

AMMONIUM MOLYBDATE: A POTENTIAL INHIBITION OF BARLEY AMYLASE

V. H. MULIMANI, R. SHASHIKALA and M. S. DHARPUR*

*Department of Chemistry, Gulbarga University, Gulbarga 585 106, India.*** Department of Biochemistry, M. R. Medical College, Gulbarga 585 106, India.*

ABSTRACT

Barley amylase is inhibited by ammonium molybdate and 50% inhibition has been obtained at 0.8 mM concentration. The inhibition was of non-competitive type with an apparent K_i values of 0.9 mM. The Hill coefficient (n) was 2 suggesting that ammonium molybdate interacts with barley alpha amylase with cooperativity.

INTRODUCTION

ALPHA amylases in plants are useful for conversion of starch into reducing sugars during seed germination. The plant amylases are of particular value in brewing, distilling and baking industries. The enzymes act in a similar manner in animal alpha amylases. The α -D-(1 \rightarrow 4) linkages in the inner and outer chains of the starch are hydrolyzed at random, whereas the α -D-(1 \rightarrow 6) and α -D-(1 \rightarrow 3) linkages cannot be hydrolyzed. Alpha amylase inhibitors which occur in plants or produced by micro-organisms can either be low MW compounds or macromolecules. Inhibitors with low MW include salicylic acid¹, caffeic acid, gallic acid², abscisic acid³, the antibiotic nojirmycin⁴ (5-amino-5-deoxy D-glucopyranose), a peptide-like compound⁵ and an oligosaccharide with MW 1500 produced by certain strains of streptomyces⁶. Novel polycation inhibits *Bacillus subtilis* α -amylase, and this type of inhibition was found to be non-competitive⁷⁻¹¹.

The inhibitory action of ammonium molybdate on some glycosidases has been known since 1949 when the inhibition of Q-enzyme was reported¹². Later the inhibitory action of ammonium molybdate on R-enzyme¹³, yeast isoamylase¹⁴ and limit dextrinase¹⁵ was reported. In the presence of 0, 0.2 and 2% (W/V) of ammonium molybdate, the activities of isoamylase after 4 hr were 78.2, 19 and 4 respectively. Furthermore the commercial ammonium molybdate $[(\text{NH}_4)_6\text{MO}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}]$ is the inhibitory substance used in the cited work.

No attempts seem to have been made to study the effect of ammonium molybdate on plant amylases. Therefore, the present investigation has been undertaken to study in detail the inhibitory action of ammonium molybdate on purified barley amylase.

MATERIALS AND METHODS

All chemicals used here are of analytical grade.

Barley amylase type VII-A was obtained from Sigma Chemical Company, USA. The enzyme was used without any further purification and stored at 0-4°C unless used immediately. Enzyme was homogenous as judged by polyacrylamide gel electrophoresis. 3,5-Dinitrosalicylic acid (DNS) was obtained from M/S Scientific Syndicate, Bombay. Maltose soluble starch and ammonium molybdate were purchased from, BDH, Bombay. Amylase activity was determined by measuring the maltose formed by the procedure of Bernfeld¹⁶. The reaction mixture consisted of 0.5 ml of 1% soluble starch, different concentrations of ammonium molybdate (1-15 mM), 0.5 ml of enzyme (3.2 units/ μ g), phosphate buffer (0.01 M) containing 0.006 M sodium chloride at PH 6.9. It was incubated for 3 min at 37°C. The assay of β -amylase was performed under identical conditions but using acetate buffer (0.01 M) of pH 4.8 instead of phosphate buffer. Enzyme unit was defined as the amount of enzyme required to liberate one μ mol of maltose per minute under assay conditions. Specific activity of the enzyme (units per mg protein) was measured by the method of Lowry *et al*¹⁷ using BSA as standard. The nature of enzyme inhibition by ammonium molybdate was evaluated according to the method of Lineweaver-Burk¹⁸. The value of apparent inhibition constant (K_i) was determined by Dixon plot¹⁹. The value of K_i was given by the point of intersection of two straight lines. From the inhibition data, the linear Hill plot of $[\log(V_0 - V_i)/V_i]$ vs log ammonium molybdate concentration was plotted according to Johnson *et al*²⁰ where V_0 and V_i are reaction velocities without and with ammonium molybdate respectively at a fixed substrate concentration.

RESULTS AND DISCUSSION

The kinetic studies on the inhibition of barley α and β amylase by ammonium molybdate show that 50% inhibition (I_{50}) for α and β amylase is obtained at 0.8

and 0.68 mM concentrations respectively. This shows that ammonium molybdate is a potent barley amylase inhibitor. The inhibition of enzyme activity increases with increase in the concentration of ammonium molybdate to the extent that there is total inhibition at 1 mM concentration (figure 1). Double reciprocal (Lineweaver-Burk) plot of initial reaction rates against the varying concentration of starch shows that the ammonium molybdate treated and controlled curves intersected at abscissa (figure 2) indicating that am-

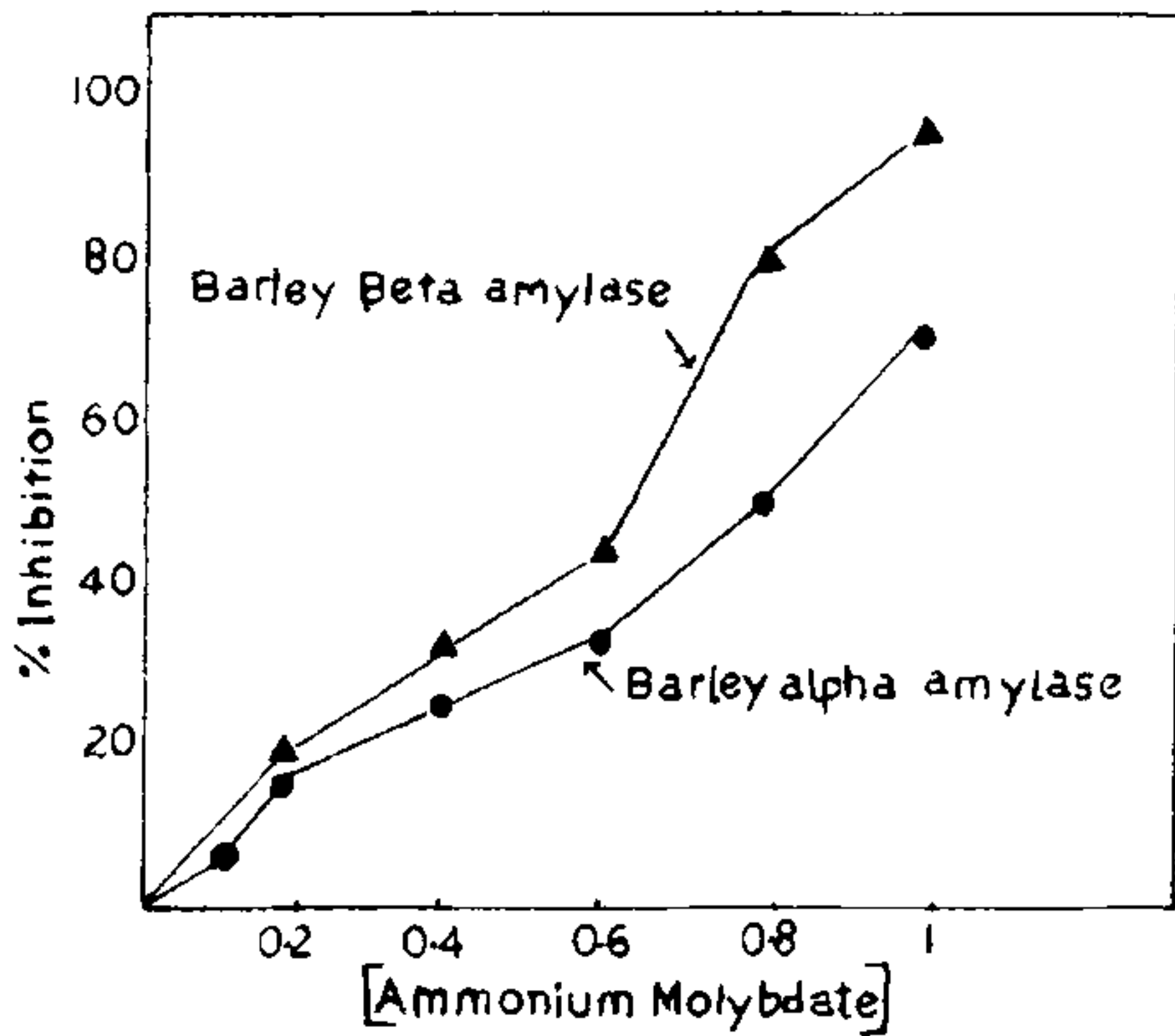


Figure 1. Inhibition of barley and amylase by ammonium molybdate.

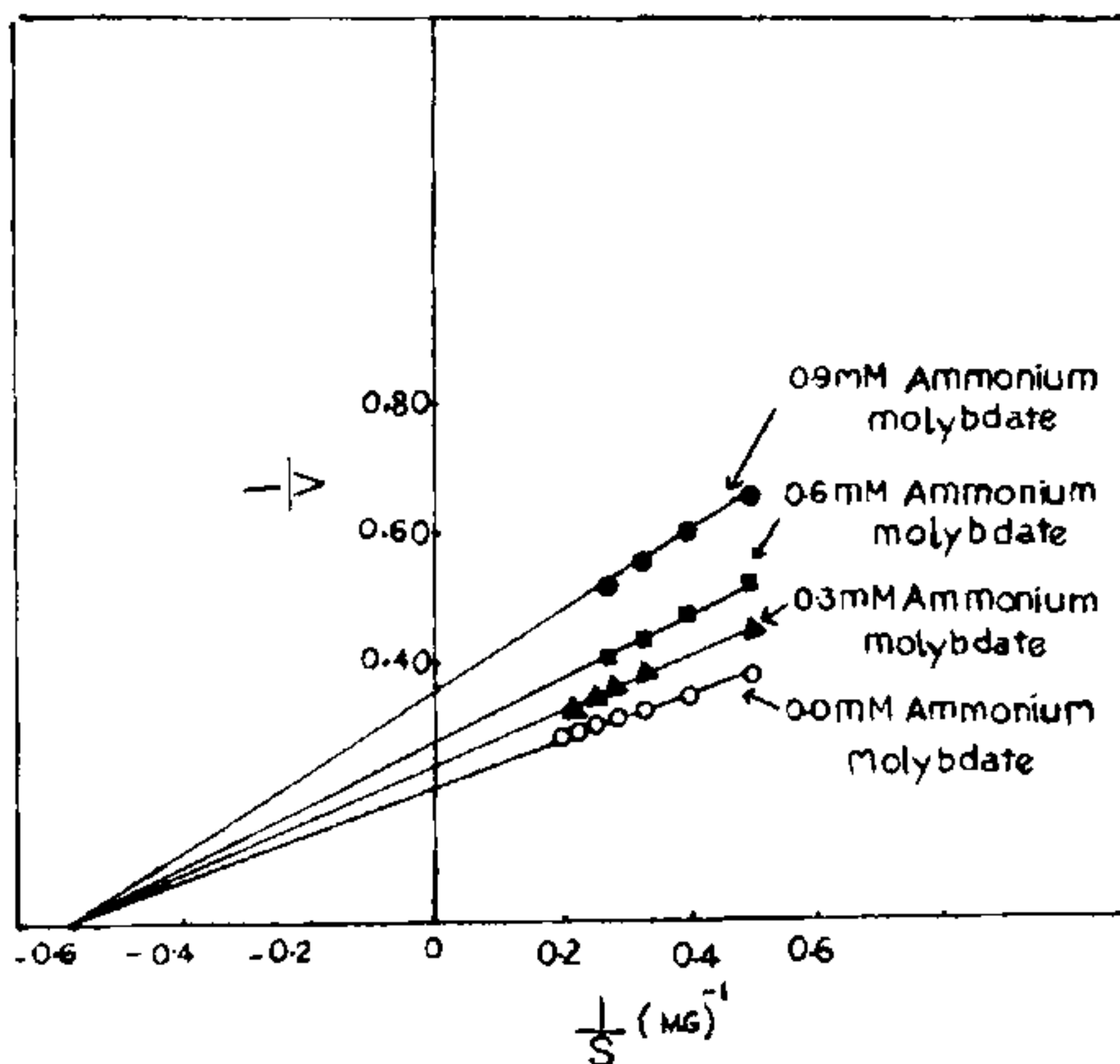


Figure 2. Lineweaver-Burk plot showing the non-competitive type of inhibition of barley alpha amylase by ammonium molybdate.

monium molybdate is a non-competitive inhibitor of the enzyme. The value of apparent K_i for α -amylase as computed from the Dixon plot obtained in the presence of different concentrations of ammonium molybdate was 0.9 mM (figure 3). The value of n , the number of ammonium molybdate units combining with one molecule of enzyme was determined from the slope of Hill plot (figure 4). This is a straight line with a slope of 2 indicating the presence of positive cooperativity phenomenon associated with most systems in-

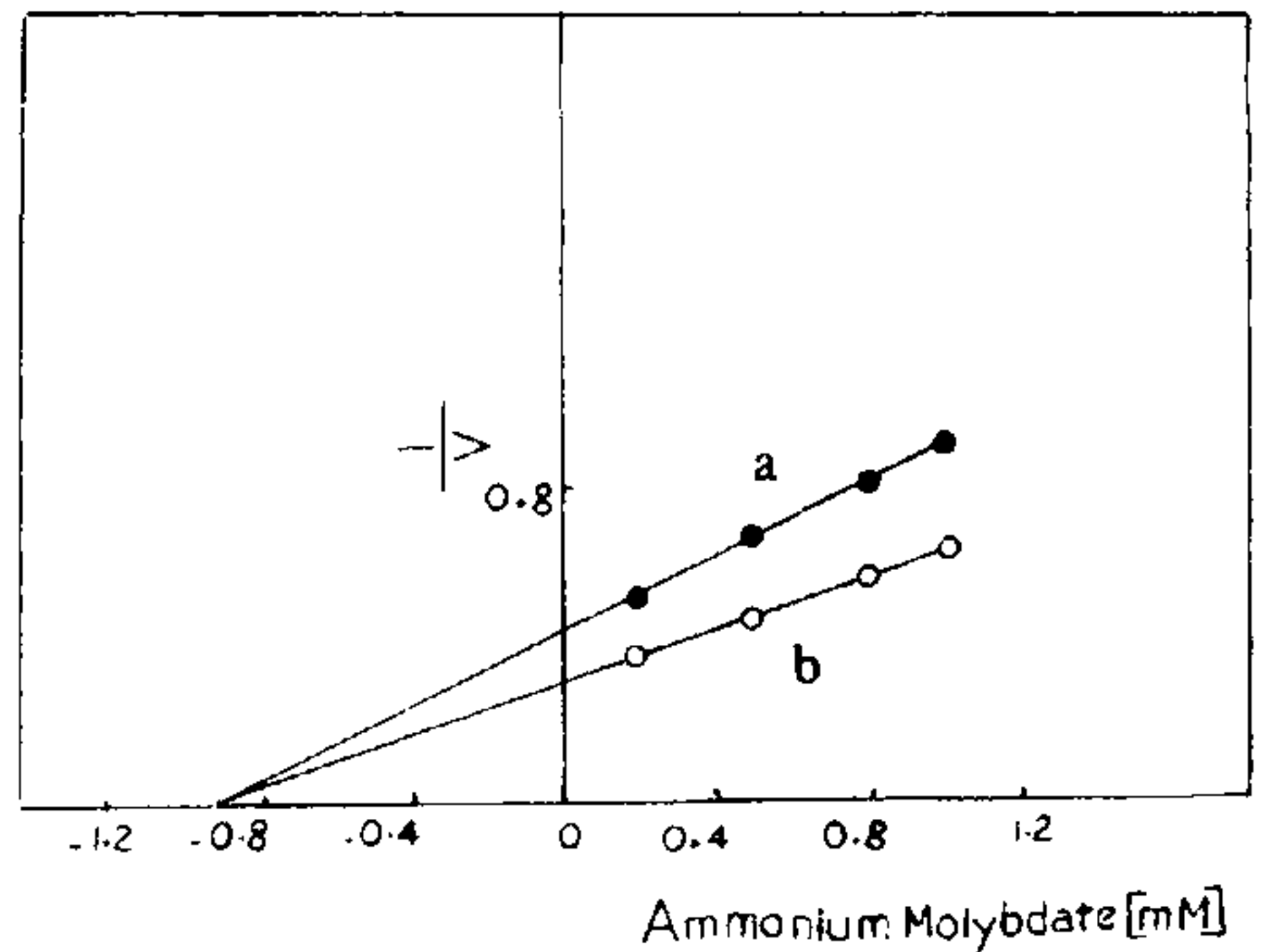


Figure 3. Plot of $1/V$ vs ammonium molybdate concentration at fixed substrate concentrations. Assays were carried out in the presence of varying inhibitor concentration (0-1 mM) at 1 and 2% (a and b) substrate concentrations. The lines of plots were extended backwards to obtain the value of K_i .

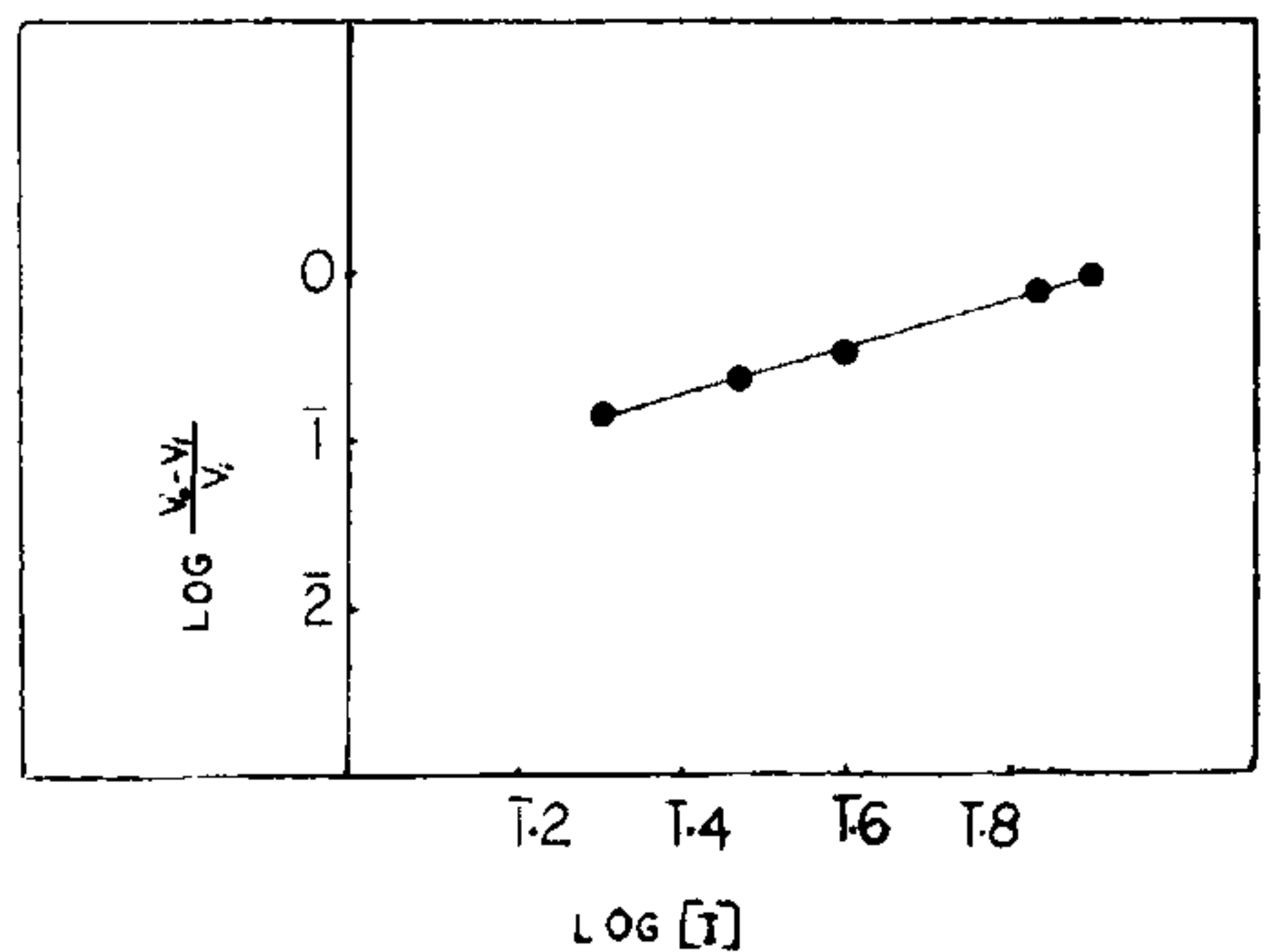


Figure 4. Plot of $\log (V_0 - V_i)$ vs \log ammonium molybdate inhibitor where V is the control activity without ammonium molybdate and V_i is the enzyme activity in presence of ammonium molybdate. Slope of this plot gives the value of n .

volving allosteric interactions²¹. The rate of inactivation of barley α -amylase by ammonium molybdate increases as the incubation period is gradually increased (Unpublished data) and maximum inhibition occurs at 40 min. Incubation longer than 40 min did not result in any further increase in the inhibition of barley α -amylase. The inhibition is not due to molybdate ion since sodium molybdate did not show any inhibitory effect. Therefore, inhibitory effect is specifically caused by the ammonium group. The mechanism of this inhibition appears to consist of a reversible binding of the ammonium molybdate to the enzyme molecule as is suggested by dialysis experiments. Similarly ammonium molybdate has been reported as an inhibitor of plant invertases²². The inhibition was of mixed type and heptamolybdate is postulated to act by a reversible binding to the enzyme.

The inhibition of barley amylase by ammonium molybdate will help in the preparation of undegraded starch from natural resources where the process of degradation may completely be prevented or kept to a minimum.

7 August 1985; Revised 23 January 1986

1. Hemberg, T. and Larson, I., *Physiol. Plant*, 1961, **14**, 861.
2. Kumar, S. Goel, A. K. and Toyal, M. S., *Curr. Sci.*, 1982, **51**, 739.
3. Hemberg, J., *Physiol. Plant*, 1975, **31**, 11.
4. Niwa, T., Inouye, S. Tsuruoka, T. Koaze, T. and Niida, T. *Agric. Biol. Chem.*, 1970, **34**, 966.
5. Veda, S. and Kaba, Y., *Agric. Biol. Chem.*, 1973, **37**, 2025.
6. Murao, S. and Dhyana, K., *Agric. Biol. Chem.*, 1975, **39**, 2271.
7. Mulimani, V. H., Day, R. A., *Curr. Sci.*, 1981, **50**, 629.
8. Mulimani, V. H. and Day, R. A. Paper presented in National Symposium on Bioorganic Chemistry, Indian Institute of Science, Bangalore 1982.
9. Dharpur, M. S. and Mulimani, V. H., Paper presented in S.B.C. Annual Meeting, V.P. Chest Institute, 1984.
10. Mulimani, V. H., Dharpur, M. S. and Shashikala, R., Paper presented in second International Symposium on 'Life Science', Shillong, 1985.
11. Mulimani, V. H. and Dharpur, M. S., Paper presented in S.B.C. Annual Meeting held in Pantnagar, G. B. Pant University of Agriculture and Technology, 1985.
12. Gilbert, G. A. and Swallow, A. J., *J. Chem. Soc.*, 1949, 2849.
13. Whelan, W. J., *Encyclopedia of plant physiology*, (ed.) W. Ruhland, Springer-Verlag, Berlin, 1958, **6**, 154.
14. Gunja, Z. H. and Manners, D. J., *Biochem. J.*, 1961, **81**, 392.
15. Macwilliam, I. C. and Harris, C. *Arch. Biochem. Biophys.*, 1959, **84**, 442.
16. Bernfeld, P., *Methods Enzymol.*, 1955, **1**, 149.
17. Lowry, O. H. Rosebrough, N. D. Farr, A. C. and Randat, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
18. Linewear, H. and Burk, D. J., *J. Am. Chem. Soc.*, 1934, **56**, 658.
19. Dixon, M., *Biochem. J.*, 1953, **55**, 170.
20. Johnson, F., Egring, H. and William, R. W. *J. Cell Comp. Physiol.*, 1942, **20**, 247.
21. Atkinson, D. E., Hathway, J. A. and Smith, E. C., *J. Biol. Chem.*, 1965, **240**, 2682.
22. Prado, F. E. Sampietro, A. R. and Vatuone, M. A., *Phytochemistry*, 1979, **18**, 1799.

NEWS

B. C. ROY AWARD

Prof. V. Ramalingaswamy, Director General of the Indian Council of Medical Research, Dr. R. K. Menda of Bombay and Dr. M. M. S. Siddhu of Lucknow have

been awarded the B. C. Roy National Award for 1985. The Awards are of the value of Rs.50,000 in cash and a silver salver.
