Inhibition of nicotinamidase activity in cell-free extracts of *Mycobacterium phlei* by 3-acetylpyridine

The inhibition of bacterial growth by sulfonic analogues of nicotinic acid and its amide has been established more than a decade ago[1-4]. These compounds were relatively non-toxic when fed to animals maintained on a niacin-deficient diet. On the other hand, 3-acetylpyridine was found to cause severe symptoms and even lead to death of niacin-depleted animals, but it did not inhibit bacterial growth[3-6]. The inhibitory effects of the afore-mentioned compounds could be overcome by addition of nicotinic acid or nicotinamide to the culture medium or to the animal diet respectively.

The general assumption derived from these findings was that the said analogues interfered with the metabolism of nicotinic acid and its amide. However, no direct evidence was available about identity of the enzymic system(s) affected by these analogues. Recently, KAPLAN AND CIOTTI[7] isolated an analogue of diphosphopyridine nucleotide (DPN) containing 3-acetylpyridine, from pig-brain preparations treated with the latter substance.

The present communication deals with the inhibitory effect of 3-acetylpyridine on the enzymic hydrolysis of nicotinamide by cell-free preparations from *Mycobacterium phlei*.

In connection with studies on the specificity of amidases in *M. phlei* we sought for a specific inhibitor of nicotinamidase activity. The following substances chemically related to the amide were tested: nicotinic acid, α-picolinic acid, trigonelline, 6-amino-nicotinamide*, *iso*nicotinic acid hydrazide, pyridine 3-sulfonic acid and 3-acetylpyridine. The bacteria and the method used for preparation of cell-free extracts have been described elsewhere[8]. The nicotinamidase activity was followed by estimation of the liberated ammonia by nesslerization.

Nicotinic acid, α-picolinic acid, trigonelline and 6-amino-nicotinamide at inhibitor/substrate ratios of 5:1–10:1 showed no effect upon the nicotinamidase activity of *M. phlei* extracts. Pyridine-3-sulfonic acid and *iso*nicotinic acid hydrazide at the same concentrations inhibited slightly the enzymic hydrolysis of nicotinamide. On the other hand, 3-acetylpyridine inhibited the system quite strongly; 50% inhibition was obtained at an inhibitor/substrate ratio of 20:1 (Fig. 1). In order to establish the nature of this inhibition, the influence of substrate concentration

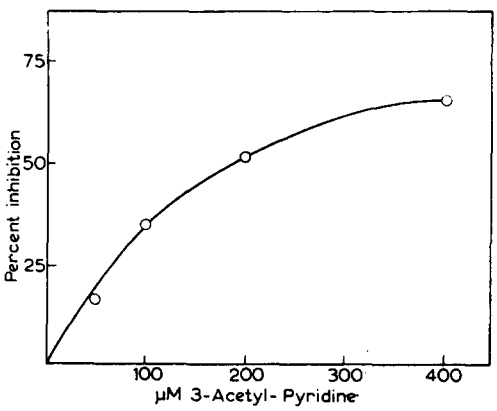

Fig. 1. Inhibition of nicotinamidase activity by 3-acetylpyridine. Reaction mixture: 10 μM nicotinamide, 3-acetylpyridine as indicated, bacterial extract corresponding to 2.7 mg protein, phosphate buffer (pH 8.2) — $M/150$ in a total volume of 1 ml. Incubation — at 37° C for 100 min. Ammonia was estimated colorimetrically by nesslerization, after previous alkalinization of the reaction mixture with borax-NaOH buffer and distillation in a water-bath at 50° C, under a constant air current for 40 min.

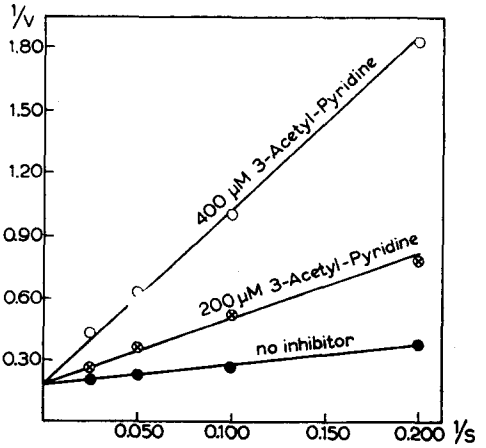

Fig. 2. Influence of substrate concentration on the inhibition of nicotinamidase activity, by 3-acetylpyridine. Reaction mixture: 5, 10, 20, and 40 μM nicotinamide respectively, 3-acetylpyridine as indicated, bacterial extract corresponding to 2.7 mg protein, phosphate buffer (pH 8.2) — $M/150$ in a total volume of 1 ml. Incubation at 37° C for 2 hours. S-substrate concentration in μmoles; V-reaction velocity — μM NH_3 in 2 hours. (Estimation of NH_3 — see explanation of Fig. 1).

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on the inhibitory effect of various amounts of 3-acetylpyridine was tested. Results are given according to LINEWEAVER AND BURK[9] (Fig. 2), showing the competitive nature of the inhibition of nicotinamidase activity by 3-acetylpyridine.

Summary: Among several nicotinamide analogues tested, 3-acetylpyridine was the only one to inhibit strongly the nicotinamidase activity of extracts from *M. phlei*. The inhibition proved to be of the competitive type.

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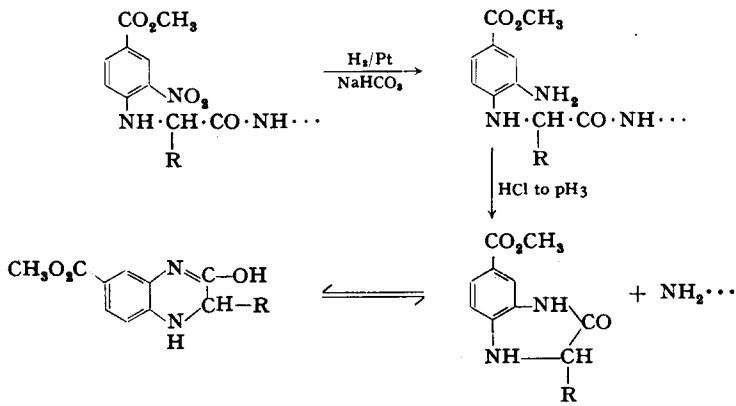
The stepwise reductive cleavage of DNP-peptides

Recently HOLLEY AND HOLLEY[1] introduced a new stepwise degradation for peptides based on the following sequence of very gentle reactions:



These conditions contrast with the strong acid used for the cleavage of the first peptide bond in previous methods[2,3] which often leads to nonspecific peptide bond fission.

An interesting variation is the substitution of 2:4-dinitrophenyl-peptides (DNP-peptides) for the 2-nitro-4-carbomethoxy-phenyl derivatives; the 2:4-diamino-phenylpeptide produced by the catalytic reduction should also lactamize readily and thereby split the first peptide bond. Identification of the amino acid at each step would be through hydrolysis of an aliquot of the DNP-peptide and characterisation of the DNP-amino acid by known methods. As HOLLEY AND HOLLEY[1] had already observed, difficulties arise because both the intermediate triamine and the amino-dihydroquinoxaline formed are very easily oxidised by oxygen. The triamine must therefore be made to lactamize in the reduction vessel in an atmosphere of hydrogen. The next reaction, condensation with fluoro-2:4-dinitrobenzene (FDNB), is carried out in nitrogen. The DNP-amino-hydroquinoxalines appear to be stable in air, can be extracted and do not seem to interfere. It has been possible to use such a method on the microscale for the degradation of two simple peptides. The recent publication by JUTISZ AND RITSCHARD[4] of work along the same lines prompts the publication of this note showing results in close agreement with these authors.