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Studies on biological activity of synthetic mannich base nitrogen mustard of 3 – aminophthalimide – 1

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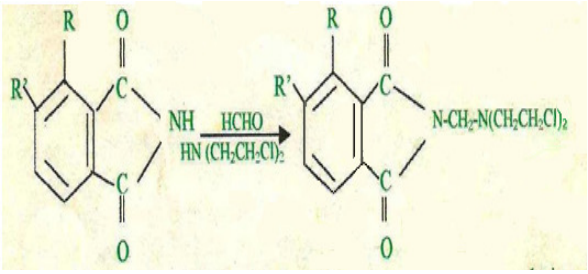
Abstract
Synthetically prepared N - bis (2 - Chloroethyl) amino – methylene - 3 - aminophthalimide (m.p. 84°) showed definite fungicidal effect on *Aspergillus* species at 5% or higher concentration of the compound in ethyl alcohol. Lower doses did not show any degree of lethality when pure culture of *Aspergillus flavns* was treated by filter paper discs of the test solutions. The compound may further be tested for its fungicidal property in vivo for its promotion as a possible anti-fungal drug and a compound active against aspergilloses.

Keywords: Nitrogen mustard, 3 - aminophthalimide, fungicidal effect, *Aspergillus sp.*

INTRODUCTION

Mannich base nitrogen mustards are known to be cyto-toxic and anti-neoplastic (Pettit and Settepani 1962). Synthetic N, N - bis (2 - chloroethyl) amine was reported to have latent activity and specificity against tumour.(Davis et al 1955; Ross et al 1955; Ross and Warwick 1956; Ross 1964). Using substituted phthalimides (3 - nitrophthalimide, 4- nitrophthalimide, 3- aminophthalimide, 4 - aminophthalimide, 3- chlorophthalimide, 4- chlorophthalimide) a number of Mannich base nitrogen mustards [N, N - bis (2-chloroethyl) amino - methylene substituted phthalimides] were synthesized on reaction with N, N – bis (2 - chloroethyl amine) and formaldehyde (Pettit and Settepani 1962; Sinha et al 1999).



3 - nitrophthalic acid and 4 - nitrophthalic acid were used to synthesize substituted phthalimides. The project aims at the screening of these nitrogen mustards for their biological activities such as bactericidal effect, fungicidal effect, cytotoxicity and anti-cancerous principles.

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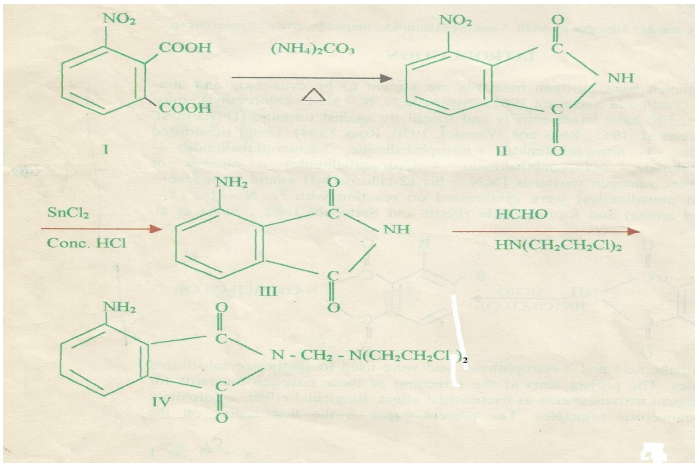
MATERIALS AND METHODS

3 - nitrophthalimide (11, m.p. 216°) was prepared by heating 3 - nitrophthalic acid (I) with ammonium carbonate. 3 - aminophthalimide (m.p. 264° d) was obtained as yellow crystals by stirring 3 - nitrophthalimide (11.20 g, 0.1 mol) with a solution of stannous chloride (84 g, 0.11 mol) in HCl (450 ml) and water (150 ml), cooling the resulting solid at 0°C , washing it with hot water and crystallized as yellow needles. Mannich base nitrogen mustard of 3 - aminophthlimide (IV m.p. 84°C) was synthesized by the reaction between N, N - bis (2 - chloroethyl) amine, 3 : 1 ethanol - formalin (37%) and 3 - aminophthlimide (III) at 40°C, yellow solids, crystallized with ethanol - ether, V - max 1760, 1700 (C=O), 1450 (CH bond), 3390 (NH), 750 (C-Cl); δ 2.4 (2H, s. N - CH2 - N), 3.5 - 3.6 (10H, m, 2 x CH_2CH_2. NH2), 7.2 - 7.3 (3H, s. ArH). M.P was determined in open capillaries and is uncorrected. Ir spectra (KBr) were taken on a Perkin - Elmer 137G spectrophotometer and pmr spectra ($CDCl_3$) on a varian A 60 D instrument using TMS as internal standard.



Spores of *Aspergillus flavus* suspended in 1ml of sterile water were mixed with 9.0 ml of lukewarm molten agar and poured into a sterile petridish. After solidification of the agar, isolated single spores were marked under a microscope on an inverted petridishe. Now, a circular agar area was cut off and aseptically transferred to PDA slants for growth at 25^{0}C. After one week of the growth, mycelial discs of 5 mm diameter were cut off from the maintained pure culture of the organism for inoculation into sterilized PDA medium in seven different petridishes for blotter disc studies. Small Whatman filter paper (no.44) discs of 15 mm soaked in 1.0%, 2.0%, 2.5%, 3.0%, 4.0% and 5.0% concentrations of 3 - aminophthalimide mustard (IV) in ethyl alcohol and sterile double distilled water were put in the middle of the inoculated petridishes one each separately. The petriplate containing the disc soaked in sterile distilled water served as the control. Paired petriplates were incubated at 25^{o}C in inverted position for 6 days for the growth of the organism after which inhibitory effect of the compound was studied. Inhibitory zone was measured in each case.

RESULTS AND DISCUSSION

Very few natural compounds have been reported to show marked antifungal activity (Priyadarshini and Tulpule 1980). Extracts of various medicinal plants have been screened for fungicidal property (Dhar et al 1968; Bilgramy et al 1980) but little or no activity was reported. Lal and Kapoor (1980) reported inhibitory effect of food preservatives like acetone, boric acid and benzoic acid on the growth of *Aspergillus* species. Reddy and Reddy (1984) evaluated the effect of some volatile compounds on the growth of *A. terreus* and found some degree of reducing effect of acetic acid and ethanol on patulin production by the organism but mycelial growth was not affected. Girisham and Reddy (1989) studied the effect of benzyl alcohol, chloroform, cyclohexane and other volatile compounds as well as some food preservatives on the vegetative growth and terreic acid production in *A.terreus* and reported that no correlation existed between mycelial growth and terreic acid production. Instead, they found that benzoic acid and sodium metabisulphate suppressed the growth of the mycelium. 1.0% concentration of 3 - aminophthalimide mustard (IV) have not shown any marked inhibition as the mycelium of the organism grew all around invading the disc (Plate - 1). Many potentially lethal compounds have been found to be effective only at higher doses (Gilman et al 1991). Inhibition of the mycelial growth started at 2% concentration of the compound but it was marked and most noticeable only at 2.5% concentration. At 3% and 4% concentrations of the compound, the inhibitory effect became increasingly pronounced but it was most lethal for mycelial growth first at 5.0 % concentration as the organism showed the sign of overall degeneration in the treated petriplate. Inhibition of growth of the mycelia by any compound may be due to some cytotoxic principle which would result into killing of mycelial cells or antimitotic principle which would arrest the mitotic process during growth of the fungus and / or due to some kind of interference with regulatory mechanisms responsible for the development and morphogenesis of the mycelia.

In all higher fungi including *Aspergillus nidulans*, it has been reported that apart from nutritional status and light factor, mycelial growth and conidiation are under the control of genetically co-cordinated regulatory pathways (Timberlake 1990). Genetic control of development process is pre-eminent and it masks the environmental control (Ebbole1996). Only when the developmental programme is inactive as due to mutation, nutritional control mechanisms can function. Hence, arrest of the growth of fungal mycelia may probably be successful only when both control systems are inactivated.

Effect of different concentrations of 3 - aminophthalimide mustard on the mycelial growth of *A. flavus*.

Plate 1. Plate 2. Plate 3.

Plate 1. Abundant mycelial growth in 1% 3 - aminophthalimide.
Plate 2. 35 – 40 mm inhibitory zone around filter paper disc soaked in 2.5 % 3 – aminophthalimide Mustard (IV).
Plate 3. 40 – 45 mm inhibitory zone and general scarce growth of mycelia under treatment by 5% 3 -aminophthalimide mustard (IV).

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