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.

N. GROSSOWICZ
Y. S. HALPERN

Dept. of Bacteriology, Hebrew University-Hadassah Medical School, Jerusalem (Israel)

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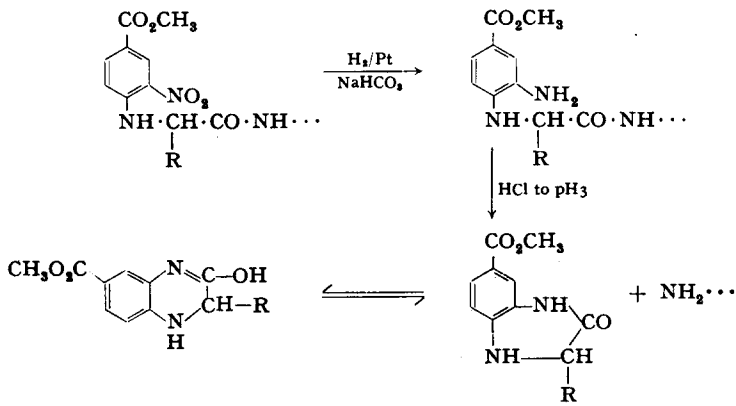
The stepwise reductive cleavage of DNP-peptides

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.

EXPERIMENTAL AND RESULTS

Degradation of DNP-diglycyl-glycine

The action of FDNB and sodium bicarbonate on diglycyl-glycine in 50% aqueous methanol gave an 80% yield of DNP-diglycyl-glycine after recrystallisation from water, m.p. 242–244° C (uncorr.). Found C, 39.3; H, 3.9; N, 19.6; $C_{12}H_{13}O_8N_5$ requires C, 40.5; H, 3.7; N, 19.7. The analysis is by Drs. WEILER AND STRAUSS, Oxford.

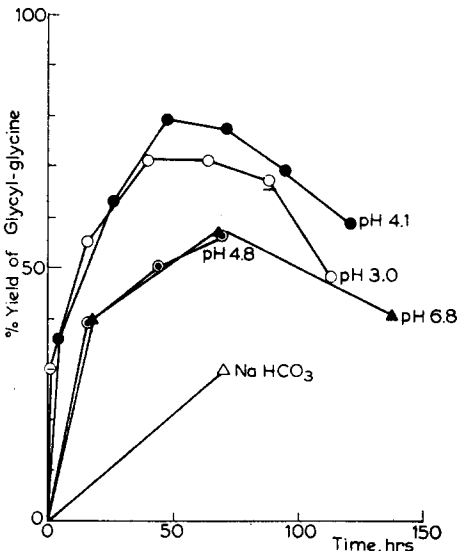


Fig. 1.

Solutions containing 50–70 μM of DNP-diglycyl-glycine in 0.5 ml ethanol and 2.0 ml of 0.2M acetate buffer of the appropriate pH were reduced by shaking with 10 mg of platinum oxide in an atmosphere of hydrogen at room temperature and pressure. Within an hour, or less, the solutions were colourless and gas absorption had ceased.

The course of lactamization was followed by withdrawing samples from time to time, without admitting air, and estimating the amount of free glycyl-glycine formed.

Three samples of 20 μl each were placed on a strip of Whatman No. 3MM filter paper which was barely wet with pyridine:acetic acid:water buffer, pH 3.6 (1:10:90). This was held between glass plates and a potential difference of 20 V/cm was applied for one hour. After drying, one of the three samples was developed with ninhydrin to locate the glycyl-glycine. The corresponding areas in the other two samples were cut out and the peptide on them estimated by the method of NAFTALIN[5]. The results at different pH values are shown in Fig. 1. Clearly the optimum pH for the reaction at room temperature is near pH 4.1 and the reaction does not give a 100% yield of glycyl-glycine, possibly owing to diketopiperazine formation.

Degradation of DL-*leucyl-glycyl-glycine*

By the action of FDNB on DL-leucyl-glycyl-glycine in aqueous dioxane containing an excess of triethylamine the crude DNP-tripeptide, 67 μM in 5 ml of water, was obtained and this was used directly without further purification. Addition of triethylamine to the aqueous solution raised the pH to 9.2 and this was then shaken in hydrogen with 10 mg of 5% palladium on alumina. After only 8 minutes the reduction was complete and the solution colourless. Addition of dilute acetic acid until a pH of 4 was reached and standing at room temperature for 17 hours caused cleavage of the first peptide bond and this was completed by warming to 50° C for 3 hours. Pure nitrogen replaced the hydrogen and the peptide was allowed to react with 0.85 ml of a 10% solution of FDNB in dioxane, 1 ml dioxane and 0.5 ml triethylamine. After two hours in the dark at room temperature the reaction mixture was diluted with an equal volume of water and extracted with ether. The yield of DNP-glycyl-glycine could be estimated by paper chromatography of small samples using the phthalate-*tert.*-amyl alcohol system of LOWTHER AND BLACKBURN[6]. The spots were eluted and the amount of DNP-peptide estimated at 360 mμ in 1% sodium bicarbonate.

The next cycle was performed in a similar manner and the DNP-glycine estimated by chromatography. No unexpected DNP-spots were noted at either stage. In the first step the yield was 87% of theory, in the second 76%, making an overall yield of 66%.

*Attempted degradation of the cytochrome-*c *haeme-peptide*

The haeme-peptide from the tryptic digestion of horse cytochrome-*c* was prepared and purified by the methods of TUPPY AND BODO[7]. After oxidation with performic acid and reaction with FDNB in the presence of triethylamine and 50% ethanol the DNP-peptide was obtained and this gave on HCl hydrolysis DNP-cysteic acid and ε-DNP-lysine as expected. Reductive cleavage and another reaction with FDNB, using the conditions described for DL-leucyl-glycyl-glycine, yielded a DNP-peptide which gave on hydrolysis DNP-alanine as the only identifiable product. DNP-alanine was expected since the N-terminal sequence of this peptide is cysteic acid-alanine. The yields of DNP-cysteic acid and DNP-alanine were, however, so low that this degradation was not pursued.

In conclusion, it appears that the reductive cleavage of DNP-peptides is useful for the

degradation of simple peptides. Complications will certainly arise when amino acids such as lysine, tyrosine or histidine form part of the molecule.

Medical Research Council Unit for the Study of the Structure of Biological Systems, Cavendish Laboratory, Cambridge (England) V. M. INGRAM

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Alkaline decomposition of β-hydroxy-α-amino acids

Serine and threonine are decomposed when heated with strong alkali with the formation, among other products, of glycine and alanine from serine, and glycine and α-amino-*n*-butyric acid from threonine[1-3]. The mechanisms of the reactions leading to the formation of glycine and of the product corresponding to $R \cdot CH_2 \cdot CH(NH_2) \cdot COOH$ by alkaline decomposition of these β-hydroxyamino acids have not been fully elucidated but plausible theories have been advanced by WIELAND AND WIRTH[2]. The work reported here was carried out to discover if the same reactions occur when other β-hydroxyamino acids are heated with strong alkali. The following amino acids were tested: serine, threonine, D-glucosaminic acid, DL-β-hydroxyaspartic acid, DL-β-hydroxyglutamic acid, DL-β-phenylserine, DL-β-*p*-hydroxyphenylserine, DL-α-amino-β-hydroxy-*n*-caproic acid (hydroxynorleucine), DL-α-amino-β-hydroxy*iso*caproic acid (hydroxyleucine), DL-α-amino-β-hydroxy-*n*-valeric acid (hydroxynorvaline) and DL-α-amino-β-hydroxy*iso*valeric acid (hydroxyvaline). Serine and threonine were obtained from British Drug Houses Ltd., DL-β-hydroxyaspartic acid (the least soluble of the two diastereoisomers obtained by the method of DAKIN[4]) from Prof. W. SHIVE, DL-β-hydroxyglutamic acid (synthesized by method of LEANZA AND PFISTER[5]) from Dr. K. PFISTER, DL-β-phenylserine and DL-β-*p*-hydroxyphenylserine (three forms synthesized by methods of BOLHOFER[6,7]) from Dr. W. A. BOLHOFER, and hydroxyleucine, hydroxynorleucine, hydroxyvaline and hydroxynorvaline (synthesized by methods of BUSTON *et al.*[8,9]) from Dr. H. W. BUSTON. D-glucosaminic acid was prepared by the method of PRINGSHEIM AND RUSCHMANN[10] modified according to WOLFROM AND CRON[11]. Paper chromatograms of these amino acids in various solvents revealed only one spot when sprayed with ninhydrin.

The procedure adopted was as follows. The amino acid (0.5 m*M*) was heated with 5*N* NaOH (2 ml) in a sealed glass tube at 105° C for 20 hours. The reaction mixture was then desalted by the method of CONSDEN, GORDON AND MARTIN[12], evaporated to dryness *in vacuo*, taken up in 1 ml of water and analysed by paper chromatography. Preliminary identification of the ninhydrin-reacting substances in the reaction products was made by two-dimensional chromatography, the chromatograms being run in one direction with phenol in an atmosphere of NH_3 and HCN and in the other with "collidine" in an atmosphere of diethylamine (DENT[13]). The "collidine" used was a mixture of equal parts of 2:4:6-collidine and the 2:4/2:5-lutidine supplied by Light and Co., the bases being redistilled before use. All chromatograms were run on Whatman No. 4 paper and sprayed with ninhydrin (0.1 %, w/v) in chloroform. Identifications were checked by marker experiments with several solvents, particularly *n*-butanol-acetic acid[14] and *tert.*-amyl alcohol-10 % (v/v) pyridine[15]. The latter separated isomeric amino acids that could not be distinguished with other solvents and proved valuable for identification of the products formed by alkaline decomposition of hydroxyvaline, hydroxynorvaline, hydroxyleucine and hydroxynorleucine. Several of the β-hydroxyamino acids could be readily distinguished from other amino acids on chromatograms by the colours of their spots with ninhydrin. For example, β-hydroxyglutamic acid gave a brownish purple spot, β-phenylserine gave a reddish brown spot, and β-hydroxyaspartic acid gave a spot that was initially greenish yellow and then changed through brown to purple. The positions of the β-hydroxyamino acids on two-dimensional phenol-"collidine" chromatograms are given in Fig. 1, and the ninhydrin-reacting substances detected on chromatograms of the products formed by heating the β-hydroxyamino acids with alkali are listed in Table I. It can be seen that all of the β-hydroxyamino acids investigated gave glycine when heated with alkali, that most also yielded the product $R \cdot CH_2 \cdot CH(NH_2) \cdot COOH$, and that some gave small amounts of other ninhydrin-reacting substances. The latter could not be identified, but the substance obtained from serine resembled glutamic acid in its behaviour on paper chromatograms and that obtained from β-hydroxyglutamic acid behaved chromatographically like