On the Reaction of Keto acids with Amino acids.

In an earlier publication[1]) from this laboratory it was reported that several amino acids in presence of α-ketoglutarate or pyruvate, formed glutamic acid and alanine respectively, when a mixture of the amino acid and the sodium salt of the keto acid was spotted on filter paper and dried at temperatures above 80° C. It was also indicated that special care should be taken in spotting the solutions for chromatography while investigating enzyme-catalysed transamination reactions, to prevent the non-enzymatic transaminations occurring on heating the paper.

In a recent note published in Naturwissenschaften, HEYNS and WALTER[2]) have commented that non-enzymatic transamination reactions have already been investigated *in extenso* by HERBST and coworkers[3–6]). We were aware of these findings.

But a careful perusal of our note would have shown that the conditions of our experiments were quite different from those employed by HERBST and coworkers. The sodium salts of α-keto-acids were used throughout and the reaction mixtures contained phosphate buffer p_H 8·2; whereas in the investigations of HERBST free keto acids were used and the reaction was carried out in boiling aqueous solutions. HERBST has specifically stated in his review on transamination reactions[3]) that addition of alkali in sufficient quantity to convert the organic acids into their salts effected complete inhibition of the reaction. In confirmation of this observation, we have also found that *no transamination* occurs when a mixture of an amino acid and the sodium salt of pyruvic acid in aqueous solutions was boiled for as long a period as 2 hours. In contrast to this, when the same reaction mixture was spotted on a filter paper and the spot dried at about 80° C. for half an hour, formation of alanine could be proved by chromatographic procedure. This further led us to investigate the effect of moisture and also cellulose on the non-enzymatic transamination reaction.

Reaction mixtures containing 0·1 c.c. of glutamic acid (0·1 M), 0·1 c.c. sodium pyruvate solution (0·1 M) and 0·2 c.c. of (M/15) potassium phosphate solution (p_H 5·0) were set up in small test tubes to which was added 0·2 gm. of cellulose powder. A control was set up in which no cellulose powder was added. The mixtures were taken to dryness by keeping in a desiccator for 48 hrs. To one of the tubes, 0·3 c.c. water was added. All the tubes were then heated at 105° for 30 minutes and after cooling, the volume in all cases was made up to 2 c.c. After centrifugation, the supernatants were analysed chromatographically. It was found that no transamination took place in presence of moisture and in the dry state the reaction proceeds only in the presence of cellulose.

Now it is quite clear that the transamination reaction between α-amino acids and the sodium salts of α-keto acids which we had reported earlier is essentially a dry reaction although just traces of moisture may be necessary to initiate the reaction. It is also in some way catalysed by cellulose. As far as we are aware this type of reaction has not been reported in the literature.

The effect of temperature on this reaction was next studied. The reaction mixture contained 0·1 c.c. of 0·1 M glutamic acid, 0·1 c.c. sodium pyruvate solution (0·1 M) and 0·8 c.c. of M/15 KH_2PO_4 solution (p_H 5·0). 20 µl aliquots of this mixture were spotted on filter paper circles (24 cms. diameter) and heated at different temperatures for 30 minutes. After cooling, the chromatograms were developed with n-butanol-acetic acid-water (40:10:50) solvent and the colour developed in the usual manner. It was found that at temperatures above 120° C., the amino acids as well as the products of the reaction were destroyed to a considerable extent. Another important observation made by us is that at temperatures above 95° C., γ-aminobutyric acid, a decarboxylation product of glutamic acid was formed (Fig. 1). In the absence of pyruvate from the reaction mixture γ-aminobutyric acid could not be detected. Similar decarboxylation was observed

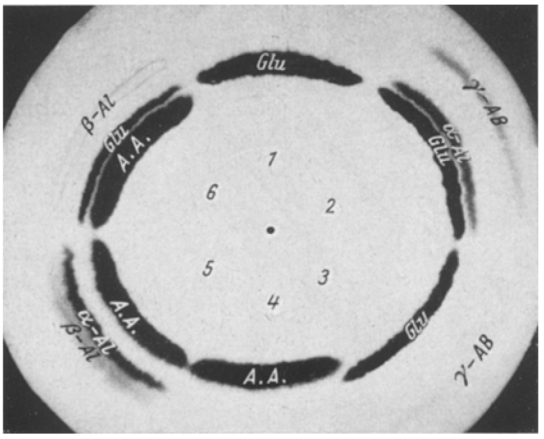

Fig. 1. Chromatogram showing the transamination and decarboxylation of aspartic and glutamic acids when heated on filter paper at 100° C. for 20 minutes in the presence of salts of α-ketoacids. *Glu* glutamic acid; *A.A.* aspartic acid; *α-Al* α-alanine; *β-Al* β-Alanine; *γ-AB* γ-amino butyric acid. *1* Glutamic acid + potassium dihydrogen phosphate solution (M/15) p_H 5·0. *2* Glutamic acid + pyruvate and potassium phosphate. *3* Glutamic acid + α-ketoglutarate + KH_2PO_4 solution. *4* Aspartic acid + potassium phosphate solution. *5* Aspartic acid + pyruvate + potassium phosphate. *6* Aspartic acid + α-ketoglutarate + potassium phosphate.

in the case of aspartic acid and pyruvate resulting in the formation of β-alanine (Fig. 1). This clearly shows that decarboxylation also occurs along with transamination.

On taking these facts into consideration, we have sufficient reason to think that the mechanism of the transamination reaction in presence of cellulose which was reported by us[1]) might be different in some respects from that of the well-known transamination reaction of HERBST.

Full details of the investigation and the quantitative data in support of this view will be published elsewhere.

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Eingegangen am 3. November 1953.

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