S.a.c.6. RAMACHANDRA SHARMA, N.–Studies on the Microbial Transformation of Alkaloids : Strychnine and 2-Nitrostrychnine–1988–Dr. P. Madhavan Pillai

Microbial transformations of natural products have been a useful method for the preparation of many biologically active compounds. Although microorganisms are capable of carrying out stereospecific and regiospecific chemical transformations on a wide variety of organic compounds, their utilization has not been fully exploited on substrates other than carbohydrates and steroids. Alkaloids as a class, have received very little attention in this respect as their transformations involving heterocyclic systems are more difficult compared to alicyclic compounds. Strychnine, the major alkaloid isolated from the seeds of the Indian tree, Strychnos nuxvomica is known to stimulate all portions of the central nervous system with preference to the spinal cord. However, being a powerful convulsant it leads to death from asphyxia. As such, neither strychnine nor any of its derivatives has any therapeutic application in the western system of medicine at present.

The microbial transformations of strychnine and one of its derivatives, 2-nitrostrychnine were extensively investigated in this work. Strychnine was subjected to the actions of a variety of microorganisms with a view to convert it into a product of less toxicity and improved pharmacological activity. Many of the cultures obtained from the National Collection of Industrial Microorganisms (NCIM) failed to convert strychnine into any useful products. Of these organisms only *Bacillus thuringiensis* 2159, *Cunninghamella blakesleeana* 687 and *C. echinulata* 691 produced the N-oxide in small amounts and this process itself is of no practical value as the same product can be obtained in better yields by chemical methods. Hence organisms that grow on strychnine were isolated from the local soil using the elective culture technique. The isolated organisms were purified and the auxoenic strains were identified upto the generic level according to Bergey's Manual of Determinative Bacteriology, based on their morphological, cultural and biochemical characteristics. The strains were identified as belonging to *Arthrobacter* species. The growth of the organism under varying physicochemical environmental factors such as temperature, pH, sodium chloride

concentration and substrate concentration were studied to arrive at the optimal conditions. Similarly, the influence of a second carbon source, glucose on the rate of transformation was also investigated. The kinetics of utilization of strychnine was studied by subjecting the fermented broth to exhaustive extraction using chloroform-methanol and estimating the concentration of the substrate in the extract at specified time intervals spectrophotometrically. The progress of strychnine utilization was also followed using thin layer chromatography. These date also gave an idea about the optimum incubation period required.

By incorporating the optimal conditions of physicochemical parameters and incubation time, experiments were designed to isolate the transformed product. The fermentation was carried out for 14-18 hours, the fermented broth was made alkaline to pH 9 using ammonium hydroxide and the solvent (water) was removed under reduced pressure. The residue was extracted with methanol and the crude product was purified by column chromatography to give about 10% of C_{16}-Hanssen acid. The identity of the product was established by comparison with an authentic sample of C_{16}-Hanssen acid obtained by the oxidation of brucine by a known procedure and also by conversion into its perchlorate and methiodide and comparison of their physical constants.

The isolated strain, *Arthrobacter* sp. ACM 1 could not produce a substantial yield of the product C_{16}-Hanssen acid because the organism was also found to degrade this product further. Also the same strain failed to act on brucine which is the dimethoxy derivative of strychnine and which occurs in *Strychnos nuvomica* seeds alongwith strychnine. The studies also revealed that the microorganism utilised strychnine alone in a mixture of strychnine and brucine and this method can therefore be used to purify the commercially available brucine. That the bacterial strain is able to utilise strychnine both as a carbon and nitrogen source indicates that it differs from the earlier reported *Arthrobacter strychnovorum*, which could utilise strychnine only in the presence of an external inorganic nitrogen source. Also the fact that it produced C_{16}-Hanssen acid and did not metabolise brucine establishes that it is different from A. *strychnophagum* also reported previously.

Transformation of 2-nitrostrychnine into 2-aminostrychnine was carried out using another organism, also isolated from the local environment. This organism was identified to the generic level and was shown to belong to *Pseudomonas* species. Here also the growth of the organism under varying physiochemical parameters were investigated to obtain the optimal conditions. These optimal conditions were incorporated and the kinetics of transformation was followed by analysing the chloroform-methanol extract of the broth using high pressure liquid chromatography. Employing the optimum physiochemical factors and incubation time, fermentation of 2-nitrostrychnine was carried out to isolate the product, 2-aminostrychnine. Its identity was established by comparison wih an authentic sample obtained by chemical reduction of 2-nitrostrychnine. This Pseudomonas strain, ACM 11 apparently contains a nitroary' reductase enzyme system because the same culture was also able to reduce aromatic nitro compounds to the corresponding ary' amines. The low yield of 2-aminostrychnine indicates that the amine formed was further broken down by the same organism.

This study, thus has led to the isolation of two new microorganism, one belonging to *Arthrobacter* species that selectively degrades strychnine into C_{16}-Hanssen acid and the other, belonging to *Pseudomonas* species that reduces

2-nitrostrychnine into 2-aminostrychnine. The characterisation of the microorganisms upto the generic level, their growth studies under different physiochemical parameters and the proof of the structures of the transformation products have been presented in detail.