Anal. Calcd. for $C_{17}H_{15}NO$: C, 81.90; H, 6.07; N, 5.62. Found: C, 81.90, 82.02; H, 6.06, 6.24; N, 5.83, 6.20.

A mixed m.p. with an authentic sample of XI, prepared by the method of Dyson and Hammick,[6] showed no depression.

Similar results were obtained in the reaction of VIII with methylmagnesium bromide in benzene solution.

Preparation of Quinaldamide from VIII.—To 200 cc. of dry ether saturated with hydrogen chloride was added 10.00 g. of VIII. The mixture was mechanically stirred as hydrogen chloride was passed in for 90 minutes. A yellow precipitate which formed was filtered. After boiling the yellow solid in water for a few minutes, 3.0 g. (45%) of quinaldamide, m.p. 131–133°, was obtained.

Anal. Calcd. for $C_{10}H_8N_2O$: C, 69.75; H, 4.68; N, 16.27. Found: C, 69.73; H, 4.65; N, 16.25.

A mixed m.p. with an authentic sample of quinaldamide showed no depression.

LAWRENCE, KANSAS RECEIVED FEBRUARY 19, 1951

[CONTRIBUTION FROM NATIONAL DAIRY RESEARCH LABORATORIES, INC.]

A Study on the Reaction of Aldoses and Amino Acids

BY G. HAUGAARD, L. TUMERMAN AND H. SILVESTRI

A new technique has been developed for the study of the reaction between aldoses and amino acids. The method has been applied to the evaluation of the reaction constants for the formation and degradation of Schiff base produced in various amino acid aldose systems under alkaline conditions. The effect of *p*H on the reaction has been studied and results indicate that its influence on the ionization of the amino acid is reflected in the extent of the reaction. The effect of temperature on the reaction has been studied and the activation energy for the primary reaction of *dl*-leucine and glucose was calculated to be 19,700 calories. Lactose and valine were found to react in equimolar proportions by an adaptation of the new technique. The specific rotation of the Schiff base produced by the reaction of *l*-leucine and gluose was found to be $[\alpha]^{20}D$ −42.6°. The relationship between the degradation of the Schiff base and the formation of a brown-colored substance is discussed. A kinetic study is presented which supports the theory that the "Browning" reaction involves condensation of amino acids with carbohydrate degradation products.

Introduction

The mechanism of the reaction between amino acids and aldose sugars has been the subject of rather extensive investigations since the original observations were published by L. C. Maillard.[1]

The possible physiological significance of amino acid–aldose interaction as well as the implication of the reaction in deteriorative changes and flavor development in foods have served to stimulate much of the recent research in this field.

A great deal of the literature concerning the reaction rate and mechanism is contradictory, a situation resulting from varying conditions of temperature, hydrogen ion and reactant concentration employed by the various investigators. In most instances the true reaction of amino acid and aldose has been obscured by secondary changes induced in either the amino acid or the aldose by elevated temperatures, and *p*H conditions far removed from neutral.

H. von Euler, *et al.*,[2] and Englis and Dykins[3] attempted to follow the course of the reaction polarimetrically. Using a cryoscopic technique, von Euler, *et al.*,[4] later attempted to demonstrate reaction between glucose and glycine and to check the results by determination of free amino nitrogen and decrease in reducing sugar. An attempt was made by Watanabe[5] to determine the course of reaction of various amino acids and sugars in 0.5 *N* sodium hydroxide, under which highly alkaline conditions quite extensive rearrangements and degradation of the carbohydrate obscured the true picture of the initial reaction.

Frankel and Katchalsky[6,7,8] published a series of papers describing the application of a potentiometric method based on the increase in acidity resulting from the formation of a Schiff base by the reaction of the aldehyde group of the reducing sugar with the amino group of the amino acid. α-Amino nitrogen decrease was determined by the van Slyke technique and data were presented to substantiate the results obtained by the potentiometric method.

Englis and Dykins[3] parallel with a polarimetric study, attempted to substantiate their findings by determination of the expected loss of α-amino nitrogen by the van Slyke nitrous acid technique, but contrary to Frankel and Katchalsky, failed to obtain any decrease even in solutions where marked sugar rotation changes were noted. They attributed this to an equilibrium reversal during the time interval required for the amino nitrogen determination. The work of Englis and Dykins was confirmed in our laboratory.

A new method has been developed, therefore, in order to circumvent the difficulties mentioned. The method is based upon changes in soluble nitrogen in an aldose solution saturated with an amino acid and equilibrated with an excess of amino acid crystals. The necessity of using a physical property, such as solubility, in order to evaluate the kinetics of a reaction of this type is dictated by the rapidity with which the reaction product dissociates into the reactants or forms other products under chemical analysis. This technique—the solubility technique—has also been used to determine the stoichiometry of an aldose–amino acid system, as well as for the determination of the specific rotation of the unstable Schiff base formed.

The following kinetic treatment of this reaction is based upon experimental evidence that aldoses

(1) L. C. Maillard, *Compt. rend.*, **154**, 66 (1912); **155**, 1554 (1912).

(2) H. von Euler, E. Brunius and K. Josephson, *J. Physiol. Chem.*, **153**, 1 (1926).

(3) D. T. Englis and F. A. Dykins, *Ind. Eng. Chem., Anal. Ed.*, **3**, 17 (1931).

(4) H. von Euler, E. Brunius and K. Josephson, *J. Physiol. Chem.*, **155**, 259 (1926).

(5) J. Watanabe, *J. Biochem. (Japan)*, **16**, 163 (1932).

(6) A. Katchalsky, *Biochem. J.*, **35**, 1024 (1941).

(7) M. Frankel and A. Katchalsky, *ibid.*, **35**, 1024 (1941).

(8) M. Frankel and A. Katchalsky, *ibid.*, **35**, 1034 (1941).

react with amino acids in equimolar ratio, the reaction product being described as a Schiff base.

$$R_1\text{—CHO} + H_2NR_2 \rightleftharpoons \underset{\displaystyle R_1}{\overset{\displaystyle H\text{—}C\text{—}NR_2}{|}} + H_2O$$

The Method Used to Study the Kinetics of the Aldose–Amino Acid Reaction.—In an aldose solution saturated with an amino acid, any reaction that consumes the dissolved amino acid will cause an equivalent amount of the crystals to go into solution, in order to re-establish saturation. The rate with which the amino acid reacts with the aldose will be reflected by the rate with which the amino acid from the solid phase dissolves to maintain the state of saturation. Consequently, the measurement of the rate of increase of soluble nitrogen in such a system affords an excellent technique for studying the reaction kinetics.

The kinetics should take a simple form if the system is saturated with an amino acid in the presence of a high concentration of aldose in which case the aldose concentration may be regarded as constant during the entire course of the experiment. The general equations will be derived under this limiting assumption and it will be demonstrated that the experimental conditions chosen permit the application of the derived equations.

Let C_N represent the concentration of compounds containing nitrogen measured as milligram equivalents of nitrogen/cc. This value is the sum total of nitrogen from the amino acid, the Schiff base and nitrogen containing degradation products of the Schiff base. Reactions in which the degradation products engage will not be considered, as they do not influence the first phase of the reaction of aldose and amino acids. Then

$$\frac{dC_N}{dt} = K_1C_CC_A - K_2C_{AC} \qquad (1)$$

where

C_C = concentration of carbohydrate
C_A = concentration of amino acid anion
C_{AC} = concentration of reaction product (Schiff base)

K_1 is the reaction constant for the formation of the Schiff base. K_2 is the constant representing the decomposition of this compound reversible with respect to the amino acid. C_C is, as pointed out previously, constant during the experiment. C_A, the concentration of the amino acid anion, is also constant during the experiment due to the applied technique. The second term of equation (1) is zero if the Schiff base is a stable compound or if the decomposition is entirely irreversible with respect to the amino acid. This would result in a linear curve. The integrated form of (1) is

$$C_{N_t} - C_{N_0} = K_1C_CC_At - K_2\int_0^t C_{AC}dt \qquad (2)$$

It is obvious that a time t_{st} must be arrived at where just as much Schiff base is formed as is decomposed. The last term of equation (2) can therefore be divided into two parts, the first part referring to a variable concentration of the reaction product C_{AC}, the last part to a constant concentration C_{ACst}

$$C_{N_t} - C_{N_0} = K_1C_CC_At - K_2\int_0^{t_{st}} C_{AC}dt - K_2C_{AC_{st}}(t - t_{st}) \qquad (3)$$

where it is understood that the last term is only to be considered valid for time values greater than t_{st}.

The curve consists of three parts, one linear part relative to the first term above, a curved portion referring to t values close to t_{st}, and finally a second line corresponding to values of t greater than t_{st}. If by some mechanism the degradation of the Schiff base could be stopped completely until a concentration would build up to that of the stationary state, and if the "brake" on the degradation process would then be released the stationary state would be established immediately. It can now be seen that this hypothetical mechanism will give two straight lines identical with the experimental lines, and that their intersection determines the amount of Schiff base at the stationary state.

For $t \ll t_{st}$

$$C_{N_t} - C_{N_0} = K_1C_CC_At \text{ or}$$
$$\text{Slope 1} = K_1C_CC_A \qquad (4)$$

For $t > t_{st}$

$$C_{N_t} - C_{N_0} = K_1C_CC_At - K_2\int_0^{t_{st}} C_{AC}dt - K_2C_{AC_{st}}(t - t_{st})$$

or for values of t_1 and t_2 both greater than t_{st}

$$C_{N_2} - C_{N_1} = K_1C_CC_A(t_2 - t_1) - K_2C_{AC_{st}}(t_2 - t_1) = K_3C_{AC_{st}}(t_2 - t_1)$$

or

$$\text{Slope 2} = \text{Slope 1} - K_2C_{AC_{st}} \qquad (5)$$

and

$$\text{Slope 2} = K_3C_{AC_{st}} \qquad (6)$$

K_3 is the reaction constant for the irreversible decomposition of Schiff base. Therefore

$$K_1 = \frac{\text{Slope 1}}{C_A \cdot C_C}$$

$$K_2 = \frac{\text{Slope 1} - \text{Slope 2}}{C_{AC_{st}}}$$

$$K_3 = \frac{\text{Slope 2}}{C_{AC_{st}}}$$

The Figs. 1A, 1B and 1C illustrate the preceding development of the kinetic equations. The mathematical interpretation presented is correct provided the concentration of the aldose can be considered constant and the change of the composition of the solution does not to any significant extent change the solubility of the amino acid.

We have distinguished between reversible and irreversible decomposition of Schiff base with respect to the amino acid, but have not been able, by means of the experimental technique which is based on nitrogen determinations, to make the same distinction with respect to the other component, the carbohydrate.

However, several experimental observations suggest that when the Schiff base decomposes reversibly with respect to the one component it also decomposes reversibly with respect to the other. Englis and Dykins[3] have shown that bound carbohydrate is recovered upon acidification of a Schiff base. The ease of hydrolysis of Schiff bases suggests a highly reversible equilibrium in aqueous systems.[9]

(9) M. L. Wolfrom, R. D. Schuetz and Liebe F. Cavalieri, THIS JOURNAL, 71, 3518 (1949).

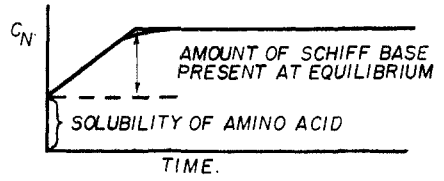

Fig. 1A.—Schiff base decomposes reversibly, or decomposes into amino acid and degradation products of carbohydrate.

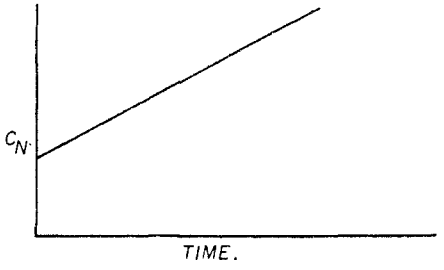

Fig. 1B.—Schiff base is either a very stable compound, or decomposes to compounds among which the original amino acid is not found.

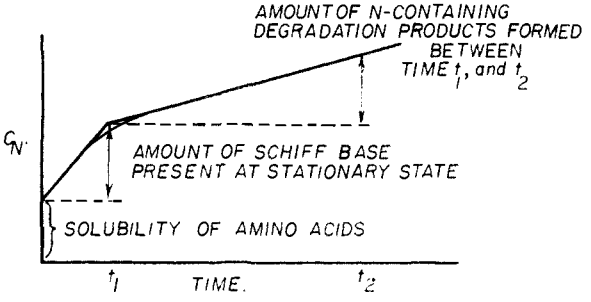

Fig. 1C.—Schiff base decomposes into degradation products, some of them containing nitrogen.

Supported by these experimental observations, one can conclude that when the decomposition of Schiff base is proven reversible with respect to the one component, it is also reversible with respect to the other.

The concentration of carbohydrate will be expected to decrease but slightly during the initial phase of the reaction. During the second phase it will stay constant if the decomposition of the Schiff base is entirely reversible. If, however, only part of the degradation takes place reversibly, the carbohydrate concentration will continue to decrease at a rate equivalent to the formation of irreversible decomposition products.

The experimental material presented here gives curves consisting of two linear parts connected by a short curved portion, demonstrating that the conditions which lead to this type of curve are essentially fulfilled. The total decrease of the carbohydrate concentration is no more than 10% in most cases. In the calculation of K_1, a slightly corrected value for the carbohydrate concentration has been applied, namely

$$C_C - {}^1/_2 \cdot C_{AC_{st}} \text{ instead of } C_C$$

The carbohydrate concentration does not enter directly into the calculation of K_2 and K_3 and no attempt has been made to correct for the slight influence of the relatively small change in the carbohydrate concentration on the values of K_2 and K_3.

Experimental Technique and Proof of the Reliability of the Solubility Method.—The success of the solubility technique depends upon the rapidity with which an aldose solution can be saturated with amino acid. A state of saturation was shown to be obtainable within acceptable time limits by the following experiment:

A saturated *dl*-leucine solution was prepared by vigorous stirring of the amino acid crystals in distilled water in a vessel immersed in a constant temperature bath at 23.9 ± 0.005°. To the vigorously stirred solution was added small increments of concentrated sodium hydroxide until the solution was adjusted to *p*H 9.5. Stirring was continued and samples of the solution were drawn at various time intervals by applying suction to a cotton plugged pipet. The filtered solutions were analyzed for total nitrogen. The data shown in Table I demonstrate that saturation is obtained in sufficiently short time to permit the application of the method to kinetic studies.

TABLE I

RATE AT WHICH DL-LEUCINE GOES INTO SOLUTION

*p*H 9.5; temp. = 23.9°

time in minutes	millimole amino acid/ml.
15	0.124
30	.127
80	.129
150	.129
400	.129
1600	.129

It was anticipated that if an aldose solution was kept saturated with an amino acid, as just described, the nitrogen content of the solution would increase steadily at a rate directly proportional to the rate of reaction of the aldose and amino acid. This was verified experimentally in the following way. A molar solution of glucose was brought to constant temperature in a water-bath. An excess of leucine (sufficient to cause saturation and provide a large excess to replace that which reacted) was added to the glucose solution which was stirred constantly. The mixture was stirred for 30 minutes at the isoelectric point of the amino acid, at which *p*H negligible reaction takes place. The *p*H of the system was then adjusted to 9.5 with concentrated sodium hydroxide and a sample was drawn. The *p*H of the system was from then on checked at frequent time intervals. Concentrated sodium hydroxide solution was added in small quantities in order to keep the *p*H constant and minimize dilution of the system. The *p*H was kept constant well within one-tenth of a unit. The normality with respect to sodium hydroxide was, after the first addition 0.0032, after 200 minutes 0.0068, and at the end of the experiment 0.0137. The effect of this small amount of electrolyte on the solubility of the amino acid can be considered insignificant.[10] Samples were drawn at various time intervals and the total nitrogen was determined by the micro Kjeldahl technique. Plotting millimoles of amino nitrogen *vs.* time in minutes (illustrated in Fig. 2) gives two straight lines as expected, showing that a stationary state is reached after the lapse of a certain time period. As the second line is not parallel to the time axis, only part of the amino acid is reversibly produced by the degradation of the Schiff base. That the experimental result is in accord with the theory brought forward is another proof of the reliability of the method.

In order to demonstrate the inactivity of a non-reducing sugar, the following experiment was performed. An excess of amino acid was added to a molar C.P. sucrose solution and the same procedure was followed as previously described. The concentration of the nitrogen in solution remained constant as shown in Fig. 2. This indicates that no reaction has occurred, a result to be expected from a non-reducing (non-aldehydic) sugar.

The Reaction Mechanism of the Consecutive Reactions.—Several systems have been studied applying the technique

(10) In this connection see: "Proteins, Amino Acids and Peptides," by E. J. Cohn and J. T. Edsall, Reinhold Publishing Corp., New York, N. Y., 1943, Chapter 11.

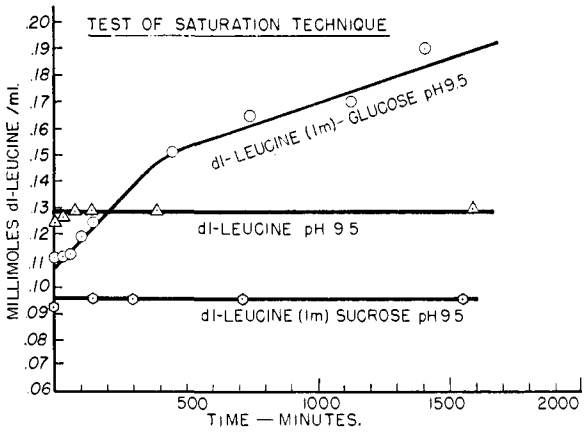

Fig. 2.

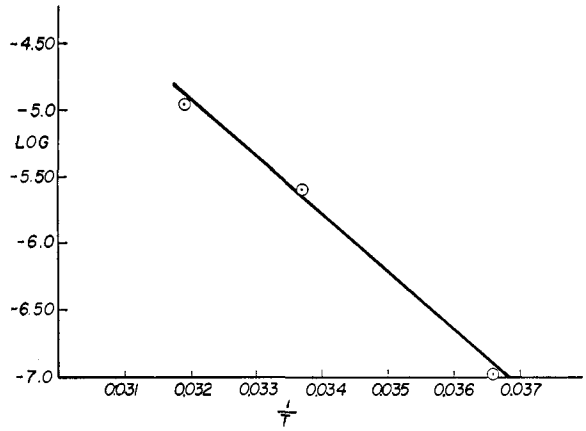

Fig. 5.

described. The experimental data are graphically presented in Figs. 3, 4, 5 and 6. The mathematical treatment described earlier has been applied with the reservations discussed. Due to the slowness of the reaction, minutes have been conveniently chosen as time units. The data used for these calculations and the results (the values of reaction constants) are listed in Table II. The data will be discussed in the order presented in this table.

Influence of *p*H.—The experiments were carried out at 9.50, 9.20 and 8.65. The temperature was 23.9°. The experimental results are presented in Fig. 3. Frankel and Katchalsky have shown that the reaction takes place between the uncharged amino group and the aldehyde group of the reducing sugar. The effect of *p*H on the rate of reaction is thus related to the concentration of amino acid anions. The Henderson–Hasselbalch equation ($pH = pK + \log(\alpha/1 - \alpha)$) has been used in the calculations of the anion concentration. The pK value of 9.62 used for leucine, however, might not be the correct value when a 1 molar glucose solution is used instead of water. The reaction constant corresponding to the formation of Schiff base, K_1, is calculated on the basis of the anion concentration (see Table II). If the true activity of the amino acid is represented by the anion concentration, the value of K_1 should be independent of the *p*H at which the constant is determined.

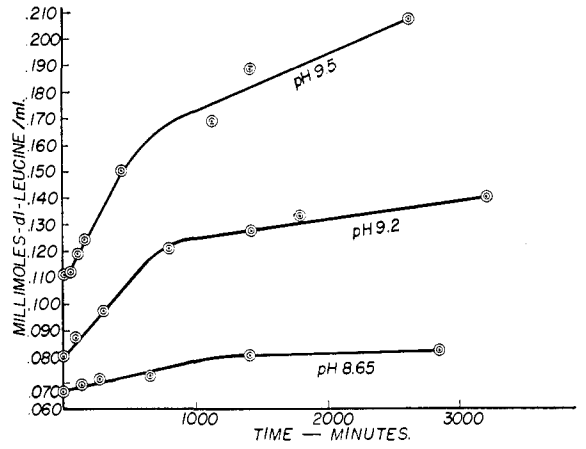

Fig. 3.

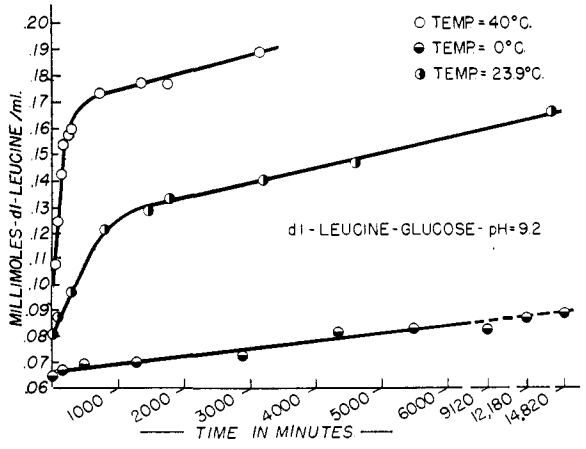

Fig. 4.

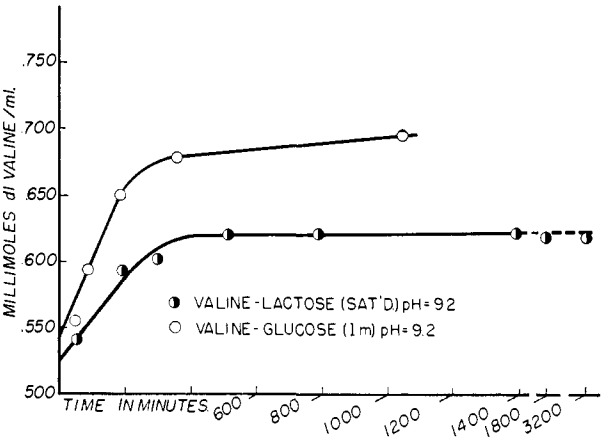

Fig. 6.

The variations in the values of K_1 are erratic but relatively small considering the difficulties described. However, the variations may reflect another factor. The assumption has been made that the entire glucose concentration represents the true activity of the carbohydrate. Since the Schiff base, however, is the product of an aldose–amine condensation, it is quite possible that the true representation of the activity of the glucose might be obtained only by considering the concentration of the free aldehyde form. Cantor and Peniston[11a] and Lippich[11b] have shown that the free aldehyde form of glucose in solution represents but a small portion of the total concentration and that a highly dynamic equilibrium exists, dependent on *p*H, the temperature and concentration. A more precise calculation of the reaction constant would have to take into consideration the extent to which the reactive form of the aldose actually exists in equilibrium under various conditions of *p*H and temperature. Unfortunately, these problems have proven insurmountable up to the present.

K_2 (the reaction constant related to the degradation of Schiff base whereby the free amino acid is formed again) and K_3 (the constant corresponding to the degradation of Schiff base leading to nitrogen-containing degradation prod-

(11) (a) L. M. Cantor and Q. P. Peniston, THIS JOURNAL, **62**, 2113 (1940); (b) Lippich, *Biochem. Z.*, **227**, 156 (1930).

TABLE II

System	pH	Temp., °C.	Initial concn. of amino acid mmoles/liter	Anion concentrations C_A mmoles/liter	Concn. of Schiff base at stationary state C_{AC}, mmoles/liter	$K_1 \times 10^{-6}$	$K_2 \times 10^{-3}$	$K_3 \times 10^{-4}$
dl-Leucine–1 *M* glucose[a]	9.50	23.9	108	47.8	57	2.04	1.30	3.68
dl-Leucine–1 *M* glucose	9.20	23.9	80	22.7	44	2.61	1.16	1.59
dl-Leucine–1 *M* glucose	8.65	23.9	67	6.8	13	1.49	0.69	1.54
dl-Leucine–1 *M* glucose	9.20	0.0	67	19.0	...	0.11	..	..
dl-Leucine–1 *M* glucose	9.20	23.9	80	22.7	44	2.61	1.16	1.59
dl-Leucine–1 *M* glucose	9.20	40.0	98	28.9	71	11.40	4.36	1.06
dl-Valine–1 *M* glucose	9.20	23.9	540	148.5	135	4.25	4.17	1.85
l-Leucine–1 *M* glucose	9.20	23.9	207	58.8	49	2.14	1.96	5.62
dl-Valine–sat. lactose[b]	9.20	23.9	526	145.0	92	4.16	3.33	0.44
dl-Leucine–1 *M* arabinose	9.20	23.9	90	25.5	32	2.72	2.06	0.63
dl-Leucine–1 *M* 2,3-dimethylglucose	9.20	23.9	73	20.7	20	0.78	0.82	0.00

[a] $C_C = C_0 - {}^1/_2 \cdot C_{AC_{st}}$. [b] A lactose solution saturated at 25° contains 560 milligram equivalents (calculated from data in "Int. Critical Tables," Vol. 2, 1927, p. 346.

ucts other than the original amino acid) are independent of the factors just discussed, as is illustrated by the equations. Only the values of slopes 1 and 2 and the concentration of Schiff base are needed for the calculations of K_2 and K_3, thus eliminating any speculation relative to the correct value of K_1 and of concentrations.

K_2 and K_3 vary directly with *p*H. The amount of Schiff base present in the stationary state may vary likewise. At *p*H 8.65 the concentration of Schiff base was 13 millimoles/liter, at *p*H 9.20, 44 millimoles/liter, and at *p*H 9.5, 57 millimoles/liter.

Influence of Temperature.—In the experiments carried out at different temperatures, the same factors which have been discussed above should be taken into consideration. The experimental data are plotted in Fig. 4. In the calculations related to the experiments carried out at three different temperatures (0°, 23° and 40°) *pK* is considered independent of temperature and the total glucose concentration is used for the calculation of K_1, as was done in the first experiment. As before, the values of K_2 and K_3 are not influenced by these considerations. In Fig. 5 log K_1 is plotted against $1/T$. It can be seen that the required linear relation between log K_1 and $1/T$ is established. With values taken from the curve, the energy of activation Q was calculated by applying the Arrhenius equation. The value of Q of the first reaction was found to be 19,700 calories.

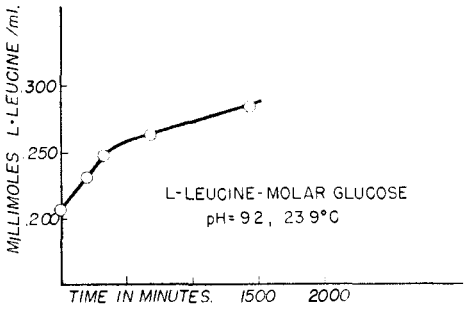

Fig. 7.

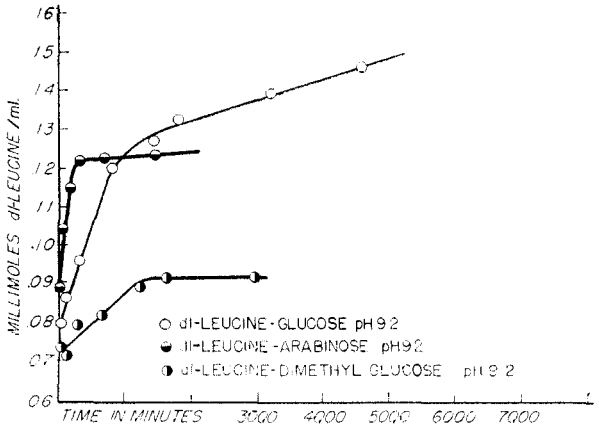

Fig. 8.

The Q values corresponding to K_2 and K_3 have not been calculated, as there is no proof that the data follow the Arrhenius equation. It is felt, furthermore, that information concerning the compound formed by the degradation of the Schiff base is necessary for the justification of the calculations.

Other systems have been studied applying the solubility technique. The results are shown in Figs. 6, 7 and 8 and in Table II.

The K_1 values, which refer to the formation of Schiff base seem to vary only slightly from system to system. *dl*-Valine–glucose gives the highest K_1 value and *dl*-leucine–2,3-dimethylglucose the lowest. The last system will be discussed later. The K_2 values are about one thousand times as large as the K_1 values. The K_3 values (the reaction constants which refer to the irreversible process by which nitrogen containing degradation products are formed) are about one hundred times as large as the K_1 values and about $^1/_{10}$ of the K_2 values.

Since enolization probably plays an important role in the process of carbohydrate degradation, it was assumed that a Schiff base formed by an amino acid and a hexose methylated in the 2,3-position would break down to the original compounds and not to nitrogen-containing fragments. A solubility experiment carried out with 1 molar 2,3-dimethylglucose and *dl*-leucine was performed and, as expected, the second line was found to be parallel to the time axis. The values of K_1 and K_2 were, respectively, 0.77×10^{-6} and 0.82×10^{-3}. On the assumption of reversibility for both components the equilibrium constant K_2/K_1 was found to be 1.2×10^3.

It would appear that although a reaction between the methylated glucose and leucine takes place, decomposition of the hexose is inhibited by the loss of enolization due to the methylation of the 2,3-positions. It is interesting to note that Wolfrom, Cavalieri and Cavalieri[12] reported a negligible browning in the refluxing of methylated glucose and amino acids. This would indicate that the reaction is also reversible with respect to the carbohydrate.

Stoichiometry of the Reaction.—We have assumed that the aldoses and amino acids react in a one to one ratio. The determination of the molar ratio of aldose and amino acid that react to form Schiff base requires the simultaneous evaluation of both nitrogen and carbohydrate concentration changes in solution. The highly reversible nature of the system requires an analytical technique that will preserve the equilibrium and give an accurate account of the nitrogen and carbohydrate content of the system. To satisfy these

(12) M. C. Wolfrom, L. F. Cavalieri and D. K. Cavalieri, THIS JOURNAL, **69**, 2412 (1947).

requirements, the previously described solubility technique was extended in the following way to a lactose–*dl*-valine system. A solution saturated with an excess of lactose was divided into two portions. One half was maintained under constant temperature and *p*H conditions and with both lactose and valine crystals in large excess, while the other half was filtered free of excess lactose crystals and was then saturated with excess valine crystals and maintained under identical *p*H and temperature conditions. In preparing these aldose solutions, the sugar was left in alkaline solution for sufficient time to permit mutarotation to go to completion and to allow the final solubility of the aldose to be established.

As previously described, samples of each solution were filtered with a plugged pipet and then analyzed for nitrogen and total aldose (by measurement of optical rotation). The results of the nitrogen analyses were identical in both cases (Fig. 6). The observed rotation differences of the two solutions represent the quantity of lactose brought into solution to replace the equivalent amount that reacted with amino acid. An advantage in determining the reacting ratios in this way is that the rotation change due to the formation of Schiff base is cancelled out and the increase of the aldose content can be measured directly. A control experiment was run to determine the effect of alkali (*p*H 9.2–9.5) on sugar stability as determined by polarimetric analysis. The results indicate that only a negligible change in the lactose rotation occurred at this range of alkalinity. Figure 9, which shows milligram equivalents of nitrogen plotted against increase in milligram equivalents of lactose, demonstrates that lactose combines in equimolar proportions with valine.

The Specific Rotation of the Reaction Product of *l*-Leucine and Glucose.—The determination of the specific rotation of the Schiff base produced by interaction of *l*-leucine and glucose can be performed with the solubility technique in the following manner: A one molar glucose solution was saturated with *l*-leucine at *p*H 9.2. Samples were drawn, as described, at various time intervals. The results summarized in Table III are those values from the linear portion of the curve that represent only the initial amino acid–aldose reaction. The column headed "rotation change due to glucose reacted" contains anticipated rotation changes calculated from the number of moles of glucose reacted. The value +52.7 was used for the specific rotation of glucose. The difference between the rotation change due to glucose consumed and the total change observed, is the rotation attributable to the Schiff base formed. These values are tabulated in the last column. Two dm. tubes were used for the measurements, and a sodium lamp was used as a light source. From these data and the value of 293 for the molecular weight of the Schiff base, the specific rotation is found to be −42.6.

TABLE III

Time, minutes	Obsd. rotation change α_1	Glucose and *l*-leucine reacted, mmoles/cc.	Rotation change due to glucose reaction α_2	Rotation change due to formation of Schiff base $\alpha_3 = \alpha_1 - \alpha_2$	Specific rotation of Schiff base $[\alpha]^{20}D$
210	−1.05	0.024	−0.45	−0.60	−42.7
330	−1.80	.041	− .78	−1.02	−42.5

Kinetic Experiments on the "Browning" Reaction

When a solution of an amino acid and a reducing sugar is refluxed for some hours a brown color develops. This process also takes place at lower temperatures. At room temperature it would take weeks instead of hours before a measurable amount would be produced.

It is most likely that the first step leading to the development of the brown color is the formation of an unstable aldose–amino acid compound. This compound may decompose into such highly reactive compounds as glyceraldehyde, pyruvic aldehyde, etc., which may be the source of the intermediates in the "Browning" reaction. Such compounds have been shown to react with amino acids to produce intensely colored, brown polymers of high molecular weight.[13] The unstable aldose–amino acid compound is most likely a Schiff base. Although only a small amount of anion is present at *p*H 6.1 (the *p*H value at which we have carried out our experiments), at the high temperature of 100° it can be expected that a compound of the Schiff base type would be produced at a rapid rate.

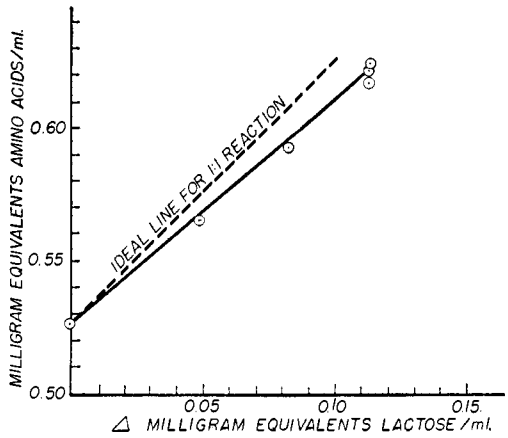

Fig. 9.

Three experiments which elucidate the mechanism of the "Browning" reaction were performed. The following solutions of glycine and glucose were prepared: Solution A and C had the same concentration of glucose, namely, 0.5 molar, but the glycine concentration was 0.2 *M* in Solution A and 0.4 *M* in Solution C. Solution B had the same glycine concentration as Solution A, but the glucose concentration was doubled to give a 1 molar solution (see Table IV). The reason for the choice of concentrations will be discussed after the presentation of the kinetic equations.

TABLE IV

Solution	Glucose, *M*	Glycine, *M*	Slope × 10^{-5}
A	0.5	0.2	0.30
B	1.0	.2	0.63
C	0.5	.4	1.42

The solutions were refluxed; a boiling water-bath was used as heat source. Samples were drawn at time intervals and cooled. log I_0/I was measured in a 1-cm. cell with a Beckman spectrophotometer at a wave length of 490 mμ. It was assumed that the concentration of the brown substance was proportional to the extinction (Beer's law).

The results of these measurements are shown in Fig. 10. The rate of production of brown substance is very small at the beginning, the curves near the origin being asymptotic to the time axis. Curves of a similar shape to these have also been reported by other investigators, for instance, Haas, Stadtman, Stadtman and MacKinney,[14] who by extraction of the intermediate products with ethyl acetate could inhibit the reaction, thus demonstrating the significance of the sugar degradation products. From light absorption curves they found

(13) Enders and Marquardt, *Naturwissenschaften*, **29**, 46 (1941).

(14) V. A. Haas, E. R. Stadtman, F. H. Stadtman and G. MacKinney, THIS JOURNAL, **70**, 3576 (1948).

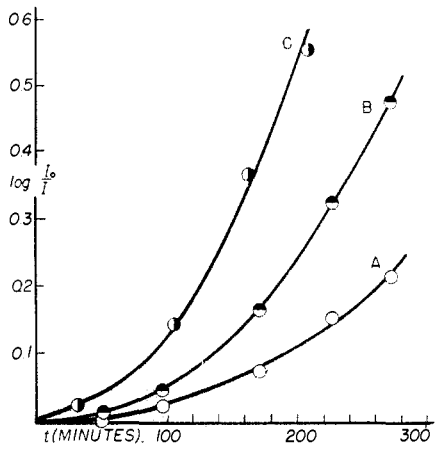

Fig. 10.

5-hydroxymethyl-2-furylaldehyde was produced by the degradation of the carbohydrate indicating that this compound enters into the reaction by which the brown substance is formed. These authors did not offer any kinetic calculations. The first period has been called the induction period, but no explanation of the significance of this and of the concave shape of the curve has hitherto been presented.

The kinetics discussed in the following appear to support the theory that the induction period and the shape of the reaction curve can be explained by the absence of one of the reactants at the start, the intermediates being formed during the refluxing. Referring to the first part of this paper it can be seen that the amount of Schiff base in the "stationary" state can be written

$$C_{AC} = K_1 \times C_A \times C_C \times t^{2s} \qquad (1)$$

where C_{AC} is the concentration of the Schiff base, C_A is the concentration of amino acid, and C_C is the concentration of carbohydrate, t_{st} is the characteristic time which elapses before the stationary state is reached, t_{st} is a constant. Combining K_1 and t_{st} in one common constant P, we have

$$C_{AC_{st}} = P \times C_A \times C_C \qquad (2)$$

As both amino acid and carbohydrate in this experiment are present in large amounts, we can consider C_A and C_C constant throughout the experiment. The rate of the irreversible degradation of Schiff base is expressed as

$$\frac{dC_{AC}}{dt} = K_3 \times C_{AC_{st}} \qquad (3)$$

or by inserting (2) in (3)

$$\frac{dC_{AC}}{dt} = K_3 \times P \times C_C \times C_A \qquad (4)$$

which in the integrated form is 5.

$$C_{D_t} = K_3 \times P \times C_A \times C_C \times t \qquad (5)$$

where C_{D_t} is the concentration of degradation products at time t.

No attempt has been made here to differentiate between the compounds formed by the degradation, but the reaction has been treated mathematically as if only one aldehyde compound were present, or as if the combined concentration of several carbonyl compounds formed were its equivalent. We can thus justify the following considerations. If the degradation products react with amino acid in a bimolecular way, the rate of reaction can be written as

$$dC_B/dt = K_4 \times C_A \times C_D \qquad (6)$$

where dC_B characterizes the increase in concentration of brown compound.[4] By inserting (5) in (6) we arrive at

$$dC_B/dt = K_4 \times K_3 \times P \times C_A^2 \times C_C \times t \qquad (7)$$

or in integrated form where Q is substituted for $K_4 \times K_3 \times P$

$$C_B = {}^1/_2 \times Q \times C_A^2 \times C_C \times t^2 \qquad (8)$$

Formula (8) would require that the concentration of the brown substance produced should be proportional to the square of the time and also that the slope of the line be proportional to the carbohydrate concentration and to the square of the concentration of amino acid. Figure 11 substantiates this. The value of the slopes is given in Table IV.

From the table it can be seen that the value of the three slopes have approximately the anticipated relationship. The close check of the results of the experiments with the theory presented seems

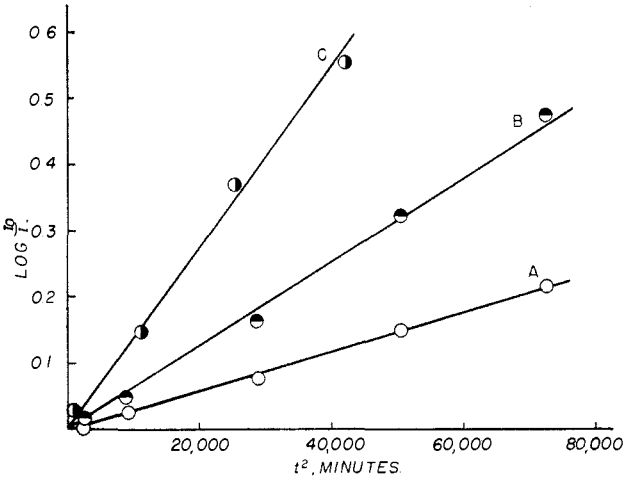

Fig. 11.

to substantiate the reaction mechanism suggested by the theory.

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