, *d*, *J* = 6.5 Hz, H-6), 7.76 (1H, *d*, *J* = 8 Hz, H-9), 7.57 (1H, *t*, *J* = ~8 Hz, H-11), 7.3 (1H, *d*, *J* = 8 Hz, H-12), 7.23 (1H, *t*, *J* = 8 Hz, H-10), 5.79 (1H, *m*, H-19), 5.38 (2H, *m*, H-18), 4.60 (1H, *br d*, H-17A), 4.40 (1H, *br t*, H-17B), 3.85 (1H, *dd*, *J* = 5.5 and 10.9 Hz, H-21A), 3.77 (1H, *dd*, *J* = 7.8 and 10.9 Hz, H-21B), 3.13 (1H, *dd*, *J* = 4.6 and 17 Hz, H-14A), 2.79 (1H, *dd*, *J* = 11 and 17 Hz, H-14B), 2.39 (1H, *m*, H-15), 2.3

(1H, *br d*, $J = 13.8$ Hz, H-16A), 2.13 (1H, *m*, $J = 5.5$ and 7.8 Hz, H-20), 1.74 (1H, *br q*, H-16B); 2D COSY spectrum: see Table 2; ^{13}CNMR: see Table 1.

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