The results of hydrolysis of the substrates by crude mouse kidney or liver extracts which exhibit usually the powerful action of acylase, were the same as those by hog kidney extract; α-chloroacetylated lysine derivative was only susceptible. In view of the increase of acidity (50 per cent splitting), the stereochemical specificity of acylase, and the lack of susceptibility of ε-benzoylamino-*n*-caproic acid, no hydrolysis of the ε-benzoylamino group is expected and the bond where the hydrolysis took place is presumed as to be the part uniting the α-chloroacetyl group and the L-isomer. This deduction agrees with the report given by Greenstein et al. as ε-chloroacetylamino-*n*-caproic acid and chloroacetyl-β-alanine show a perfect resistance and the rate of hydrolysis of ε-carbobenzoxy-α-chloroacetyl- and α, ε-dichloroacetyl-DL-lysine are very slow. ε-Benzoylamino-*n*-caproic acid, ε-benzoylamino-DL-α-bromocaproic acid or α-formyl, α-acetyl, α-chloroacetyl or α-benzoyl derivatives of ε-benzoyl-DL-lysine were incubated with aniline in the presence of ficin (milky sap of the fig tree). Only with α-benzoyl derivative α, ε-dibenzoyl-L-lysine anilide precipitates out quantitatively and α, ε-dibenzoyl-D-lysine remains in the solution.

Thus this asymmetric synthesis of the anilinde has been employed successfully in the resolution of DL-lysine. DL-Lysine or its ε-benzoyl derivative was converted to the α, ε-dibenzoyl-DL-lysine. Its sodium salt in 0.1 M citrate buffer solution (pH 4.5) was kept at 37° for 24 hours or more with aniline in the presence of ficin. The separated anilide of L-derivative was refluxed with 6 N KCl for 10 hours. After removal of benzoic acid, the filtrate was concentrated in vacuo to a thick syrup. The resindue was taken up in a small amount of hot 100% alcohol and acetone and was added. The precipitate was analytically pure L-lysine dihydrochloride (87 per cent; m. p. 201–202°, $(\alpha)_D^{23} = +15.5°$(3% in H_2O); N; calculated, 12.8, found 12.6. The filtrate from the enzymatic synthesis of L-anilide was heated to boiling to coagulate the proteins, and the filtrate was concentrated in vacuo. After acidification to Congo red, the α, ε-dibenzoyl-D-lysine separated. In the same manner as the L-anilide the precipitate was hydrolyzed with 6 N HCl and produced D-lysine dihydrochloride with m. p. 201–202°, $(\alpha)_D^{23} = -15.6$ (3% in H_2O) and 76% yield (N; calculated 12.8,. found 12.7).

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34. Studies on the Manufacture of Diastase. (III)

Hideo Katagiri, Toyozo Shibutani and *Teruhisa Mugibayashi.*

In the previous papers,[1] we have determined several conditions for patent No. 126296 and the experimental results obtained after these conditions are given in this report.

(8) Diastase was precipitated by tannic acid from crude extract [I] of dried malt, and this precipitate was washed by alcohol [II] according to the patent. Further purification [III] was carried out as follows: the filtrate of aqueous solution of [II] was dried under reduced pressure (1/100~2/100 mm Hg) at −70°C.

When these preparations are compared with the precipitate [IV] obtained by alcohol as is generally performed, it will be seen in the Table that the preparation [III] showed the highest diastatic power and preparation [II] is superior to preparation [IV] both in the yields and diastatic power.

Preparation	Diastase unit of pharmacopoeia	Yield (%)	Saccharifying power		Starch liquefying power	Proteoclastic power
			α-Amylase	β-Amylase		
I	0.3	100	0.12	1.00	1.0	1.0
II	14.3	59	6.24	48.13	42.7	29.3
III	22.0	50	8.64	85.36	64.0	58.2
IV	9.0	54	5.25	41.26	45.7	19.4

(9) The enzyme compositions of these preparartions were compared with each other in the following ways: α- and β-amylase were determined by Kneen and Sandstedt's method,[2] starch liquefying power was observed by viscosimeter with potato starch solution and proteoclastic power upon gelatin was determined by formol titration method. It will be pointed out that, in the above Table, the highest powers of these enzymes were found in preparation [III], and preparation [II] revealed higher enzymatic powers with little liquefying power when it was compared with preparation [IV].

No remarkable difference in the ratio of saccharifying power to liquefying power was observed among all these preparations, however proteoclastic power was found to decrease by the treatments of precipitation especially with alcohol.

1) Katagiri, Shibutani and Mugibayashi: This Bulletin **18**, 45 (1949), **19**, 61 (1949).
2) Kneen and Sandstedt: Cereal Chem. **16**, 712 (1939), **18**, 273 (1941).

35. Manufacture of Gluconic Acid by Fermentation under Aeration.

On the Industrial Studies of Oxidising Fermetation

Hideo Katagiri and *Koji Itagaki.*

In order to prepare gluconic acid from glucose, several experimets were caried out as to determine a suitable source of nitrogen substances, and wheat bran Koji extract was found to be practically useful among various kinds of materials, such as wheat bran, soy bean cake, yeast and beer spent Koji.