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PYRROLE-3-CARBAMIDINE: A LETHAL PRINCIPLE FROM *NIEREMBERGIA HIPPOMANICA*

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Key Word Index—*Nierembergia hippomanica*; Nicotianeae; Solanaceae; lethal principle; structure elucidation; pyrrole-3-carbamidine.

Abstract—Pyrrole-3-carbamidine has been isolated and identified as the lethal constituent of *Nierembergia hippomanica*.

INTRODUCTION

Nierembergia hippomanica Miers. is an Argentinian plant toxic to livestock. Since the last century there are records of the plant being poisonous to cattle, sheep, goats, horses and rabbits in Argentina. Attempts to identify the toxin and pharmacological tests with guinea-pigs, dogs, toads, pigeons and gasteropods have been reviewed [1]. However no success was achieved in the isolation and identification of the toxic constituent(s).

Death may occur some hours after eating the plant and is preceded by symptoms of diarrhoea, midriasis, locomotor ataxia, excitement, weakened heart action, dyspnoea, and strong convulsions. On autopsy, acute cases showed evidence of gastro-intestinal irritation and hyperaemia of brain and meninges. Some of these symptoms may be explained by the identification of sympathomimetic β-phenethylamines [2], pentacyclic triterpenes [2] and a parasympatholytic tropane alkaloid [3] which we have previously reported. But none of these and other compounds we isolated [4–7] accounted for the lethality observed by ingestion of the plant.

In the present paper the novel pyrrole-3-carbamidine **1** is reported as the lethal principle of this plant. The structure was elucidated by chemical and spectroscopic methods. Toxicity was monitored by i.p. injection in mice.

RESULTS AND DISCUSSION

The methanolic extract of whole plants of *N. hippomanica* was toxic to mice when injected i.p. Therefore, this extract was successively percolated on polyamide with chloroform, water and methanol. Only the aqueous percolate was lethal to mice. Fractionation based on toxicity led to an Ehrlich positive fraction that was further chromatographed on a Bio-Gel P-2 column [8]. Further purification led to compound **1**. Upon alkaline hydrolysis of **1**, pyrrole-3-carboxylic acid and ammonia were obtained.

The ^{1}H NMR spectrum of **1** showed an ABX system of the pyrrolic protons (J_{AX} = 1.4, J_{BX} = 1.4 and J_{AB} = 2.8 Hz). ^{13}C NMR spectral data were in complete agreement with the structure **1**, on the basis of published chemical shifts of related pyrrolic compounds [9]. Both spectra suggested a 3-substituted pyrrole and the latter indicated the presence of an amidine carbon (160.3 ppm). Moreover, the mass spectrum of **1** showed the molecular ion at *m/z* 109 and main fragments at *m/z* 93, 66 and 43 (Scheme 1) indicative of an amidine group. This fact was

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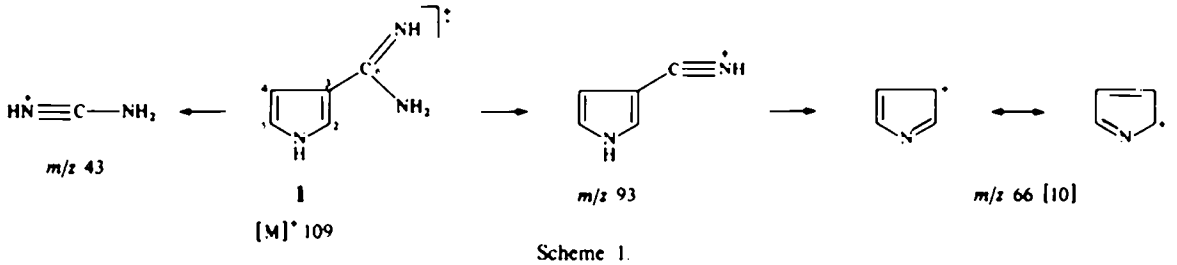

Scheme 1.

confirmed by comparison of MS of **1** and deuterated **1** (2:1 relation between no cyclic NH protons). The structure of **1** was finally confirmed through synthesis based on the addition of sodium amide [11] to pyrrole-3-carbonitrile. Further synthesis of **1** for large scale work is being studied in our laboratories.

Although detailed studies on the biological activity of this toxic principle will be published elsewhere [12], when administered to mice in sublethal doses, it showed CNS effects.

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR spectra were obtained at 100 MHz and ^{13}C NMR at 25.2 MHz. IR spectra were recorded from KBr discs. MS were determined at 70 eV by direct inlet.

Plant material. Whole plants of *Nierembergia hippomanica* Miers. were collected in different provinces of Argentina: La Pampa, Santa Fé, Entre Rios and Córdoba (Herbarium: INTA No. 1324 and IPNAYS No. 121). The samples were studied separately.

Bioassay of the toxin **1**. Throughout this work white mice (15–20 g) were used as test animals to detect and assay toxicity. Doses were injected i.p.

Extraction and isolation of **1**. Dried ground whole plants of *N. hippomanica* were successively extracted with $CHCl_3$ (5.5% of dried plant) and MeOH (17.9% of dried plant). Only the latter was found to be toxic to mice. Therefore, it was further percolated on polyamide with $CHCl_3$ (21.5% rel. to MeOH extract), H_2O (51.2% rel. to MeOH extract) and finally MeOH (28.0% rel. to MeOH extract). Only the aq. percolate was toxic to mice.

Fractionation of the aq. percolate was performed on a polyamide column using $CHCl_3$–MeOH H_2O (3:1:0.1) as eluent. Fractions were monitored using TLC and bioassay with mice. Those containing the toxin (Ehrlich positive test) were combined and further chromatographed on a Bio-Gel P-2 column. Elution with H_2O produced selective retention of the toxin which was then eluted from this column with 0.3 M HOAc–Pyr buffer (pH 6.5), further purified on an alumina column (eluant: $CHCl_3$ MeOH H_2O, 20:7:1) and its structure was elucidated.

Identification of pyrrole-3-carbamidine **1**. Hygroscopic compound; R_f 0.20 (silica gel TLC, $CHCl_3$ MeOH H_2O, 20:7:1; Ehrlich: violet; anisaldehyde sulphuric acid: orange). IR (film) cm^{-1}: 3500, 3400, 3100, 1650, 1600, 1500, 1450, 1390, 1260, 1130, 1090, 1050, 950, 780, 730. ^{1}H NMR (DMSO-d_6): δ6.84 (1H, *m*, H-4, *v*A), 6.94 (1H, *m*, H-5, *v*B), 7.94 (1H, *m*, H-2, *v*X), 8.1–9.9 (4H, *br s*, NH, exchangeable with D_2O). ^{13}C NMR (DMSO-d_6) δ107.4 (*d*, C-4), 111.0 (*s*, C-3), 120.2 (*d*, C-5), 123.4 (*d*, C-2), 160.3 (*s*, C-6). MS *m/z* (%): 109 ([M]$^+$, 100.0), 94 (10.4), 93 (99.0), 92 (20.3), 81 (9.4), 67 (7.3), 66 (33.3), 65 (6.7), 64 (2.3), 60 (55.3), 58 (4.0), 54 (5.9), 52 (3.1), 45 (34.2), 44 (8.4), 43 (35.2). MS (deuterated **1**): *m/z* (%): 112 (2.9), 111 (21.3), 110 (78.3), 109 (100), 95 (4.8), 94 (38.3), 93 (97.2), 92 (11.4), 81 (5.7), 68 (1.8), 67 (8.7), 66 (20.8), 65 (4.8), 64 (1.8), 61 (7.6), 60 (44.7), 58 (5.3), 54 (3.8), 52 (2.3), 46 (5.2), 45 (32.3), 44 (7.3), 43 (37.4).

Picrate derivative of **1**. Mp 247–249° (EtOH). ^{1}H NMR (acetone-d_6): δ6.03 (1H, *m*, H-4), 7.80 (1H, *m*, H-5), 8.20 (1H, *m*, H-2), 8.40 and 8.80 (2 *br s*, NH and picric acid protons).

Alkaline hydrolysis of **1**. This was carried out in a sealed tube with 10% NaOH for 4 hr at 100°. An acidic compound was extracted with Et_2O, further purified on a silica gel column with $CHCl_3$ MeOH (19:1) and identified as pyrrole-3-carboxylic acid [13] (mp, MS and ^{1}H NMR coincident with those of a standard). Ammonia was also detected.

Preparation of **1** was carried out by addition of $NaNH_2$ [11] to pyrrole-3-carbonitrile under reflux in toluene for 6 hr. The latter was previously prepared according to lit. [14]. R_f and spectral data of the synthetic compound were identical with those of the natural product **1**.

Analysis of N. hippomanica *samples from other areas.* Plants from four areas of Argentina (Provinces of La Pampa, Santa Fé, Entre Rios and Córdoba) were analysed as above for the presence of the toxin. The samples were gathered when plants were at the same stage of growth. All contained the toxic **1**.

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N_b-DEMETHYLALSTOPHYLLINE OXINDOLE, AN OXINDOLE ALKALOID FROM THE LEAVES OF *ALSTONIA MACROPHYLLA*

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Key Word Index *Alstonia macrophylla*; Apocynaceae; N_b-demethylalstophylline oxindole; NMR.

Abstract - An oxindole alkaloid, N_b-demethylalstophylline oxindole has been isolated from the leaves of *Alstonia macrophylla*. Its structure has been elucidated on the basis of spectroscopic studies.

INTRODUCTION

Alstonia macrophylla has been introduced to Sri Lanka as a forest tree and has rapidly become naturalized in the moist region up to an elevation of 1200–1500 m [1]. An alkaloid, N_b-demethylalstophylline oxindole (1), has been isolated from its leaves and its structure elucidated on the basis of mass spectrometry and ^{1}H NMR including two-dimensional NMR (2D *J*-resolved, COSY 45), ^{13}C NMR (BB and DEPT) and NOE difference studies.

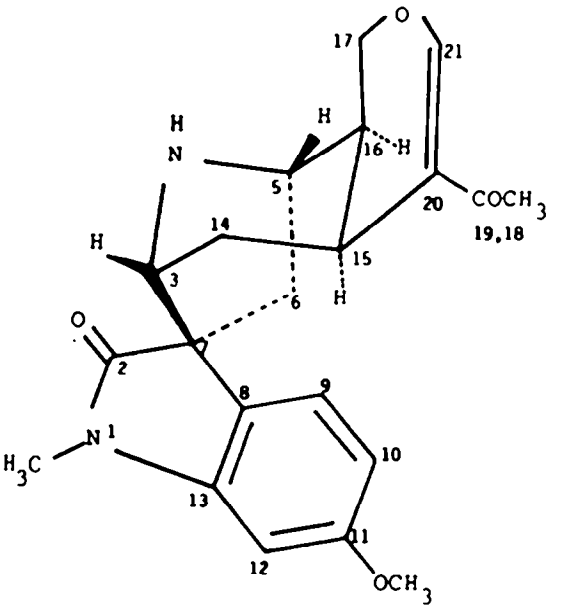
RESULTS AND DISCUSSION

The methanolic extract of the dried leaves was concentrated under reduced pressure and the crude alkaloids were isolated by acid–base extraction. A combination of CC and prep. TLC afforded the new alkaloid named *N*-demethylalstophylline oxindole. It was obtained as a white amorphous solid, $[\alpha]_D^{27}$ +76° ($CHCl_3$, *c* 0.25).

The UV spectrum displayed characteristic absorptions for the oxindole system, with λ_{max}^{MeOH} nm (log ε) 223 (4.29), 256 (3.98), 286 sh (3.44), 294 sh (3.31) and λ_{min}^{MeOH} nm (log ε) 243 (3.91). The IR spectrum showed typical bands at $\nu_{max}^{CHCl_3}$ cm^{-1} 1650 (conj C=O) and 1705 (lactam C=O).

The mass spectrum of **1** showed a $[M]^+$ at *m/z* 368 and the high resolution spectrum showed the exact mass of this peak to be 368.1738 in agreement with the molecular formula $C_{21}H_{24}N_2O_4$ (calcd. 368.1736). Similar measurements on some of the prominent ions served to establish the mass fragmentation pattern of **1**. N_b-Demethylalstophylline oxindole displayed a base peak at *m/z* 179.0949. As reported for other oxindole alkaloids [2, 3] this ion is formed due to cleavage of the spiran ring. The accompanying peaks at *m/z* 161.0837 and 136.0765 are associated with the loss of water and an acyl radical (CH_3CO^+) from the base peak. Further decomposition of the base peak results in the formation of the second intense fragment at *m/z* 56.0503. The retro Diels–Alder type fragmentation of ring D gives rise to the indole-containing fragment at *m/z* 244.1211. Another fragment with *m/z* 190.0868 bears the indole skeleton.

The 300 MHz (PFT) ^{1}H NMR spectrum of **1** in $CDCl_3$ showed three methyl singlets at δ 2.24, 3.17 and 3.85. These signals were assigned to the acetyl methyl, N_a-methyl and methoxy groups, respectively. The rather low field value of



(1)