firm that pK_2 and pK_3 represent the phenolic and pyridinium group ionizations, respectively.[28] Spectrophotometric measurements of absorbancy *vs.* *p*H at 294 and 324 mμ were used to calculate $pK_2 = 3.86$ and $pK_3 = 8.02$ with a precision of ±0.03 *p*H unit.[29]

Formation of Threonylglycine from Glycylglycine.—A solution 0.05 *M* in glycylglycine, 0.1 *M* in acetaldehyde, 0.01 *M* in pyridoxal, 0.002 *M* in potassium aluminum sulfate and 0.1 *M* in *p*H 6 maleate buffer was allowed to stand 2 weeks at room temperature. A well-defined band moving more slowly than threonine but faster than glycylglycine or glycine was separated on a column of Dowex 50 and represented 23% of the original glycylglycine on the basis of its color yield with ninhydrin. When chromatographed on paper and sprayed with ninhydrin the compound gave a single zone with an initial yellow color typical of peptides and reacted at a rate similar to serylglycine. In 77% ethanol it migrated to a position intermediate between glycine and threonine. Hydrolysis with 6 *N* hydrochloric acid for 8 hr. at 110° followed by separation on the ion exchange column and paper chromatography revealed that 66% of the peptide was split to give equal quantities of glycine and a mixture of threonine and allothreonine; the remainder was not yet hydrolyzed. The separated band containing the threonine and allothreonine was assayed microbiologically and by comparison with the ninhydrin color was calculated to contain about 37% of allothreonine. The unhydrolyzed peptide was as active microbiologically as the threonine-allothreonine isolated after hydrolysis; glycylglycine is without activity in this assay.

In view of its manner of formation, its hydrolysis to equal amounts of threonine–allothreonine and glycine, and its microbiological activity in the threonine assay the identity of the product as a mixture of threonylglycine and allothreonylglycine is definite.

Formation of Threonylphenylalanine from Glycylphenylalanine.—A reaction mixture identical with that used above, but with glycylphenylalanine replacing glycylglycine was heated at 100° for 30 minutes. Separation on the Dowex 50 column showed the formation in 8.1% yield (ninhydrin color) of a new compound which was collected separately. Unlike glycylphenylalanine, the compound possessed threonine activity in the microbiological assay both before and after acid hydrolysis, migrated on paper as a single zone before hydrolysis, but yielded threonine–allothreonine and phenylalanine in equal quantities (as determined on paper chromatograms) upon hydrolysis. Its identification as a mixture of threonyl- and allothreonylphenylalanine therefore appears definite.

Formation of Serylglycine from Glycylglycine and Formaldehyde.—A reaction mixture similar to that used for formation of threonylglycine, but with formaldehyde replacing acetaldehyde was heated at 100° for 30 minutes. By the same techniques used for separation and identification of the threonine peptides, serylglycine, formed in 18% yield, was separated from the reaction mixture. The peptide showed microbiological activity in the serine assay both before and after acid hydrolysis.

Formation of β-Hydroxyaspartic Acid from Glycine and Glyoxylic Acid.—Glycine (0.2 mole), sodium glyoxylate (0.3 mole), pyridoxal hydrochloride (0.01 mole) and alum (0.005 mole) in 200 ml. of 0.1 *M* acetate buffer, *p*H 5.0, were heated at 100° for 100 minutes. The mixture was concentrated in vacuum to a small volume and transferred to a column of Dowex 50 (2.2 × 40 cm.) with 150 ml. of water. The β-hydroxyaspartic acid was eluted with 1.5 *N* HCl, and appeared in the fraction between 125 and 160 ml. of the acid effluent. This fraction was concentrated in vacuum; the sirup dried further over potassium hydroxide, and crystallized from a mixture of acetic acid and ethanol. The white product (250 mg.) was recrystallized by dissolving in one equivalent (8.4 ml.) of 0.2 *N* sodium hydroxide followed by the addition of 0.9 equivalent of 0.2 *N* hydrochloric acid. The product was washed with water and dried in vacuum over potassium hydroxide.

Anal. Calcd. for $C_4H_7NO_5$: C, 32.2; H, 4.73; N, 9.40. Found: C, 32.15; H, 4.81; N, 9.34.

This product was quite insoluble in water, and appears to be the *para* isomer described by Dakin.[30] The more soluble diasteromer[30] is also formed and can be obtained from the mother liquors of the first crystallization.

In the absence of added pyridoxal, the same reaction product is obtained, but in significantly lower (approximately 50%) yield.

(28) S. A. Harris, T. J. Webb and K. Folkers, THIS JOURNAL, **62**, 3198 (1940).

(29) Similar calculations on some other compounds of the vitamin B_6 group have recently been reported by O. K. Linn and R. A. Morton in *The Analyst*, **77**, 718 (1952).

(30) H. D. Dakin, *J. Biol. Chem.*, **48**, 273 (1921).

AUSTIN, TEXAS

[CONTRIBUTION FROM THE BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF TEXAS, AND THE CLAYTON FOUNDATION FOR RESEARCH]

Transamination of Pyridoxamine and Amino Acids with Glyoxylic Acid[1]

BY D. E. METZLER,[2] JOANNE OLIVARD AND ESMOND E. SNELL

RECEIVED SEPTEMBER 25, 1953

Pyridoxamine undergoes rapid transamination with glyoxylic acid in aqueous solution, *p*H 5, and 79–100° to yield pyridoxal and glycine. Many α-amino acids transaminate directly with glyoxylate in the *p*H range 4–10 to yield glycine and the corresponding keto acids. Each of these reactions is catalyzed by aluminum, iron(III) or copper(II) salts, and the equilibrium strongly favors the conversion of glyoxylate to glycine. This probably explains the fact that while enzymatic transamination between glycine and keto acids has not been observed, that between glyoxylate and amino acids does occur. Other biochemical implications of this fact also are discussed. Use of the reaction for preparation of α-ketoglutaric acid and sodium α-ketoisocaproate is described.

The metal-ion catalyzed transamination reactions 1 and 2 occur with most amino acids in aqueous solution at *p*H 3–8 and 100°.[3] Glycine reacts very slowly with pyridoxal, however, and little or no glyoxylate is produced.[3]

$$\text{Pyridoxal} + \alpha\text{-amino acid} \underset{(2)}{\overset{(1)}{\rightleftharpoons}} \text{pyridoxamine} + \alpha\text{-keto acid}$$

However, the reverse reaction of glyoxylate with pyridoxamine is rapid (Fig. 1). The disappearance of a mole of glyoxylate is accompanied by the formation of 0.6–0.7 mole of pyridoxal. Chromatographic experiments show that glycine is formed simultaneously. Thus the predominant reaction is transamination and, like previously studied transamination reactions, it is catalyzed by aluminum salts.

An uncomplicated measurement of the equilibrium position of this transamination reaction cannot be made because of interfering side reactions. The sum of glyoxylate and pyridoxal concentra-

(1) Supported in part by a grant from Sharp and Dohme, Inc.

(2) Department of Chemistry, Iowa State College, Ames, Iowa.

(3) D. E. Metzler and E. E. Snell, THIS JOURNAL, **74**, 979 (1952).

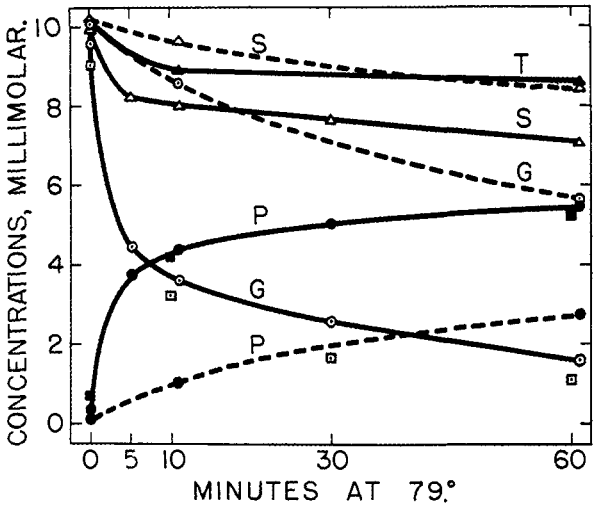


Fig. 1.—Transamination between pyridoxamine and glyoxylate. Reactant and product concentrations are plotted *vs.* time during the reaction between 0.01 *M* pyridoxamine and 0.01 *M* glyoxylate at *p*H 5, 0.1 *M* acetate buffer: P = pyridoxal, G = glyoxylate, S = sum of pyridoxal and glyoxylate, T = total vitamin B_6 concentration (pyridoxal + pyridoxamine) measured spectrophotometrically. Solid lines are for reaction catalyzed by 0.001 *M* alum [$KAl(SO_4)_2 \cdot 12H_2O$]. Dashed lines are for reaction without added metal salts. Squares represent pyridoxal and glyoxylate concentrations observed in the alum-catalyzed reaction when 0.10 *M* glycine also was present initially.

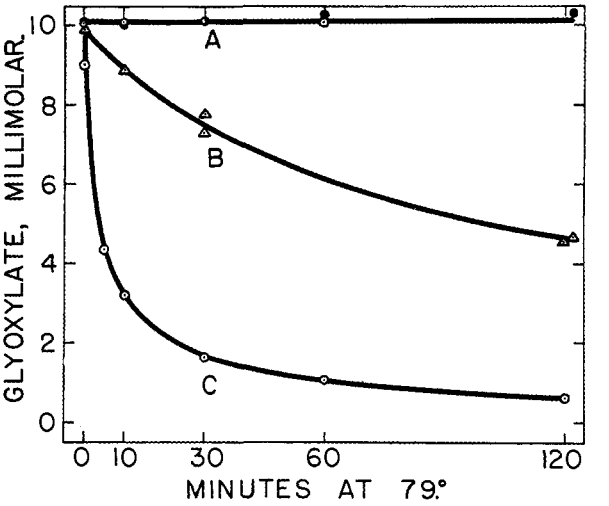


Fig. 2.—The reaction of glyoxylate with glycine: curve A: ⊙, 0.01 *M* glyoxylate heated with 0.001 *M* alum; ●, 0.01 *M* glyoxylate heated with 0.10 *M* glycine; curve B: 0.01 *M* glyoxylate heated with 0.10 *M* glycine and 0.001 *M* alum; curve C: 0.01 *M* glyoxylate heated with 0.10 *M* glycine, 0.01 *M* pyridoxamine and 0.001 *M* alum. All solutions were 0.01 *M* in acetate buffer, *p*H 5.

tions decreases to about 70% of the initial value in two hours, and none of the concentrations reach stationary values with time. Although the pyridoxal concentration appears to approach a constant value suggestive of an equilibrium concentration, the same amount of pyridoxal is formed in the presence of a tenfold excess of glycine (Fig. 1) which would alter the equilibrium position greatly. Furthermore the glyoxylate disappears more rapidly when the excess of glycine is present.

The two most important side reactions appear to be (1) the reaction of glyoxylate with glycine, and (2) the reaction of pyridoxal with glycine and alum. Figure 2 shows that glyoxylate is stable when heated with either potassium aluminum sulfate or glycine, but that when both alum and glycine are present, a rapid loss takes place. When pyridoxamine is also present a much more rapid loss of glyoxylate occurs due to transamination. The products of the alum-catalyzed reaction of glyoxylate with glycine include brownish products and β-hydroxyaspartic acid.[4] The latter gives a positive ninhydrin test and migrates on paper chromatograms with an R_f value lower than that of glycine in several chromatographic solvents. The nature of the reaction is discussed separately.[4]

When 0.1 *M* glycine and 0.01 *M* pyridoxal are heated at *p*H 5 in 0.1 *M* acetate buffer with 0.001 *M* alum at 79–100° only 0.002–0.003 *M* glyoxylate (or keto acids) is produced in two hours. The apparent pyridoxal concentration decreases by about 10% in the first 10 minutes and by about 25% in two hours. Some transamination probably occurs and the low glyoxylate concentration is in part due to its further reaction with glycine. The occurrence of the latter reaction in the pyridoxal–glycine mixture was confirmed by the appearance on paper chromatograms of the slow-moving ninhydrin-positive compound (β-hydroxyaspartic acid[4]) previously observed in glycine–glyoxylate mixtures.

The loss of pyridoxal is due partly to the formation from glycine, pyridoxal and alum of an insoluble compound which begins to crystallize in fine needles from the reaction mixtures after about 5 minutes of heating. The nature of this product, which is a metal chelate containing pyridoxal, aluminum(III) and β-pyridoxylserine, is discussed separately.[4] Since the alum is limiting and the compound formed gave some color in the ethanolamine procedure for pyridoxal determination, at most about 10% of the pyridoxal appeared to be lost by this reaction.

The remaining loss of pyridoxal when heated with glycine is slight compared to its rapid and extensive production by the reaction between pyridoxamine and glyoxylate. The equilibrium in this transamination reaction, therefore, strongly favors glycine and pyridoxal rather than glyoxylate and pyridoxamine.

Since with equal reactant concentrations, the equilibrium position in most transamination reactions between pyridoxal and amino acids lies not far from 50% reaction,[3] the equilibrium in transamination between glyoxylate and amino acids should favor almost complete conversion to glycine and keto acid. Although the direct transamination between amino and keto acids described by Herbst[5] is slow and accompanied by decarboxylation, it was found that glyoxylate reacted rapidly with amino acids at 100° to form glycine and the corresponding keto acids without decarboxylation. Like the

(4) D. E. Metzler, J. B. Longenecker and E. E. Snell, THIS JOURNAL, **75**, 2782 (1953); **76**, 639 (1954).

(5) R. M. Herbst and L. L. Engel, *J. Biol. Chem.*, **107**, 505 (1934).

transamination reactions of pyridoxal, those of glyoxylate with amino acids are metal-ion catalyzed (Fig. 3). With copper and iron salts, the glyoxylate–glutamate reaction is complete in less than ten minutes. Magnesium salts were not catalytic.

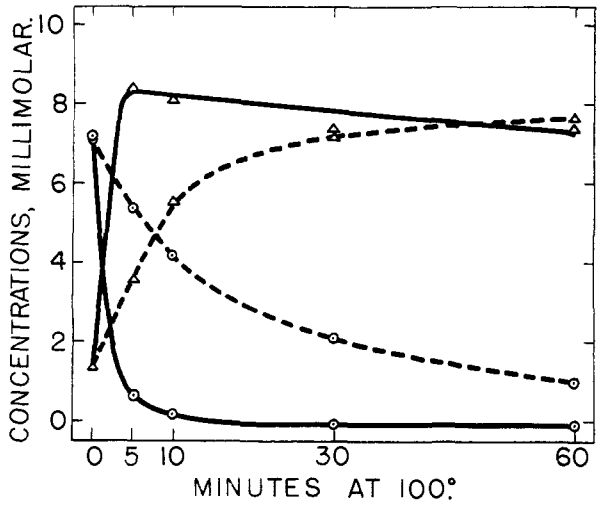


Fig. 3.—Transamination between glyoxylate and glutamate; 0.01 M reactants in 0.1 M acetate buffer, pH 5.0, were heated at 100°: ⊙, glyoxylate; △, α-ketoglutarate. Solid lines are for reaction catalyzed by 0.001 M alum; dashed lines are for reaction without added catalyst. Since no attempt was made to exclude trace quantities of metal ions, the reaction also may be metal-catalyzed in the latter case.

TABLE I

R_f VALUES AND ABSORPTION MAXIMA OF DINITROPHENYLHYDRAZONES OF KETO ACIDS FORMED BY TRANSAMINATION BETWEEN AMINO ACIDS AND GLYOXYLATE

Reaction mixtures 0.01 M in both amino acid and glyoxylate, 0.001 M in alum or copper sulfate and 0.1 M in acetate buffer, pH 5, were heated 10–120 min. at 100°. The 2,4-dinitrophenylhydrazones of the keto acids formed were chromatographed and R_f values and positions of absorption maxima of the eluted spots determined. See text for details.

Keto acid corresponding to:	Slower moving zone[a] R_f	Slower moving zone[a] Absorption maximum, mμ pH 7.4	Slower moving zone[a] Absorption maximum, mμ 0.5 N NaOH	Faster moving zone[a] R_f	Faster moving zone[a] Absorption maximum, mμ pH 7.4	Faster moving zone[a] Absorption maximum, mμ 0.5 N NaOH
Glycine	0.40	366	452	0.57	371	371
Alanine	.55	370	445	.72	380	395
α-Aminobutyric acid	.65	370	439	.77	380	400–430[e]
Valine				0.85	378	428
Leucine	0.73	371	444	.84	379	426
Isoleucine				.85	380	426
Glutamic acid				0.17–0.40	382	390–394[e]
Methionine[b]	0.68	371	448	0.77	381	400
Tyrosine[c]	.68	370	442	.78	375	405
Serine[d]	.46	372	450	.54	378	382

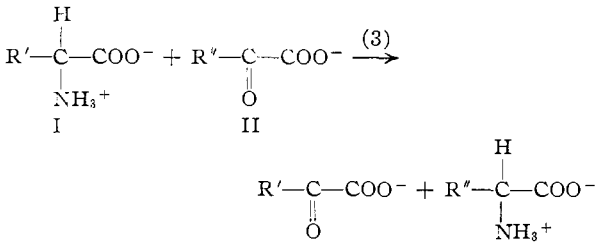
[a] The keto acids corresponding to valine, isoleucine and glutamic acid each gave only one zone. The data were placed in the first or second column of the table on the basis of similarities in the spectra. [b] Pyridoxal (0.001 M) was added to reaction mixture as a catalyst. Other weak zones of R_f 0.35 and 0.52 were present. [c] Weak zones of R_f 0.25 and 0.29 and a fluorescent zone of R_f 0.24 also were present. [d] The keto acid was not obtained by transamination; data are included for comparison only. [e] Plateau, range indicated.

The reaction goes rapidly between pH 4 and 10; lower and higher pH values were not checked.

The rates of reaction of glyoxylate with other amino acids were estimated from the rates at which the corresponding keto acids (determined from chromatograms of their 2,4-dinitrophenylhydrazones) appeared in the reaction mixtures. These rates decrease in the order glutamic acid > alanine = α-aminobutyric acid = leucine > tyrosine > isoleucine > valine > methionine. The R_f values and absorption maxima in neutral and alkaline solution of the 2,4-dinitrophenylhydrazones of the keto acids formed by transamination with glyoxylate are given in Table I.

The foregoing results suggested use of sodium glyoxylate for the preparation of α-keto acids from amino acids. Preparations of α-ketoglutaric acid and sodium α-ketoisocaproate proved rapid and convenient though the yields of purified product were somewhat low (Experimental section). Preparation of other keto acids and improvement of yields should be possible. A lower reaction temperature would probably be advantageous or even necessary in some cases.

In the transamination reaction 3 a small free energy change is expected for most R groups from



structural considerations. For the enzyme-catalyzed reaction where I is glutamic acid and II is pyruvate, ΔF^0 is -0.06[6,7] and ΔH^0 is 0 kcal. per mole[8] at 25°. Where I is glutamate and II is oxaloacetate, ΔF^0 is -1.2 kcal. per mole at 25°.[6,7] The equilibrium positions of non-enzymatic transamination reactions[3] with pyridoxal and amino acids at 100° confirm the small free energy change for transamination between glutamate and pyruvate, and show that it must also be small for R′ and R″ corresponding to any of the following: alanine, glutamate, leucine, isoleucine, norvaline, methionine, phenylalanine and tyrosine. When I is glycine, however, the free energy change is positive, probably by 2 kcal. or more at 79°, and undoubtedly at temperatures of biological importance as well.

The positive free energy change for transamination reactions with glycine may explain a number of observations in biological systems. Though glycine can be oxidatively deaminated to glyoxylate by some animal tissues,[9] Weinhouse and Friedmann were unable to demonstrate conversion of glycine to glyoxylate in the intact rat but did demonstrate a rapid conversion of glyoxylate to glycine.[10] Glyoxylate

(6) H. A. Krebs, *Biochem. J.*, **54**, 82 (1953).

(7) K. Burton and H. A. Krebs, *ibid.*, **54**, 94 (1953).

(8) S. Darling, *Acta Physiol. Scand.*, **10**, 150 (1945), from *C. A.*, **40**, 20639 (1946).

(9) S. Ratner, V. Nocito and D. E. Green, *J. Biol. Chem.*, **152**, 119 (1944).

(10) S. Weinhouse and B. Friedmann, *ibid.*, **191**, 707 (1951).

also replaces glycine in supporting growth of a *Neurospora* mutant.[11] Transaminases have usually been reported to catalyze little or no reaction between glycine and keto acids.[12–15] Glyoxylate, on the other hand, reacts rapidly with aspartic and glutamic acids, asparagine and glutamine in rat liver enzyme preparations to form glycine.[14]

Glycine is one of the compounds which becomes radioactive early during photosynthesis in the presence of $C^{14}O_2$ and may arise by transamination. The transamination equilibrium would favor a relatively low glyoxylate concentration which may explain the failure to detect radioactive glyoxylate.[16] An active glycine–ketoglutarate transaminating system in plants has been reported,[17] but the equilibrium position was not determined.

These observations suggest a widespread biological importance for transamination reactions with glyoxylate.

Experimental

Chemicals.—Most chemicals, stock solutions and reagents have been described elsewhere.[3]

Sodium Glyoxylate Monohydrate.[18]—Tartaric acid (11 g.) was dissolved in 10 ml. of water and 13.8 g. of periodic acid (H_5IO_6) in 30 ml. of water. Both solutions were chilled to 0° and the solution of periodic acid was added gradually to that of tartaric acid keeping the temperature below 5°. The glyoxylic acid was separated from the reaction mixture by continuous extraction with ether. The extracts were concentrated in vacuum to about 15 ml. of a thick sirup. An equal volume of water was added, the solution filtered and chilled to 0°. The *p*H was adjusted to between 5 and 6 by the slow addition of concentrated (50% by weight) sodium hydroxide solution. An equal volume of acetone was then added and the mixture was allowed to stand overnight in the cold. The crystalline sodium glyoxylate was collected by suction filtration, washed with 50% aqueous acetone, then with anhydrous acetone. The yield was 75% of theory, on the assumption that one mole of periodate gives two moles of glyoxylate. The salt was recrystallized by dissolving in 4.5 ml. of warm water per g., adding 2.5 ml. of acetone, and cooling. The product was dried in air or in vacuum with P_2O_5. Further recrystallizations led to no increase in the color yield with 2,4-dinitrophenylhydrazine in the quantitative procedure for keto acids.[3]

Anal. Calcd. for $C_2H_3O_4Na$: Na, 20.17. Found: Na, 20.09. Stock solutions (0.05 *M*) were prepared immediately before use.

Procedure.—Aqueous solutions of the reactants were prepared and heated in sealed glass tubes as previously described.[3]

Analytical Methods.—Measurement of pyridoxal, pyridoxal + pyridoxamine, and keto acid concentrations were made according to the methods used in earlier work.[3] Glyoxylic acid was determined by the "keto acid" procedure[3] when pyridoxal was the only other carbonyl compound present. The calibration constant

$$K = \frac{\text{micromoles of glyoxylate per 3-ml. sample}}{\text{absorbency, } L}$$

was 1.07 with the Evelyn number 515 filter.

For the simultaneous determination of glyoxylic and ketoglutaric acids a 6-ml. sample was allowed to react with 1 ml. of dinitrophenylhydrazine reagent, neutralized and extracted as in the regular keto acid method. Duplicate 5-ml. portions of the extracted sample were treated with 5 ml. of 2.5 *N* and 0.1 *N* sodium hydroxide, respectively. The samples were read in the Evelyn colorimeter with filter number 490 after 10 minutes. The calibration constants

$$K = \frac{\text{micromoles per 6-ml. sample}}{\text{absorbency, } L}$$

were glyoxylic acid: 1.31 in 0.05 *N* and 1.23 in 1.25 *N* sodium hydroxide; ketoglutaric acid 11.4 in 0.05 *N* and 1.60 in 1.25 *N* sodium hydroxide. The concentrations were calculated by successive approximation, taking as the first approximation that the color in 0.05 *N* sodium hydroxide represented only glyoxylic acid.

Keto acids were identified by chromatography of their 2,4-dinitrophenylhydrazones as previously described[19] using the upper layer of the system butanol 50, water 40, ethanol 10 (parts by volume) as the solvent. Average R_f values are given in Table I. These values were obtained with authentic samples of the keto acid analogs of glutamic acid, alanine, serine, glycine, valine and leucine as well as with the products from transamination with glyoxylate. Samples of the other keto acids were not available and identification was based on the R_f values and absorption spectra of the 2,4-dinitrophenylhydrazones obtained. The zones from paper chromatograms were eluted with 0.05 *M* phosphate buffer, *p*H 7.4, and the solutions filtered. Three-ml. aliquots were placed in 1-cm. cells and the absorption spectra measured. Sodium hydroxide (0.75 ml. of 2.5 *N* solution) was then added to each cell, the solution stirred, allowed to stand 10 min., and the spectra again measured as rapidly as possible. The positions of the absorption maxima are useful in differentiation of zones of similar R_f values. Most keto acids gave two zones of different R_f values which are probably the *syn* and *anti* isomers of the hydrazones.[19] The slower moving zone was the denser of the two for the keto acid analogs of glycine, alanine, methionine, aminobutyric acid and tyrosine. Spectrophotometric data for crystalline 2,4-dinitrophenylhydrazones in neutral and alkaline solution have also been reported from another laboratory.[20]

Amino acids were identified by chromatography on Whatman #1 filter paper using the ascending technique.

Preparation of Keto Acids by Transamination with Glyoxylate. A. α-Ketoglutaric Acid.—A mixture of 0.025 mole of sodium glyoxylate, 0.025 mole of glutamic acid and 0.0025 mole of potassium aluminum sulfate was dissolved in 500 ml. of water and the *p*H adjusted to 5.0 with sodium hydroxide. The solution was heated in a boiling water-bath to 100°, held 10 min., and cooled. The *p*H was adjusted to 1 with sulfuric acid and the solution subjected to continuous ether extraction. The ether extract was evaporated in vacuum, the residue taken up in boiling nitromethane, cooled, and held overnight in the refrigerator. A first crop of 0.52 g. of α-pyrrolidonecarboxylic acid separated. Concentration of the mother liquors in vacuum and cooling yielded 1.01 g. (27%) of α-ketoglutaric acid, m.p. (cor.) 110–111° (literature[21] 109–110°) which before recrystallization gave 94–95% of the color of pure α-ketoglutaric acid in the keto acid analysis,[3] was ninhydrin-negative, and free of glyoxylate as determined chromatographically.

B. Sodium α-Ketoisocaproate.—Sodium glyoxylate (0.05 mole), DL-leucine (0.055 mole) and potassium aluminum sulfate (0.001 mole) were dissolved in 1 l. of water. The *p*H was adjusted to 5.0 with sodium hydroxide and nitrogen bubbled through the solution to expel oxygen. The solution was heated for 1.5 hours in a boiling water-bath, then cooled to room temperature, adjusted to *p*H 4.5, and the solution concentrated in vacuum to 270 ml. It was then stirred at 50° with 1 g. of activated carbon (Darco G-60), filtered, adjusted to *p*H 1.0 and the solution extracted 8 times with 150-ml. portions of ether. The keto acid present in the extract was distilled and converted to the sodium salt by the procedure of Meister.[22] The yield was 19% of theory.

An authentic sample of sodium α-ketoisocaproate was prepared for comparison by the D-amino oxidase method.[22]

(11) B. E. Wright, *Arch. Biochem. Biophys.*, **31**, 332 (1951).

(12) P. P. Cohen, *J. Biol. Chem.*, **136**, 565 (1940).

(13) L. J. Feldman and I. C. Gunsalus, *ibid.*, **187**, 821 (1950).

(14) A. Meister, H. A. Sober, S. U. Tice and P. E. Fraser, *ibid.*, **197**, 319 (1952).

(15) A. E. Braunshtein, in *Advances in Protein Chemistry*, **3**, 1 (1947).

(16) M. Calvin, *J. Chem. Ed.*, **26**, 639 (1949).

(17) K. W. King, D. G. Wilson and R. H. Burris, *Federation Proc.*, **12**, 230 (1953).

(18) This preparation was developed in part by Dr. Norman Radin, Veterans Administration Hospital, Hines, Illinois.

(19) D. E. Metzler and E. E. Snell, *J. Biol. Chem.*, **198**, 353 (1952).

(20) D. Cavallini, *Ricerca Sci.*, **20**, 803 (1950), from *C. A.*, **45**, 3295 (1951).

(21) L. Friedman and E. Kosewer, *Org. Syntheses*, **26**, 42 (1946).

(22) A. Meister, *J. Biol. Chem.*, **197**, 309 (1952).

The free acids both distilled at 85–88° at 10 mm. The recrystallized sodium salts gave the same color yield in the keto acid procedure with 2,4-dinitrophenylhydrazine. Samples of the 2,4-dinitrophenylhydrazones were prepared in aqueous hydrochloric acid solution and tested without recrystallization. Each melted at 142° with immediate resolidification and final melting at 158–159°. No depression of melting point resulted from mixing the two samples, and the R_f values of the dinitrophenylhydrazones on paper chromatograms were identical.

AUSTIN, TEXAS

[CONTRIBUTION FROM THE BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS, AND THE CLAYTON FOUNDATION FOR RESEARCH]

A General Mechanism for Vitamin B_6-catalyzed Reactions[1]

BY DAVID E. METZLER, MIYOSHI IKAWA AND ESMOND E. SNELL

RECEIVED SEPTEMBER 25, 1953

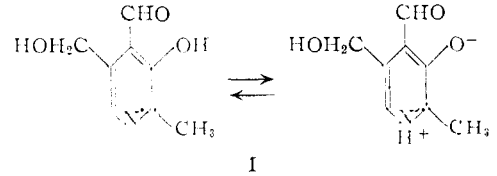
The similarity between reactions catalyzed by pyridoxal phosphate enzymes and those catalyzed by pyridoxal and metal salts is stressed. The structural features of the pyridoxal molecule essential for catalysis of these non-enzymatic reactions are the formyl group, the phenolic group and the heterocyclic ring nitrogen arranged in the 4,3- and 1-positions, respectively, as in pyridoxal, or in the electronically equivalent structure, 2-formyl-3-hydroxypyridine. The formyl group functions in formation of a Schiff base with the amino acid, the latter intermediate being stabilized to the necessary degree by chelation with the catalytic metal ion *via* the nitrogen of the resulting azomethine linkage, the phenolic group, and probably the carboxyl group of the amino acid residue. The resulting planar system of conjugated double bonds provides a mechanism for the displacement of an electron pair from any of the bonds of the α-carbon atom of the amino acid toward the strongly electrophilic heterocyclic nitrogen, a displacement that is intensified by attraction toward the strongly electronegative chelated metal ion. It is shown how such displacement can result in the observed reactions catalyzed by such systems. These include racemization, decarboxylation, transamination and elimination of an α-hydrogen together with a β-substituent of the amino acid. The reverse of the latter process also can occur, as shown by the catalysis of tryptophan formation from serine and indole.

Many different reactions of amino acids are catalyzed by pyridoxal phosphate-containing enzymes.[1a] These include transamination,[2] racemization,[3] decarboxylation,[4] elimination of the α-hydrogen together with either a β-substituent (*e.g.*, serine and threonine dehydration,[5,6] cysteine desulfhydration,[7] the tryptophanase reaction[8]) or a γ-substituent (*e.g.*, homocysteine desulfhydrase[7]), reactions that can be formulated[6] as an α,β-desaturation followed by an addition to the double bond (*e.g.*, formation of tryptophan from serine and indole[9]), and probably the reversible cleavage of β-hydroxyamino acids to glycine and carbonyl compounds.[10,11]

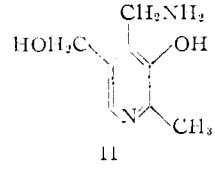
Most of these enzymatic reactions have been duplicated by non-enzymatic model reactions in which pyridoxal[11–18] or other appropriate aldehydes[18,19] and a suitable metal salt serve as catalysts. There is little doubt, therefore, that the catalytic potentialities of pyridoxal phosphate enzymes are, to an exaggerated degree, those of their prosthetic group, and that the non-enzymatic and enzymatic reactions proceed by closely similar mechanisms.

Each of these varied reactions can be visualized as resulting from the intermediate removal of an electron pair from one of the bonds to the α-carbon of the amino acid. The necessary structural features of pyridoxal that permit such reactions, together with a suggested mechanism for them, are considered herein.

In pyridoxal I there are five groups to be considered both as to presence in the molecule and relative position on the ring: (1) heterocyclic nitrogen atom, (2) methyl group, (3) phenolic hydroxyl group, (4) formyl group and (5) the hydroxymethyl group.



I



II

The primary importance of the formyl group is known from the role of pyridoxal in transamination whereby it is converted to pyridoxamine (II),[12,13]

(1) Supported in part by a grant from Sharp and Dohme, Inc.

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