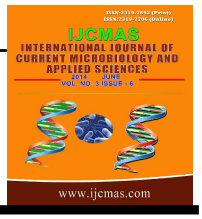
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 6 (2014) pp. 182-188**
http://www.ijcmas.com



Original Research Article

Antimicrobial activities of the oxidized product of Skatole by peroxodisulphate (PDS) oxidant Using Ethanol Medium

J.Stephen[1*], K.Subramani[2], and P.Mani[3]

[1]MAM College of Engineering, Siruganur, Trichyi, Tamil Nadu, India
[2]Department of Chemistry, Islamiah College, Vaniyampadi, Tamil Nadu, India
[3]Department of Biotechnology, TUK arts and science college, Karanthai, Tannjavur, Tamil Nadu, India
Corresponding author

Keywords

Oxidation, 3-methylindole (3-MI), Peroxo-disulphate

A B S T R A C T

Kinetics studies of 3-methylindole (3-MI) by peroxo oxidant peroxodisulphate (PDS) using ethanol medium have been investigated. The final product was identified as 3-methyloxindole and then examined for antimicrobial activity. The antibacterial of the synthesized product was determined by agar diffusion method using Nutrient Agar medium. The antifungal activity of the synthesized product was evaluated by agar diffusion method using potato dextrose agar

Introduction

Indoles possess significant biological activity[1]. Oxidation of indoles has received much attention due to the involvement of their resulting products in significant biological processes[2]. 3-MI a degradation product of tryptophan formed in the rumen of cattle and goats and in the large intestine of humans is well-known as a highly selective pulmonary toxicant for ruminants[3]. Metabolism and bioactivation of 3-MI by human liver microsomes have been reported that a total of eight oxidized metabolites were detected in human liver microsomes. Five of the metabolites were confirmed as 3-methyloxindole, 3-hydroxy-3-methylindolenine, 3-hydroxy-3-methylindole, 5-hydroxy-3-methylindole and 6-hydroxy-3-methylindole. Among the three newly identified metabolites, one is either 4- or 7-hydroxy-3-methylindole and the other two were derived from subsequent oxidation on the phenyl ring of 3-methyloxindole[4].

It had recently reported that the oxidation of 3-methylindole by peroxomonosulphate

(PMS) in ethanol medium[5]. In view of this, the present work involves mechanistic investigation of oxidation of 3-methyindole by peroxodisulphate (PDS) and antimicrobial studies of the oxidized product.

Materials and Methods

Several techniques, both kinetic and chemical are employed in organic chemistry to delineate the mechanism of a reaction under examination. Kinetic studies were carried out in 50% (v/v) aqueous ethanol medium under pseudo first-order conditions with a large excess of 3-MI over PDS in the temperature range of 303-323 K. The reaction was followed by estimating the unreacted PDS as a function of time by using the iodometric method. The liberated iodine was titrated against standard sodium thiosulphate solution by using starch as indicator.

Various kinds of experiment were carried out varying the concentration of the substrate [3-MI] by keeping constant concentrations of Oxidant, Solvent, [H+], μ and vice-versa. A total second order, first order each with respect to [3-MI] and [PDS] has been observed. The reaction was unaffected by increase of $[H^+]$. Increase of percentage of ethanol decreases the rate. Variation of ionic strength had no influence on the rate.

To find out the stoichiometry, Solutions of 3-MI containing an excess of PDS were kept overnight at room temperature. Titrimetric estimation of the concentration of PDS consumed and assuming that all the 3-MI taken had reacted, the stoichiometry of 3-MI: PDS was found to be 1:2. Moreover no polymer formation was observed when a freshly distilled acrylonitrile monomer was added to the deaerated reaction mixture indicating the absence of free radical intermediates. For the product analysis, slight excess of PDS was added with 3-MI dissolved in ethanol and kept the reaction mixture for a day. The product was separated and analysed by IR and found to be 3-methyloxindole. So the proposed mechanism is as follows:

This results suggest that the reaction proceeds through an electrophilic attack of the oxidant (PDS) exists as $S_2O_8^{-2}$ ion in solution at the nucleophilic site C3 of 3-MI by a mechanism involving displacement of sulphate ion to form compound (1) as the rate determining step. Compound (1) undergoes intramolecular rearrangement[6] to give 2-hydroxy-3-methylindole (4) through a cyclic intermediate (2). The second attack of PDS ion on compound (4) gives Compound (5) which finally loses HSO_4^- to give 3-methyl-2-oxoindole as the product.

Antimicrobial studies of Oxidized product 3-Methyloxindole

Antibacterial study

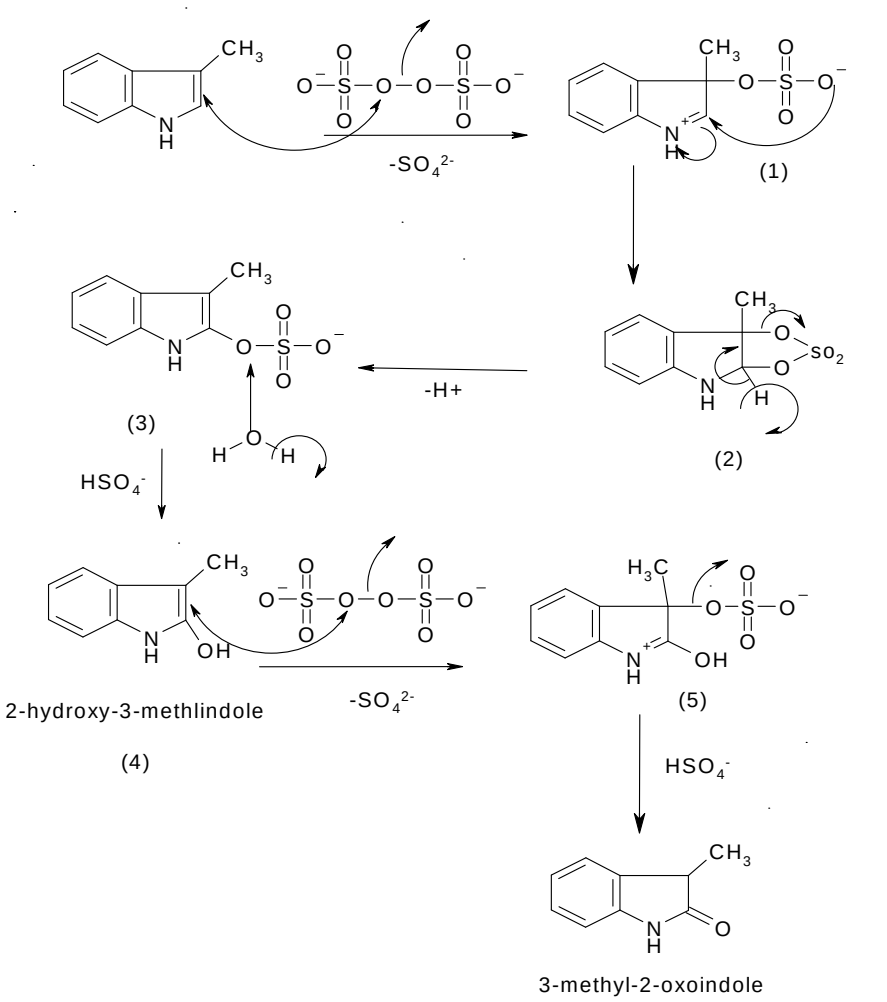
The antibacterial activity was performed by agar cup plate method.

Preparation of medium

Suspended 28.0 grams in 1000 ml distilled water. Heated to boiling and dissolved the medium completely. Sterilization was done by autoclaving at 15 Ibs pressure (121˚c) for 15 minutes. Mixed well and poured into sterile Petri plates.

Antifungal study

The antifungal activity was performed by agar cup plate method.



Preparation of medium

Suspended 39.0 grams in 1000 ml distilled water. Heated to boiling and dissolved the medium completely. Sterilization was done by autoclaving at 15 Ibs pressure (121˚c) for 15 minutes. Mixed well before dispensing in specific work, when pH 3.5 is required; acidified the medium with sterile 10 % tartaric acid. The amount of acid required for 100 ml of sterile cooled medium was approximately 1 ml. The medium was not heated after the addition of acid.

Microorganisms

Bacteria as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Bacillus subtilis* and fungi as *Candida albicans, Aspergillus flavus, Aspergillus niger* and *Aspergillus oryzae* were the microorganisms used and they were obtained from the Microbiology Laboratory of the Thanjavur Medical College Hospital, Thanjavur. These microorganisms were identified and confirmed by Microbiologists, Department of Microbiology, Thanjavur Medical College, Thanjavur.

Preparation of 24 hours pure culture

A loop full of each of the microorganisms was suspended in about 10 ml of physiological saline in a Roux bottle. Each of these was streaked on to the appropriate culture slants and was incubated at 37ºC

for 24 hours for bacteria and for fungi which was incubated at 25ºC for 24-48 hours. After completion of incubation period, the growth was observed and the tubes were kept into 2-8˚C until use.

Preparation of solutions for the experiment

The sample solution (30 mg/ml distilled water) as 50 µl, 100 µl and 150 µl were used for the experiment. Standard antibiotic solution as Chloromphenical and Nystatin for fungi (10mg/ml distilled water- 30 µl (30 µg / disc) (positive control) used to compare the sample.

Preparation of dried filter paper discs

Whattman filter paper (No:1) was used to prepare discs approximately 6 mm in diameter, which were placed in hot air for sterilization After sterilization, the discs were loaded with different concentrations of prepared sample solutions again kept under refrigeration for 24 hrs.

Application of discs to inoculated agar plates

Previously prepared paper discs were dispensed onto the surface of the inoculated agar plate. Each disc was pressed down firmly to ensure complete contact with the agar surface. The discs were placed on the medium suitably apart and the plates were incubated at 5°C for 1 hr to permit good diffusion and then transferred to incubator at 37°C for 24 hrs. After completion of 24hrs, the plates were inverted and placed in an incubator set to respective temperature for 24 hrs.

Antimicrobial assay

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka et al., 2007). Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 minutes. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab was dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing bacteria were spread on Nutrient agar plates and fungi were spread on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the sample (50 µl, 100 µl, 150 µl and 30µl for standard) were laid down on the surface of inoculated agar plate. The plates were incubated at 37˚C for 24 h for the bacteria and at room temperature (30±1) for 24-48 hrs. for yeasts strains. Each sample was tested in triplicate.

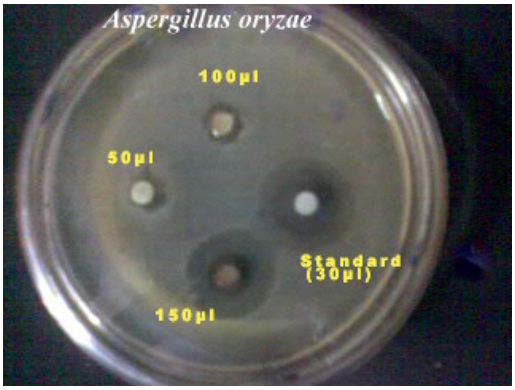
Measurement of zone of inhibition

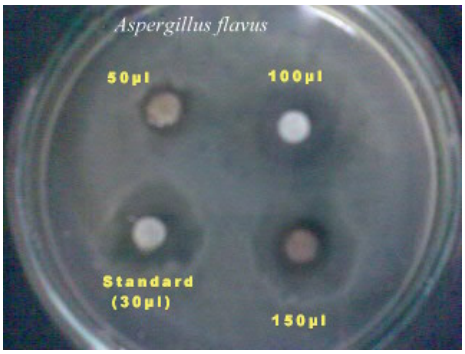
The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the sample were measured using a millimeter scale. The diameter sizes in mm of the zone of inhibition are shown in the table 1 and 2.

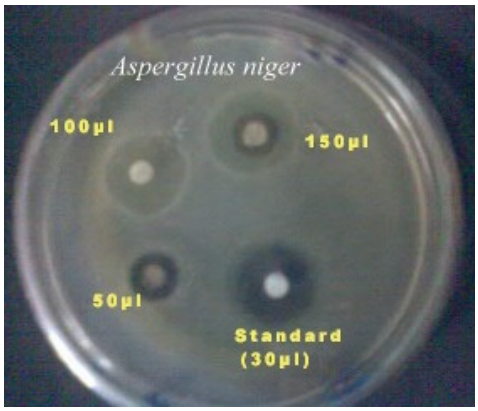
Table.1 Antifungal activity of sample: Zone of Inhibition

Sample	*Aspergillus flavus (mm)*	*Aspergillus niger (mm)*	*Aspergillus oryzae (mm)*	*Candida albicans (mm)*
50 µl	3 ± 0.02	4 ± 0.03	2 ± 0.03	-
100 µl	7 ± 0.04	6 ± 0.04	5 ± 0.04	8 ± 0.03
150 µl	18 ± 0.09	16 ± 0.06	17 ± 0.09	13 ± 0.07
Standard (30 µl) (Nystatin)	17 ± 0.07	12 ± 0.05	18 ± 0.10	8 ± 0.03

Figure.1 Antifungal activity of sample







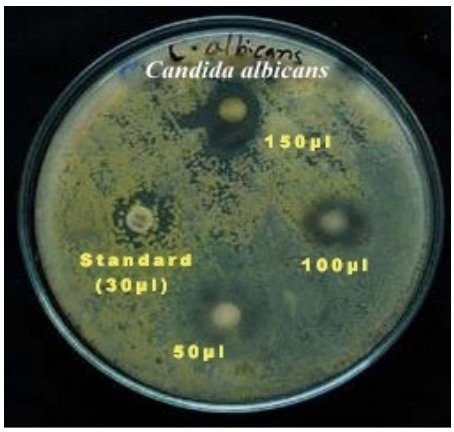
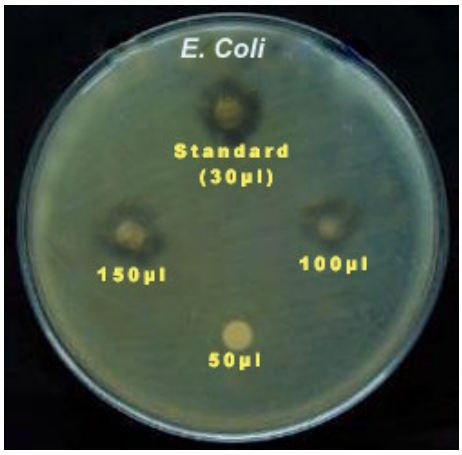


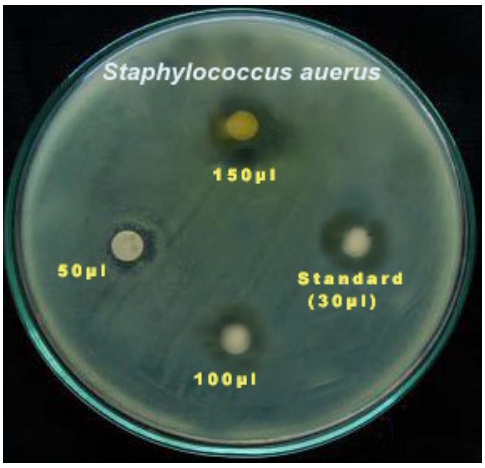
Table.2 Antibacterial activity of sample: Zone of Inhibition

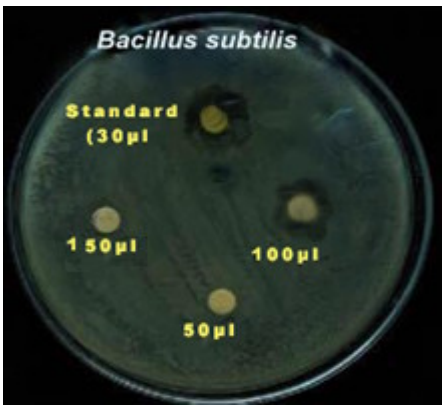
Sample	*Escherichia Coli (mm)*	*Staphylococcus auerus (mm)*	*Klebsiella pneumonia (mm)*	*Bacillus subtilis (mm)*
50 µl	-	2 ± 0.01	2 ± 0.03	-
100 µl	3 ± 0.02	5 ± 0.03	7 ± 0.04	-
150 µl	6 ± 0.04	14 ± 0.08	15 ± 0.06	7 ± 0.07
Standard (30 µl) (Chloromphenical)	6 ± 0.04	11 ± 0.06	9 ± 0.05	10 ± 0.08

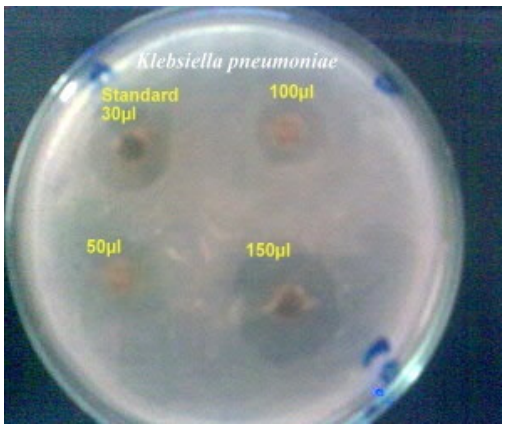
.

Figure.2 Antibacterial activity of sample









It has been observed that the product 3-methyl-2-oxindole shows good inhibitory activity against the fungal pathogen *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans* than the standard Nystatin. The product also shows good inhibitory activity against the bacterial pathogen *Staphylococcus auerus* than the standard Chloromphenical and shows same inhibitory activity against *Escherichia coli*. We hope that our discussion will prove helpful in further

development and progress of the pharmacological applications of our oxidized product.

Acknowledgment

We are very thankful to MAMCE- trichy, TUK college and Bharathiar university for giving this opportunity to do our research project in their esteemed organisation.

References

Al-Kazwini, A. T.; O'Neill, Peter; Adams, G. E.; Cundall, R. B.; Lang, G.; Junino, A. J Chem Soc, Perkin Trans 2, 1941 (1991)

Carlson, J. R,; Yokoyama, M. T. and Dickinson, E. O.; Science 176, 298-299 (1972).

Jackson A. H. and Lynch P. P. 1987. J. Chem. Soc., Perkin Trans 2, 1215.

Shyadligeri, A. S.; Gadaginamath, G. S. Indian J Chem, Sect B: Org Chem Incl Med Chem 34, 1059 (1995).

Stephen J,; Subramani K,; Muniyappan K and Chandramohan.G. 2013. International Journal of Current Research, Vol. 5, Issue, 09, 2566-2569.

Zhengyin Yan,; LaHoma M. Easterwood,; Noureddine Maher,; Rhoda Torres,; Norman Huebert and Garold S. Yost,; Chem. Res. Toxicol., 20, 140-148 (2007).