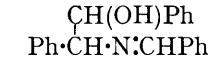
48. *Equilibria between Amino-acids and Aromatic Aldehydes. Part I.*

By J. Masson Gulland and Thomas H. Mead.

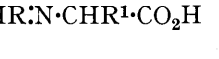
The extensive use of Sörensen's method of " formol titration " of amino-acids, peptides, and proteins (*Biochem. Z.*, 1907, **7**, 45) has led many chemists to study the nature of the equilibria existing in aqueous solutions of formaldehyde and these substances, but analogous equilibria between aromatic aldehydes and amino-acids have received little attention.

Erlenmeyer and his collaborators (*Annalen*, 1894, **284**, 36; 1899, **307**, 79, 113; 1904, **337**, 212, 307) showed that benzaldehyde (2 mols.) condensed with glycine (1 mol.) in presence of sodium hydroxide (2·5 mols.) to yield the sodium salt of *N*-benzylidenephenylserine (I) and the non-acidic *N*-benzylidene derivative of αβ-diphenylhydroxyethylamine (II). Erlenmeyer and Bade (*ibid.*, 1904, **337**, 222) found that *o*-methoxybenzaldehyde readily underwent similar transformations with glycine, although anisaldehyde, *p*-isopropylbenzaldehyde, salicylaldehyde, vanillin, and piperonal reacted only very slightly. Phenylalanine (*ibid.*, p. 215), tyrosine, aspartic acid, and leucine (Erlenmeyer, *Ber.*, 1897, **30**, 2896) reacted with benzaldehyde to give only the benzylidine derivative (II) and none of the sodium salt corresponding to (I).

(I.)



(II.)



(III.)

Gerngross (*Biochem. Z.*, 1920, **108**, 89) and Gerngross and Zühlke (*Ber.*, 1924, **57**, 1482) obtained the sodium salts of *N*-arylidene derivatives of amino-acids (III) by interaction of the aldehyde with the amino-acid ester in presence of sodium ethoxide, and showed that these salts are hydrolysed by water into the aldehyde and amino-acid. Bergmann, Ensslin, and Zervas (*Ber.*, 1925, **58**, 1034) and Bergmann and Zervas (*Z. physiol. Chem.*, 1926, **152**, 282) prepared Schiff's bases (III) by condensing benzaldehyde, salicylaldehyde, furfuraldehyde, *p*-nitrobenzaldehyde, and chloral with a representative selection of α-amino-acids in presence of baryta, barium acetate, or brucine or other alkaloids.

Apart from the interest of the equilibria involved, we decided to investigate the nature of these reactions partly because of the divergences reported above and partly because the progress of other researches demanded a knowledge of the relative capacities of aromatic aldehydes for condensation with amino-acids, in which Erlenmeyer and Bade (*loc. cit.*) had found marked variations.

First, Erlenmeyer's observations on the interaction of benzaldehyde and glycine were confirmed, and phenylserine, αβ-diphenylhydroxyethylamine, and their benzylidene derivatives (I and II) were isolated as described by him.

Secondly, repetition of the work of Bergmann and his collaborators confirmed that glycine condenses with benzaldehyde and with salicylaldehyde in presence of baryta and barium acetate respectively to yield the barium salts of the corresponding Schiff's bases.

In addition, the *barium* salts of 2-*hydroxy*-3- and -5-*methoxy*-N-*benzylideneglycine* and of their 2-*methyl ethers* (all of type III) were prepared by similar methods.

In contrast to these results, protocatechualdehyde, its two monomethyl ethers, its dimethyl ether, and *p*-dimethylaminobenzaldehyde all failed to yield sparingly soluble barium salts when treated with glycine and barium hydroxide or acetate, and were recovered unchanged.

It was thus evident that there is no discrepancy between the results of Erlenmeyer and those of Bergmann, and that the nature of the product in reactions of this type is influenced by the conditions prevailing. On the other hand, further investigation was required of the fact that some aromatic aldehydes appear to condense with amino-acids, whereas others do not.

There are two major objections to employing the isolation of the products of condensation to investigate the equilibria between aldehydes and amino-acids. Failure to isolate a compound might be due solely to its greater solubility, and if in different examples the solubilities of the products were not the same, the yields would bear no relation to the affinities for condensation, because the active concentration of the products would become constant when precipitation occurred, and the reactions would then cease to be true equilibria.

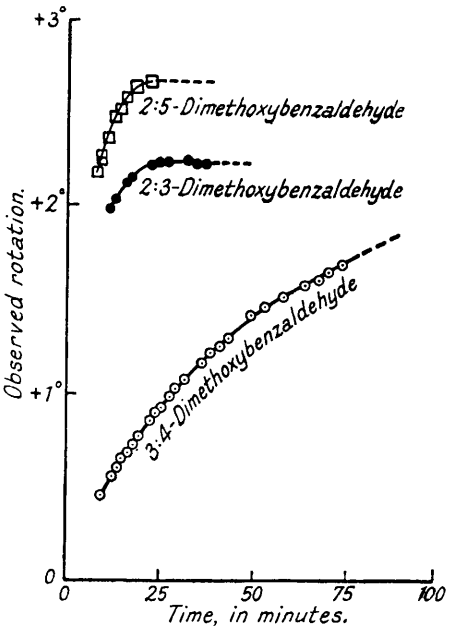
Holden and Freeman (*Austral. J. Exp. Biol.*, 1931, **8**, 189) used the van Slyke method to estimate the decrease in amino-nitrogen during the condensation of formaldehyde with amino-acids. This procedure was unsuccessful in experiments with aromatic aldehydes, because hydrolysis of the Schiff's bases occurred in the acetic acid required in the method.

Polarimetric investigation of the reactions offered the great advantages of continuous examination of their progress and the absence of disturbance of the equilibria when these were established. Holden and Freeman (*loc. cit.*) observed only small changes in the optical activity of amino-acids in presence of formaldehyde and alkali, a fact which is in harmony with Levy's conclusion (*J. Biol. Chem.*, 1933, **99**, 767) that the products have the structure

$$R{\cdot}CH[N(CH_2{\cdot}OH)_2]{\cdot}CO_2H.$$

With aromatic aldehydes, on the other hand, large changes in rotation take place, as is described below.

FIG. 1.



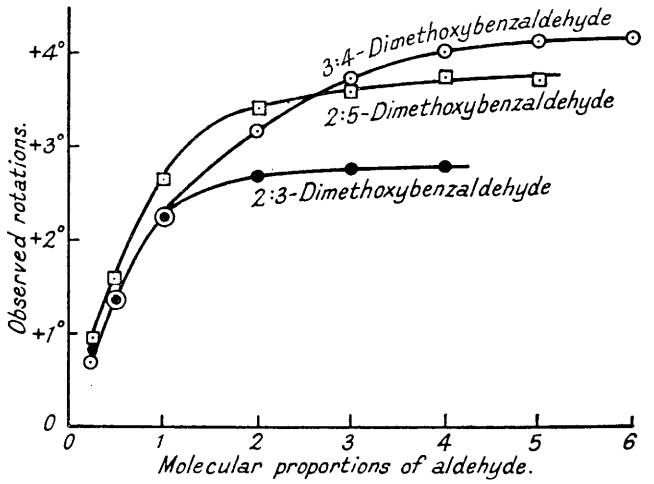
Solutions containing 0·125 *millimol. of each of* d-*phenylalanine, sodium hydroxide, and dimethoxybenzaldehydes.*

The use of the polarimetric method imposed certain restrictions. With very few exceptions, condensations between aldehydes and amino-acids only occur in alkaline media. Moreover, the specific rotations of amino-acids vary widely with the p_H of the solutions owing to differences between the optical rotatory powers of the " zwitterion," anion, and kation. This necessitated control of the alkalinity of the reaction mixtures, and attention has been restricted in the first instance to 2 : 3-, 2 : 5-, and 3 : 4-dimethoxybenzaldehydes, since the use of acidic phenolic aldehydes would have introduced complications difficult to control. The first two aldehydes condensed readily with barium aminoacetate; the last did not appear to do so. *d*-Phenylalanine was selected as the amino-acid, since it is moderately soluble, has a relatively high specific rotation ($[\alpha]_D$ + 35°) which is still measurable in alkaline solutions, and is readily prepared synthetically (Harington and McCartney, *Biochem. J.*, 1927, **21**, 852; Lamb and Robson, *ibid.*, 1931, **25**, 1231; Fischer and Schoeller, *Annalen*, 1907, **357**, 1). 50% Ethyl alcohol was the most suitable solvent, and since buffer-

ing could not be satisfactorily effected in this, approximately constant conditions were realised by the use of one molecular proportion of sodium hydroxide.

The observed rotations of mixtures of equimolecular proportions of *d*-phenylalanine, sodium hydroxide, and 2 : 3-, 2 : 5-, or 3 : 4-dimethoxybenzaldehyde in 50% alcohol increased ultimately to a constant value (Fig. 1); equilibrium was more rapidly attained with the first two aldehydes than with the third. Hence, in alkaline solution *d*-phenylalanine combined with the aldehydes to yield compounds of high specific rotation; but no other conclusions could be drawn, since the values of these rotations were unknown and could not be directly determined. Even if the products could have been isolated in pure state, and attempts to do this were unsuccessful, they would have decomposed in solution owing to alterations of the conditions of equilibrium (cf. Gerngross and Zühlke, *loc. cit.*). Approximate values of the observed and specific rotations of the condensation products were obtained indirectly as follows. Fig. 2 shows the observed rotations of solutions of *d*-phenylalanine at equilibrium with 1 mol. of sodium hydroxide and various molar proportions of the three aldehydes. In each case the rotation rose on the addition of more

FIG. 2.



Rotations of solutions containing d*-phenylalanine* (0·125 *millimol.*) *at equilibrium with varying proportions of dimethoxylbenzaldehydes.*

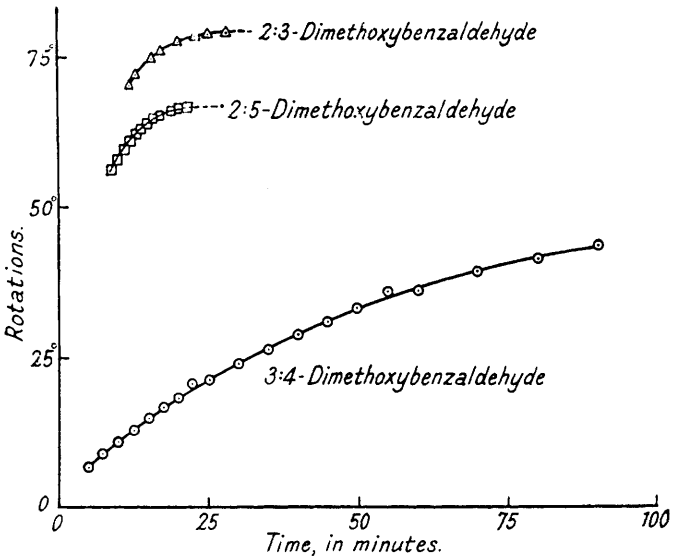
aldehyde, owing to closer approach of the equilibrium to complete condensation. On the probable assumption (see below) that the rotations are modified but slightly and to the same extent by the presence of the uncombined excess of the aldehydes, the maximum rotations obtained by extrapolation of the curves have been taken as approximate values of the observed rotations of the reaction products under the conditions of the experiments.

These deductions are only valid so long as the reactions remain as reversible equilibria. If, *e.g.*, any appreciable amount of arylidene derivative of diarylhydroxyethylamine (as II) were formed, as might happen by analogy with Erlenmeyer's work, the observed rotation would be lower, since these bases are optically inactive when formed in this way. This question was examined as follows. Solutions containing 1 mol. each of *d*-phenylalanine and sodium hydroxide and 3 mols. of the aldehyde were allowed to attain equilibrium, and were then adjusted to p_H 4 with acetic acid in order to hydrolyse the condensation product. The observed rotations fell to the same value, which was also the same, within the limits of experimental error, as that observed with solutions at p_H 4 which contained all the reactants but had not been allowed to become alkaline at any stage. This value was very slightly higher than the observed rotation of the *d*-phenylalanine alone at p_H 4, the difference being due presumably to the influence of the uncombined aldehyde in the solution. In the same series of experiments, the aldehyde and amino-acid were separated quantitatively, and were recovered as 2 : 4-dinitrophenylhydrazones and as α-naphthyl-

carbamyl or *p*-toluenesulphonyl derivatives respectively. No difference could be detected, in weights or purity of the materials recovered, between experimental and control solutions. It was concluded, therefore, that the reactions were true equilibria, and are thus unlike Erlenmeyer's reactions, the differentiating factor being the high concentration of sodium hydroxide used by him.

In the light of the experiments just described, comparable rate of reaction curves (Fig. 3) were obtained by plotting the rotations in Fig. 1 as percentages of the observed rotations of the corresponding pure condensation products as deduced by extrapolation of the curves in Fig. 2. It is evident that, under similar conditions, 2 : 3- and 2 : 5-dimethoxybenzaldehydes condensed both more rapidly and to a greater extent with *d*-phenylalanine than did the 3 : 4-isomeride, a conclusion which harmonises with the results of the experiments on the isolation of barium salts of Schiff's bases of glycine.

FIG. 3.



Rates of reaction between d-*phenylalanine and dimethoxybenzaldehydes. Rotations are expressed as percentages of the values deduced for the corresponding phenylalanine–aldehyde compounds.*

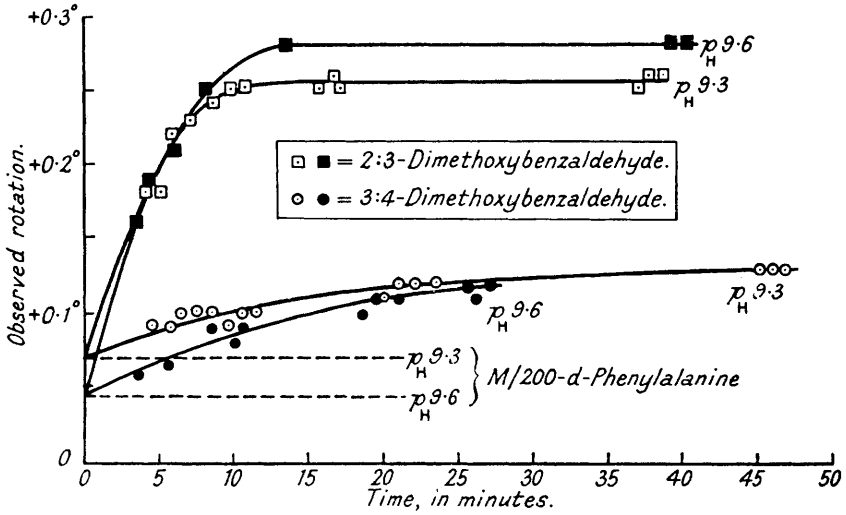
The use of 50% alcohol as solvent allowed relatively high concentrations of the aldehydes to be reached, but no accurate control of the p_H was possible. Measurements were therefore made in buffered aqueous solutions at different p_H values, and Table I summarises the equilibrium rotations of buffered aqueous solutions of *d*-phenylalanine both alone and at equilibrium with 1 mol. of 2 : 3- and 3 : 4-dimethoxybenzaldehydes. Fig. 4 shows the results obtained at p_H 9·3 and 9·6. It is clear that in both cases reaction was slight at p_H 8, that the effect of increasing the hydroxyl-ion concentration was to raise both the rate of attainment of equilibrium and the extent of the condensation, and that here again the 2 : 3-isomeride condensed more quickly and to a greater extent than did the 3 : 4-isomeride. Table I also shows that the depression of the ionisation of the amino-group of phenylalanine becomes appreciable over the range p_H 8·5—10, and this is in harmony with the p_{K_2} values of monoaminomonocarboxylic acids. It is over this region also that the extent of condensation with the aldehydes increased greatly, and this implies that the aldehydes condense with the un-ionised amino-group of the amino-acid anion. This conclusion is in agreement with the views of others on the condensation of formaldehyde with amino-acids. The greater extent of condensation in the more alkaline solutions is presumably due to the higher concentration of undissociated amino-groups; the greater rapidity with which equilibrium is reached may possibly be attributable in part to hydroxyl-ion catalysis.

As regards the structure of the products of condensation, it is doubtful whether (VI) would account for the enhanced rotatory power, although (III), (IV), or (V) might do so.

$$\underset{\text{(IV.)}}{Ar\cdot CH(OH)\cdot NH\cdot CH{<}^{CH_2Ph}_{CO_2H}} \qquad \underset{\text{(V.)}}{Ar\cdot CH\left[NH\cdot CH{<}^{CH_2Ph}_{CO_2H}\right]_2} \qquad \underset{\text{(VI.)}}{[Ar\cdot CH\cdot OH]_2N\cdot CH{<}^{CH_2Ph}_{CO_2H}}$$

It is well known that double bonds in juxtaposition to an asymmetric atom, as in (III), increase its rotatory power. Further, the relationship between phenylalanine and (V) is not widely different from that between cysteine, with its small rotation, and cystine, with greatly increased optical activity. In addition, (IV) contains a new asymmetric carbon atom which might be optically active by analogy with the asymmetric synthesis of optically active hydroxynitriles in presence of alkaloids (Bredig and Minaeff, *Biochem. Z.*, 1932, **249**, 241). It is not possible to decide between these structures from polarimetric measurements. By using the slow-reacting 3 : 4-dimethoxybenzaldehyde in buffered aqueous solution, indications were obtained that the condensation involved 2 mols. of

FIG. 4.



d-*Phenylalanine and dimethoxybenzaldehydes in buffered aqueous solutions.*

amino-acid and 1 mol. of aldehyde, but in general the reactions are too rapid to permit sufficiently accurate determinations, and retardation of the rate by dilution of reactants or lowering of temperature would introduce practical difficulties, such as small observed rotations and low solubilities of aldehydes. Possibly a decision on the structures might be reached by titration methods, as used by Levy (*J. Biol. Chem.*, 1933, **99**, 767) in the case of formaldehyde.

EXPERIMENTAL.

Schiff's Bases of Barium Aminoacetate.—(i) *Phenolic.* A solution of the aldehyde (3 g.; 1·5 mols.) in 50% alcohol (10 c.c.) was added to glycine (1 g.; 1 mol.) and barium acetate (3·0 g.) in warm water (6 c.c.) and kept over-night at 0°; if the product had not crystallised, the mixture was cooled further, and the material which separated was collected, washed with 50% alcohol and ice-water, dried in a vacuum desiccator, and stirred into dry ether to remove any free aldehyde.

Barium 2-hydroxy-3-methoxybenzylideneglycine was deposited in the reaction mixture as a yellow crystalline powder, which could be recrystallised from 50% alcohol, or better, by adding 2 vols. of acetone to the aqueous solution and cooling the mixture in ice. It formed canary-yellow, spherical aggregates of minute needles (Found: N, 5·0; Ba, 23·1. $C_{20}H_{20}O_8N_2Ba$ requires N, 5·1; Ba, 24·8%), which did not melt, and underwent hydrolysis when treated with acids or warm water.

The 5-*methoxy*-salt was obtained as small, orange, hair-like crystals by cooling the reaction mixture in a freezing mixture. It was hydrolysed by warm water (Found : N, 5·0; Ba, 24·0. $C_{20}H_{20}O_8N_2Ba$ requires N, 5·1; Ba, 24·8%).

(ii) *Non-phenolic.* A mixture of the aldehyde (2·2 g.; 1 mol.) in 50% alcohol (12 c.c.), glycine (1 g.; 1 mol.), and barium hydroxide octahydrate (2·1 g.; 0·5 mol.) in water (4 c.c.) was warmed until it became clear, and then cooled. After some hours at 0°, the crystalline precipitate of the barium salt of the Schiff's base, which had separated slowly, was collected, washed with 50% alcohol and ice-water, dried in a desiccator, and finally washed with dry ether. The non-phenolic Schiff's bases are more readily hydrolysed than their phenolic analogues.

Barium 2 : 5-*dimethoxybenzylideneglycine* formed almost colourless, small, irregular plates, which were hydrolysed in cold water and decomposed partly when dried at 75° in a vacuum (Found : N, 4·8; Ba, 23·6. $C_{22}H_{24}O_8N_2Ba$ requires N, 4·8; Ba, 23·6%).

The 2 : 3-*isomeride* formed almost colourless plates, which became oily when washed with water, owing to partial hydrolysis (Found : N, 4·8; Ba, 25·3. $C_{22}H_{24}O_8N_2Ba$ requires N, 4·8; Ba, 23·6%).

Attempts to condense Certain Other Aldehydes with Glycine.—Vanillin. Experiments were made in similar conditions to those described above. With barium acetate, practically all the vanillin was recovered unchanged (m. p. and mixed m. p.). With barium hydroxide, faintly yellow, crystalline barium vanillin was isolated (Tiemann and Haarmann, *Ber.*, **1874**, **7**, 615) (Found : Ba, 29·3. Calc. for $C_{16}H_{14}O_6Ba$: Ba, 31·2%), which yielded vanillin and barium sulphate when treated with dilute sulphuric acid.

iso*Vanillin.* After an attempted condensation using barium acetate, about 75% of the *iso*vanillin used separated in pure condition when the mixture was left over-night at 0°.

Veratraldehyde. No precipitate was formed when barium hydroxide was used under the foregoing conditions, and on concentrating and cooling the solution, veratraldehyde separated. *p*-Dimethylaminobenzaldehyde also failed to condense in presence of barium acetate.

Rate of Reaction of d-*Phenylalanine with Dimethoxybenzaldehydes in* 50% *Alcohol in Presence of Sodium Hydroxide : Reactants in Molecular Proportion.—Solutions.* A 0·25*N*-sodium hydroxide solution in 50% ethyl alcohol, kept in hard glass and protected from carbon dioxide, remained of constant strength throughout the experiments. An aqueous solution of *d*-phenylalanine ($[\alpha]^{20°}_{5461} = +37·4°$, $[\alpha]^{20°}_{D} = +35·2°$, both in water) (0·2062 g. in 10·0 c.c.; 1 c.c. = 0·125 millimol.) was prepared at frequent intervals; and absolute alcoholic solutions were prepared containing 0·0519 g. of 2 : 3-, 2 : 5-, or 3 : 4-dimethoxybenzaldehyde in 2·5 c.c. (1 c.c. = 0·125 millimol.).

Procedure. The aldehyde solution (1·0 c.c.) was added rapidly to the phenylalanine solution (1·0 c.c.) and sodium hydroxide solution (0·5 c.c.), the time of addition being noted as the start of the experiment, and the solutions being at 20°; the resulting solution contained 0·125 millimol. of each reactant in 2·5 c.c. of 50% alcohol, the slight contraction on mixing being neglected. The solution was at once transferred to a 1-dm. polarimeter tube, and rotations observed at definite times. The results are given in Fig. 1, four concordant experiments having been made with 3 : 4-dimethoxybenzaldehyde and two with each of the other two aldehydes.

In a control experiment in which absolute alcohol (1·0 c.c.) replaced the aldehyde solution, the observed rotation was 0·12°; this remained unchanged for 17 hours. Under the conditions of these experiments, therefore, the *d*-phenylalanine had $[\alpha]_{5461} = +14·5°$. The mercury green line was used throughout this work.

Determination of the Specific Rotation of the Products of Condensation using Excess of the Aldehydes.—Solutions. The phenylalanine and sodium hydroxide solutions were those used in the preceding experiments. The aldehyde solutions were prepared by weighing into a graduated flask sufficient aldehyde to contain the desired molecular proportion in 1·0 c.c. of alcoholic solution.

Procedure. Reaction mixtures containing 1·0 c.c. of each of the phenylalanine and aldehyde solutions and 0·5 c.c. of the sodium hydroxide solution were kept at room temperature overnight in stoppered flasks to allow them to reach equilibrium. The rotations were then measured in a 1-dm. tube, and again after some hours in order to ensure that equilibrium had been attained. The results are given in Fig. 2, and by extrapolation the following approximate values for the observed rotations of the condensation products from the isomeric dimethoxybenzaldehydes were obtained : 2 : 3-, + 3·0°; 2 : 5-, + 4·0°; 3 : 4-, + 4·3°. If it is assumed that condensation has proceeded to completion in presence of the large excess of the aldehydes, with the production of one of the substances (III), (IV), (V), or (VI), then the following would be the specific rotations of the sodium salts of these products respectively in 50% alcohol when formed from each of the

three dimethoxybenzaldehydes : 2 : 3-, + 192°, 181°, 126°, 121°; 2 : 5-, + 256°, 242°, 168°, 161°; 3 : 4-, + 276°, 258°, 180°, 173°.

Interaction between d-*Phenylalanine and Dimethoxybenzaldehydes in Buffered Aqueous Solutions.—Solutions.* Buffer solutions were prepared according to Clark (" Determination of Hydrogen Ions," 1920, p. 69, Williams and Williams, Baltimore).

The required aldehyde (0·0519 g.) was dissolved in 50·0 c.c. of buffer solution at the required p_H; 40·0 c.c. of this solution contained 0·0415 g. (0·25 millimol.) of aldehyde. *d*-Phenylalanine (0·0619 g.) was dissolved in 15·0 c.c. of buffer solution at the required p_H; 10·0 c.c. of this solution contained 0·0413 g. (0·25 millimol.).

Procedure. The aldehyde solution (40·0 c.c.) and phenylalanine solution (10·0 c.c.) were rapidly mixed, and the time noted as the start of the experiment. The mixture was quickly transferred to a 4-dm. polarimeter tube and polarimetric observations were begun at once. The p_H of the reaction mixture, determined colorimetrically before and after the reaction, remained constant.

The rotations of 0·005*M*-*d*-phenylalanine over the range p_H 7—10 were determined in a series of experiments in which the procedure was similar to that described above, except that 40 c.c. of the appropriate buffer solution replaced the aldehyde solution.

Table I shows the observed rotations at different p_H values of 0·005*M*-*d*-phenylalanine alone and at equilibrium with 1 mol. of 2 : 3- and 3 : 4-dimethoxybenzaldehydes.

TABLE I.

[α = observed rotation; T = time (mins.) required to reach equilibrium; I = increase in α due to condensation.]

	Phenylalanine	2 : 3-Dimethoxybenzaldehyde.			3 : 4-Dimethoxybenzaldehyde.		
p_H.	alone.	α.	T.	I.	α.	T.	I.
7	+ 0·14°	—	—	—	—	—	—
8	0·12	+ 0·17°	5	0·05°	+ 0·12°	—	0
9	0·09	0·20	6	0·11	0·13	10	0·04°
9·3	0·07	0·25	9	0·18	0·13	>45	0·06
9·6	0·04	0·28	14	0·24	0·12	>27	0·08
10·0	0·03	—	—	—	—	—	—

Proof of the Reversibility of the Condensation of Dimethoxybenzaldehydes with Phenylalanine.—(i) *With* 1 *mol. of aldehyde.* A mixture of *d*-phenylalanine (0·25 millimol.), 2 : 5- or 3 : 4-dimethoxybenzaldehyde (0·25 millimol.), and sodium hydroxide (0·25 millimol.) in 50% ethyl alcohol (5·0 c.c.) was shaken until clear and then allowed to attain equilibrium as shown by a constant rotation; the portion not in the polarimeter tube was exposed simultaneously to the light of the mercury arc, in case this catalysed the formation of arylidenediarylhydroxyethylamine (cf. Erlenmeyer, *Annalen*, 1899, **307**, 118).

The whole solution was then brought to about p_H 4 (B.D.H. capillator) by the addition of 4 drops of glacial acetic acid, added, as in all these experiments, from a standard-drop pipette so as to ensure constant conditions. The observed rotation of the acidified solution was + 0·20°, (l = 0·5), whereas in a control experiment without aldehyde the rotation of *d*-phenylalanine alone was + 0·19°.

(ii) *With* 3 *mols. of aldehyde.* Control 1. Glacial acetic acid (4 drops) was added to a solution of *d*-phenylalanine (41·25 mg.; 0·25 millimol.) and sodium hydroxide (0·25 millimol.) in 50% ethyl alcohol (5·0 c.c.). The p_H of the solution was about 4, and the observed rotation was + 0·40° (l = 1).

Control 2. Glacial acetic acid (4 drops) and then sodium hydroxide (0·25 millimol.) in 50% alcohol were added in that order to a mixture of *d*-phenylalanine (0·25 millimol.) and one of the three dimethoxybenzaldehydes (0·75 millimol.) in 50% alcohol (3·75 c.c.). When the solid had completely dissolved, the solution was diluted to 5·0 c.c. with 50% alcohol. The observed rotation was + 0·58° (l = 1). Hence the presence of 124·5 mg. of aldehyde raised the rotation by + 0·18°.

The actual experiment was carried out as described above, except that the order of addition of the sodium hydroxide and acetic acid was reversed, and time was allowed for the attainment of equilibrium before acidification. The observed rotations for the acidified solutions were : 2 : 5-dimethoxybenzaldehyde, + 0·57°; 2 : 3- and 3 : 4-dimethoxybenzaldehydes, + 0·58°.

Identification of recovered phenylalanine. (i) The acidified control solution No. 2 or experimental solutions were mixed with 2*N*-hydrochloric acid (0·5 c.c.) and water (15 c.c.). The mixture was extracted with ether in a continuous-extraction apparatus for 2 hours to remove the

aldehyde, and the residual aqueous layer was mixed with 2*N*-sodium hydroxide (0·6 c.c.), *p*-toluenesulphonyl chloride (200 mg.), and some ether, and shaken mechanically whilst being kept just alkaline by the addition of successive portions of 0·1 c.c. of 2*N*-sodium hydroxide until it remained alkaline for more than 1 hour. The alkaline solution was concentrated to small volume under reduced pressure, treated with a little charcoal, filtered, and acidified with excess of hydrochloric acid; its volume was then adjusted to 16 c.c., if necessary, by addition of water. The toluenesulphonyl derivative crystallised immediately, and after remaining at 0° for some time, was collected, washed with water (2 c.c.), and dried in a vacuum desiccator. The derivatives from the control experiment and from those with 2 : 5- and with 3 : 4-dimethoxybenzaldehydes weighed respectively 70·7, 70·2, and 69·6 mg., having crude m. p.'s 151°, 150°, and 149°, and raised by recrystallisation from 50% ethyl alcohol to 164°, 163°, and 163° (all corr.) respectively, in each case with previous softening; the theoretical yield is 79·7 mg., and Fischer and Lipschitz (*Ber.*, 1915, **48**, 369) give m.p. 164—165° (corr.) with previous softening.

(ii) Solutions were prepared from phenylalanine (61·9 mg.; 1 mol.) in water (3·0 c c.), sodium hydroxide (1 mol.) in 50% alcohol (1·5 c.c.), and 2 : 5-dimethoxybenzaldehyde (3 mols.) in alcohol (3 c.c.) or, in the control experiment, alcohol (3 c.c.) alone. When the mixture containing the aldehyde had come to equilibrium, 2*N*-hydrochloric acid (0·5 c.c.) was added to each mixture, and they were extracted three times with ether. 2*N*-Sodium hydroxide (0·6 c.c.) and α-naphthyl *iso*cyanate (0·74 c.c.) were then added, and the solutions were kept alkaline and shaken for 30 minutes, and filtered. The filtrates were cloudy, and after several days naphthylamine had separated from both solutions. This was removed, and the clear filtrates were acidified. The carbamido-acid separated from both solutions in a gelatinous condition, but was ultimately collected, washed with water, and dried over phosphoric oxide. The yields were 82·6 mg. from the experimental and 79·6 mg. from the control solutions (theory, 125·2 mg.). When recrystallised once from aqueous alcohol, both specimens weighed 65—70 mg., and both melted at 152° (corr.), with previous softening. α-2-Naphthylcarbamido-β-phenylpropionic acid melts at 155° after softening at 150° (Neuberg and Rosenberg, *Biochem. Z.*, 1907, **5**, 456).

Identification of recovered 2 : 5-*dimethoxybenzaldehyde.* 2 : 5-Dimethoxybenzaldehyde (186·7 mg.; 3 mols.) in alcohol (3 c.c.) was added to phenylalanine (61·9 mg.; 1 mol.) in water (3 c.c.) and sodium hydroxide (1 mol.) in 50% alcohol (1·5 c.c.). This solution was allowed to attain equilibrium, and simultaneously a control solution was prepared in which the amino-acid solution was replaced by water (3 c.c.). Both solutions were acidified with 2*N*-hydrochloric acid (0·2 c.c.), and extracted with ether in a continuous-extraction apparatus. The extracts were concentrated, and the residues dissolved in alcohol (15 c.c.) and added to 2 : 4-dinitrophenylhydrazine (0·35 g.) in a mixture of concentrated sulphuric acid (0·7 c.c.) and alcohol (5 c.c.). After standing over-night at 0°, the 2 : 4-dinitrophenylhydrazones were collected, washed with alcoholic sulphuric acid, then with alcohol, and dried. The yields of crude product were 98% and 97% from the experiment and the control respectively, and when crystallised from xylene the two products formed orange prisms, m.p. 199—201°, undepressed on admixture.

THE LISTER INSTITUTE, LONDON, S.W. 1. [*Received, December 20th*, 1934.]