Phytochemistry, 1977, Vol. 16, pp. 1399–1400. Pergamon Press. Printed in England

2-AMINOIMIDAZOLE IN SEEDS OF *MUNDULEA SERICEA*

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(*Received* 5 *April* 1977)

Key Word Index—*Mundulea sericea*; Leguminosae: 2-aminoimidazole; isolation.

Abstract—An amine present as *ca* 0.9% of the fr. wt of the seeds of the legume *Mundulea sericea* has been isolated and characterised as 2-aminoimidazole on the basis of chemical tests, PMR and MS. The structure has been confirmed by synthesis.

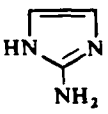
INTRODUCTION

The leguminous shrub *Mundulea sericea* (Willd.) A. Chev., (syn. *M. suberosa*, Benth.), is distributed throughout tropical Africa and parts of S. Africa, extending to Madagascar, India and Indonesia [1]. Depending on local custom, the seeds, leaf, root and bark are employed in these areas as fish poisons or insecticides and several non-nitrogenous toxic compounds, including rotenone, are reported in the plant [1, 2]. In the course of our investigations on tropical legumes for nitrogenous compounds with potential physiological activity, the seeds of *Mundulea sericea* were found to contain a non-volatile compound behaving as a strong base during paper ionophoresis and which gave colour reactions suggesting an imidazole and a guanidine derivative. This paper describes the isolation of the amine (**MSX**) as its hydrochloride, its characterisation as 2-aminoimidazole and the preparation of a reduced and a monoacetyl derivative.

RESULTS AND DISCUSSION

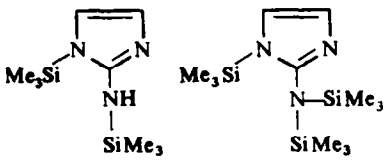
MSX proved to be indistinguishable from authentic 2-aminoimidazole on the basis of PMR, MS, elemental analysis, PC and paper ionophoresis, and spot colour tests. Both were stable to acid hydrolysis, being unaffected by 6 M HCl at 100° for 15 hr, but were degraded by M NaOH at room temperature. (This contradicts an early report that 2-aminoimidazole is unaffected by alkali [3]). The PMR spectrum in DMSO-d_6/$CDCl_3$ shows 3 singlets of equal intensity, two of which disappear when the sample is shaken with CD_3OD. Elemental analysis indicates an empirical formula of $C_6H_{12}N_6SO_4$ for the sulphate and hence a MW of 83 for the free base.

The MS of **MSX**-sulphate obtained on the probe showed an abundant ion at *m/e* 83 which lost 27 a.m.u. (HCN) to give an ion at *m/e* 56 (metastable ion at *m/e* 37.8). A mass measurement (at a resolution of ~2000) of the *m/e* 83 ion with respect to *m/e* 78.0469 of benzene and *m/e* 92.0626 of toluene gave a value of *m/e* 83.049 ± 0.002. Only two possible formulae fit this experimental value, $C_3H_5N_3$ or C_5H_7O; the second may be disregarded because it does not contain N and has an odd valency. This empirical formula is consistent with the stable positive ion (**1**), which is the M^+ of 2-aminoimidazole formed by decomposition of the original salt on the heated probe of the mass spectrometer.



1

Treatment of **MSX**-sulphate with *N,O-bis*trimethylsilyltrifluoracetamide gave two peaks upon subsequent GC–MS analysis. These two components were poorly resolved on a 3% OV1 column and corresponded to compounds (**2**) and (**3**) by examination of their MS.



2 **3**

MSX is readily reduced with H_2 and platinum black, absorbing 1 mol H_2 per mole amine. The PMR spectrum of the reduced compound consists of 3 singlets in a ratio of 2:1:1 and indicates that the additional protons are attached to C atoms. The signals attributed to N protons disappear on shaking with CD_3OD. Reaction of **MSX** with acetic anhydride leads to the formation of a monoacetyl derivative. This absorbs in the UV with a maximum at 238 nm (in contrast to **MSX** and dihydro-**MSX** which do not absorb above 210 nm), resulting from conjugation of the acetyl carbonyl and the guanidine moiety [4]. Treatment with M HCl at 100° for 30 min leads to regeneration of the parent amine. The PMR spectrum consists of two singlets in the ratio of 3:2, attributed to the MeCO-protons and the olefin protons respectively.

MSX was most readily detected on chromatograms of

plant extracts by the Pauly reagent, which can detect as little as 1 μg on paper. For quantitative estimation the Bratton–Marshall reagent was used as described in [7]. Alkaline reagents are unsuitable owing to the lability of **MSX**.

2-Aminoimidazole occurs in certain marine sponges [5, 6], and has been shown to be the precursor of the antibiotic 2-nitroimidazole (azomycin) in *Streptomyces eurocidicus* [7]. To our knowledge it has not been reported previously in plants, although the 4,5-dihydro derivative is found in *Cassia absus* [8] but combined with a monoterpene in the alkaloid chaksine. It is noteworthy that certain acyl derivatives of 2-aminoimidazole have been synthesised as potential local anaesthetics [9]. The isolation of **MSX** is yet another example of the occurrence of a N-rich low MW compound in seeds which might combine the roles of chemical defence (i.e. as an antimetabolite to a potential predator) and of a readily mobilised source of N for use on germination [10].

EXPERIMENTAL

The 90 MHz PMR spectra were determined in DMSO-d_6 or DMSO-d_6 + $CDCl_3$ using TMS as internal standard and frequency lock signal. MS were obtained at 70 eV with a trap current of 60 μA. For routine examination of samples descending PC using Whatman 3 MM and *n*-BuOH–HOAc–H_2O (12:3:5) was used. High voltage paper ionophoresis using buffers at pH 1.9 and 3.6 was used as described elsewhere [12]. For location of spots on paper ninhydrin, pentacyanoaquoferriate (PCF) and diazotised sulphanilic acid (Pauly reagent) were used as in [13], the Bratton–Marshall reagent as in [7] and pentacyanoammineferriate (PCAF) as in [14].

Isolation of **MSX**. Seeds of *M. sericea* (230 g) were ground to a fine powder and transferred to a Soxhlet extraction apparatus, capacity 1 l.). The powder was then extracted for successive periods of 16 hr with CH_2Cl_2 (2 l.), *n*-heptane (2 l.) and MeOH (2 l.). The first two extracts did not contain any **MSX** and were discarded. The MeOH extract was evapd almost to dryness on a rotary evaporator at 40°, the residue suspended in 200 ml of 50% MeOH, filtered and then applied at 5 ml/min by gravity feed to a column (15 × 4.5 cm) of Amberlite CG-50 (H^+ form) which had been previously equilibrated in 50% MeOH. (A small pre-column, (2 × 1 cm), of the same resin was interposed between the extract and the main column to trap any brown oil which otherwise interfered with the subsequent elution of **MSX** from the main column). When all the extracts had been applied, the main column was washed exhaustively with 50% MeOH until the effluent was colourless, then with 500 ml of H_2O and finally with 0.1 M HCl. The acid eluate was collected in 10 ml fractions which were monitored for **MSX** by means of spot tests with Pauly reagent. Fractions containing the amine were pooled and fed at 3 ml/min directly onto a column of Dowex 50W-X8, 20–50 mesh, H^+ form (20 × 4.5 cm), previously equilibrated in H_2O. The column was washed with H_2O (1 l.), then eluted with successive 250 ml aliquots of 0.4 M, 0.6 M, 0.8 M and 1 M HCl. The eluate was again collected in 10 ml fractions which were monitored for **MSX** using Pauly reagent. Amine-containing fractions (No. 16 onwards) were pooled and evapd to dryness at 60° under vac. The residue was suspended in H_2O and re-evapd. This procedure was repeated until all excess HCl had been removed. Finally the residue was suspended with gentle warming in a minimum vol. of EtOH and *ca* two vols hexane were added. A two-phase system was obtained with most of the amine in the upper phase and most of the coloured impurities in the lower phase. The upper phase was evapd to dryness at 30° and the residue again taken up in a minimum vol. of warm EtOH. A few drops of Et_2O were added and the mixture was left at 4° to crystallise. White hygroscopic crystals of the amine HCl were obtained (350 mg) which were then stored in a desiccator. The HCl salt proved to be too hygroscopic for accurate elemental analysis and a portion was therefore converted to the sulphate by means of Ag_2SO_4. Cal. for $C_6H_{12}N_6SO_4$ C, 27.30; H, 4.54; N, 31.80; SO_4, 36.36. Found: C, 27.35; H, 4.52; N, 31.76; SO_4 36.30. PMR (DMSO-d_6 + $CDCl_3$) δ 6.63 (4,5 CH); 7.22 (2C-NH_3); 11.88 (1.3 NH); integral ratios (1:1:1). MS (direct insertion using a temp.-programmed glass probe) *m/e* 83 (100, M^+), 56 (26, M^+-HCN), 55 (13. M^+-HCN-H), 54 (2), 53 (3), 43 (8), 41 (5), 40 (4). PC R_f 0.45; ninhydrin (yellow); PCF (green → magenta); PCAF (green); Pauly (yellow → purple); Bratton–Marshall (magenta).

Preparation of reduced derivative of **MSX**. **MSX**-HCl (20 mg) was dissolved in H_2O (1 ml) and a small amount of Pt black added. H_2 was then bubbled through until the soln was negative to Pauly reagent, but gave a strong blue-purple with PCF reagent, (*ca* 4 hr). The Pt black was removed by filtration and the filtrate lyophilised. Yield *ca* 19 mg white hygroscopic crystals of reduced **MSX**-HCl (This was also converted to the sulphate for elemental analysis). Calculated for $C_6H_{16}N_6SO_4$: C, 26.86; H, 5.97; N, 31.34; SO_4, 35.82. Found: C, 26.76; H, 5.85; N, 31.40; SO_4, 35.90. PMR (DMSO-d_6) δ 3.54 (4,5 CH_2); 7.82, (2C-NH_2); 7.93, (1,3 NH); integral ratio (2:1:1). MS (probe) *m/e* 85 (74, M^+), 84 (100, M^+-H), 83 (17, M^+-2H), 57 (23), 56 (62, M^+-CH_2 = NH), 55 (21), 43 (40). PC R_f 0.45; ninhydrin (yellow); PCF (blue-purple); PCAF (pale blue-purple); Pauly (negative); Bratton–Marshall (negative).

Preparation of monoacetyl derivative of **MSX**. **MSX**-HCl (14 mg) was suspended in 0.2 ml of Ac_2O in a closed vial and heated at 100° for 1 hr. The amine dissolved slowly and colourless crystals of the acetylated derivative pptd out. These were washed with a little Ac_2O and recrystallised from EtOH–Et_2O. Calc. for $C_5H_8N_3OCl$: C, 37.15; H, 4.95; N, 26.00; Cl, 21.98. Found: C, 37.05; H, 4.95; N, 25.80; Cl, 22.16. PMR (DMSO-d_6 + $CDCl_3$), δ 2.29 (Me); 6.99 (4,5 CH); integral ratio (3:2). MS (probe) *m/e* 125 (28, M^+), 110 (2, M^+-Me), 83 (100, M^+-CH_2CO), 56 (35), 55 (12), 43 (37). PC R_f 0.65; ninhydrin (weak yellow); PCF (pale green); PCAF (negative); Pauly (red); Bratton–Marshall (negative).

Synthesis of 2-aminoimidazole sulphate. This was prepared by the method of ref. [11]. The sulphate could be converted to the chloride (e.g. to facilitate the preparation of the monoacetyl derivative) by means of $BaCl_2$.

Acknowledgements—We wish to thank the Wellcome Trust and the Science Research Council for financial support. The seeds were kindly supplied by Drs. B. A. Krukoff and K. Mwauluka.

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