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REVISION OF THE STRUCTURE OF STRYCHNOFLUORINE, AN ALKALOID OF *STRYCHNOS GOSSWEILERI*

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Key Word Index—*Strychnos gossweileri*; Strychnaceae; root bark; indole alkaloid; strychnofluorine; 18-hydroxynorfluorocurarine; 2D-NMR; cytotoxic activity.

Abstract—The structure of strychnofluorine, an indole alkaloid isolated from the root bark of *Strychnos gossweileri* has been revised. Strychnofluorine proved to be identical with 18-hydroxynorfluorocurarine. Furthermore, it has a low cytotoxic activity on B16 melanoma cells.

INTRODUCTION

Strychnofluorine is an indoline alkaloid which was isolated for the first time from the root bark of *Strychnos gossweileri* Exell in 1980 [1, 2]. From partial spectroscopic data, mainly UV, IR and mass spectra, structure **1** was proposed for this fluorescent alkaloid (hence the name). In the course of reinvestigation of the content of a new batch of *S. gossweileri*, we isolated a number of alkaloids including further amounts of strychnofluorine. In order to confirm this hypothetical structure, we decided to perform high resolution ^{1}H and ^{13}C NMR spectra. Furthermore, we realized a preliminary study of its potential cytotoxic activity on cancer and non-cancer cell lines.

RESULTS AND DISCUSSION

The ^{1}H NMR spectrum (Table 1) showed that, besides the expected signals of an aldehydic proton (δ9.32) and four benzenoid protons, some peaks did not fit with the proposed structure. In particular, we could not detect any methyl signal but we observed a triplet at δ5.53 indicative of a hydroxyethylidene side chain and two mobile protons at δ10.37 and 2.07. The presence of a hydroxyl function was suggested by acetylation at room temperature. High resolution ^{13}C and 2D-NMR experiments (COSY and X-H Corr.) confirmed that strychnofluorine was different from structure **1**. A more detailed understanding of the molecular structure of strychnofluorine was gained from its 2D-COSY spectrum (Table 1). In addition to the expected spin system related to the hydrogens respectively bonded to C-5/C-6 and C-3/C-14/C-15, we can assemble another useful fragment. A convenient entry point in this system is afforded by the two equivalent C-18 protons resonating at δ4.24, a chemical shift indicative of a hydroxymethyl group. The vicinal and homoallylic connectivities provide the means of assembling the following substructure: HO–(C-18)H_2–(C-19)H=(C-20) [–C-15(H)]–(C-21)H_2. All these considerations suggested possible identity of strychnofluorine with 18-hydroxynorfluorocurarine (**2**). All the other NMR signals were fully supportive of this structure as compared with data for related compounds [3, 4]. 18-Hydroxynorfluorocurarine has been isolated as a minor alkaloid from *Strychnos ngouniensis* in 1983 but only some ^{1}H NMR data were provided [5]. Furthermore, the published UV and IR spectra were different from those of strychnofluorine [2]. In order to confirm the identity of the two compounds, co-TLC were carried out in different solvent systems. They showed that both substances had the same R_f and fluorescence but also that the sample isolated from *S. ngouniensis* was slightly contaminated by another derivative, explaining the differences in the UV and IR spectra. Although the name strychnofluorine is earlier than 18-hydroxynorfluorocurarine [1], it is desirable to retain the latter name as it is in line with the names of a number of other alkaloids isolated from various *Strychnos* species.

The CD spectrum, negative in the 340 nm region and positive in the 250 nm region is indicative of a H-7β(7*R*) configuration in the vicinity of the main chromophoric group [6]. Comparison with the CD spectrum of fluorocurarine taken under the same experimental conditions confirms the stereochemistry of both compounds. The H-15α (15*R*) and the H-3α (3*S*) configurations are strictly dependent on the configuration of C-7 and are in close agreement with the biogenetic hypothesis [7].

The cytotoxicity test we performed showed that strychnofluorine has no significant effect on non-cancer mouse 3T3 fibroblasts even at the highest tested concentration, but is toxic in B16 melanoma cells apart from 200 μg ml^{-1} (treated: 55 ± 2.7%, controls: 100 ± 2.5%). This biological activity is relatively low as compared to

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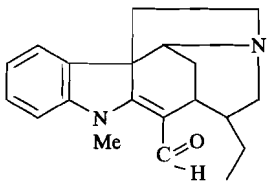

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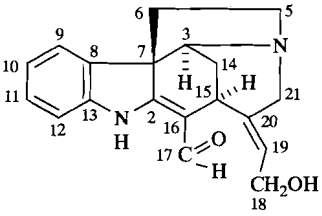

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Table 1 ^{1}H NMR spectral data of compound **2** (in $CDCl_3$)

H	2	H/H correlations (*J* in Hz) observed in the 2D-COSY spectrum
3	4.09 *s*	14A, 14B, 15
5A	3.32 *td*	5B (12.4), 6A (12.4), 6B (5.2)
5B	3.09 *dd*	5A (12.4), 6A (6.5)
6A	2.41 *td*	5A (12.4), 5B (6.5), 6B (12.4)
6B	1.86 *dd*	5A (5.2), 6A (12.4)
9	7.30 *d*	10 (7.4)
10	7.00 *t*	9 (7.4), 11 (7.4)
11	7.21 *td*	10 (7.4), 12 (7.7)
12	6.93 *d*	11 (7.7)
14A	2.57 *dq*	3 (2.1), 14B (13.6), 15 (2.8)
14B	1.29 *dd*	3, 14A (13.6), 15
15	3.78 *br s*	3, 14A, 14B, 18, 19, 21A
17	9.32 *s*	
18AB	4.24 *d*	15, 19 (6.2), 21A
19	5.53 *t*	15; 18 (6.2), 21A
21A	4.00 *d*	15, 18, 19, 21B (15.8)
21B	2.97 *d*	21A (15.8)
NH	10.37 *br s*	
OH	2.07 *br s*	

other indole alkaloids tested previously [8]. However, the difference observed as far as the sensitivity of cancer (B16) and non-cancer (3T3) cells is concerned is interesting to note. Further studies have now to be performed with other cancer and non-cancer cell lines in order to confirm such an apparent selectivity of action.

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 extraction of strychnofluorine followed the procedure recently described [9]. Strychnofluorine was present in the upper aq. phase of fraction A. It was first fractionated on a Lobar® column (LichroPrep RP-8) with Me_2CO–H_2O (1:9) and secondly by medium pressure liquid chromatography (Superformance®) on silica 60 using as solvent system $CHCl_3$ with increasing concns of MeOH. Fractions containing strychnofluorine were then purified by prep. TLC on silica gel with EtOAc–*iso*-PrOH–1.5% NH_4OH (9:2:3). Strychnofluorine was finally eluted from silica with MeOH. This sample of strychnofluorine was compared with the reference compound and was identical in all aspects (IR, UV and co-TLC) [2].

Cytotoxicity tests. Screening microtests were performed in NUNC 96-well plates as previously described [8] on B16 mouse melanoma cells (1000 cells per well) and Flow mouse 3T3 fibroblasts (8000 cells per well). The effects of strychnofluorine at 5, 10, 25, 50, 100 and 200 $\mu g\,ml^{-1}$ on the number of living cells were measured using the MTT test [9]. The percentages of living cells were calculated by comparison with the controls considered as 100%. For comparing control values to those obtained for treated cells, the non-parametric Mann–Whitney test was applied. *P* values lower than 0.01 were considered as significant. All these analyses as well as the calculation of the standard errors were performed on a PS2 (model 60) computer with Statgraphic software.

Strychnofluorine (*18-hydroxynorfluorocurarine*). UV λ_{max}^{MeOH} (log ε) nm: 365 (4.01), 299 (3.38), 291 (3.32), 243 (3.76) [2]. CD (MeOH; *c* 0.01): $\Delta\varepsilon_{376}-6.6$, $\Delta\varepsilon_{361}-3.7$, $\Delta\varepsilon_{344}-6.9$, $\Delta\varepsilon_{293}+1.8$, $\Delta\varepsilon_{273.5}+0.5$, $\Delta\varepsilon_{239}+15$. IR ν_{max}^{KBr} cm^{-1}: 2950, 1635, 1600, 1590, 1550, 1480, 1460, 1415, 1390, 1335, 1295, 1270, 1230, 1180, 1160, 1130, 1105, 1060, 1040, 1020, 980, 930, 900, 880, 840, 800, 760 cm^{-1} [2]. MS (70 eV, 200°): *m/z* (rel. int.): 308 ($[M]^+$, 25), 295 (27), 281 (28), 277 (23), 263 (21), 261 (21), 249 (15), 247 (17), 221 (75), 207 (39), 194 (31), 180 (43), 168 (58), 167 (55), 156 (50), 154 (39), 154.5 ($[M]^{2+}$), 144 (63), 143 (73), 137 (62), 130 (54), 123 (100), 105 (58), 94 (82), 93 (100), 92 (100) [2]; FAB-MS: *m/z* (rel. int.) 309 ([M+1], 100), 291 (9), 261 (5), 247 (7), 234 (10), 218 (16), 206 (20), 204 (21), 194 (21), 180 (32), 168 (52), 167 (31), 156 (18), 154 (20), 149 (15), 144 (22), 130 (28), 128 (21), 115 (25), 106 (14), 96 (5), 91 (18). ^{1}H NMR spectrum: see Table 1, ^{13}C NMR (100 MHz, CD_3OD): δ188.2 (C-17), 169.7 (C-2), 142.9 (C-13), 141.8 (C-20), 137.1 (C-8), 131.1 (C-11), 125.9 (C-19), 122.6 (C-10), 121.3 (C-9), 111.4 (C-16), 110.9 (C-12), 61.8 (C-3), 58.6 (C-18). 56.9 (C-21), 56.8 (C-5), 46.6 (C-6), 31.5 (C-15), 31.1 (C-14).

C-Fluorocurarine. (Authentic sample available in our laboratory [3]): CD (MeOH; *c* 0.01): $\Delta\varepsilon_{378}-8.4$, $\Delta\varepsilon_{358}-1.1$, $\Delta\varepsilon_{337}-10.7$, $\Delta\varepsilon_{290}+4.3$, $\Delta\varepsilon_{272}$, $+2.6$, $\Delta\varepsilon_{237}+21.1$.

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