of 1,3,4,6-tetraacetyl-β-D-glucosamine in 75 ml. of ethyl acetate. The mixture was kept at room temperature for 24 hours. During this time 2.0 g. of the product (IIb) separated from the reaction mixture as needles which, after two recrystallizations from 95% ethanol, melted at 201–202° and had $[\alpha]^{22}D$ +14.85 (*c* 5, pyridine). The filtrate was extracted twice with *N* hydrochloric acid, twice with a 5% solution of potassium bicarbonate and twice with water, filtered through a dry filter paper and evaporated to dryness *in vacuo*. Recrystallization of the residue from 95% ethanol gave an additional 3.0 g. of the product, bringing the total yield to 64%.

*Anal.* Calcd. for $C_{27}H_{37}O_{12}N_3S$: C, 51.66; H, 5.94; N, 6.70. Found: C, 51.69; H, 5.97; N, 7.12.

**Identification of β-Methylmercaptopropionaldehyde as a Product of the Acid Hydrolysis of IIb.**—A mixture of 0.5 g. of IIb and 10 ml. of 5% sulfuric acid was boiled under reflux for one hour. Liquid was then distilled from the mixture until it was apparent that no more oil phase was distilling over (total distillate about 5 ml.). This distillate was added to a mixture of 260 mg. of 4-hydroxycoumarin and 10 ml. of water in a boiling water-bath. After 15 minutes the reaction mixture was cooled, scratched with a glass rod, then reheated to boiling, and filtered with suction. The product (38 mg.) was washed on the funnel with several portions of boiling water. After four recrystallizations from 95% ethanol it melted at 151–154°. The melting point was not depressed by mixture of this substance with a sample of 3,3′-β-methylmercaptopropylidinebis-(4-hydroxycoumarin) (V) prepared from authentic β-methylmercaptopropionaldehyde in a like manner.

*Anal.* Calcd. for $C_{22}H_{18}O_6S$: C, 64.38; H, 4.42. Found: C, 64.50; H, 4.67.

**N-(1,5-Dicarbobenzoxyamido-*n*-pentylcarbamyl)-1,3,4,6-tetraacetyl-β-D-glucosamine (IIa).**—A solution in chloroform of dicarbobenzoxy-L-lysine azide, prepared from 13 g. of the hydrazide by the method of Bergmann, Zervas and Greenstein,[11] was added to a cold solution of 10.4 g. of 1,3,4,6-tetraacetyl-β-D-glucosamine in 100 ml. of chloroform. Keeping the solution for 5 hours in an ice–salt-bath and 36 hours at room temperature, it was extracted twice with *N* hydrochloric acid, twice with 5% potassium bicarbonate solution and twice with water, dried over anhydrous sodium sulfate and evaporated to dryness *in vacuo*. The residue was dissolved in hot dioxane and a little water added. The product separated as a sirup which crystallized on cooling; yield 14.2 g., 63%. After one recrystallization from 95% ethanol, it melted at 190–191° and had $[\alpha]^{20}D$ +10.9 (*c* 4, pyridine). The analytical sample melted at 191–192°.

*Anal.* Calcd. for $C_{36}H_{45}O_{14}N_4$: C, 56.98; H, 6.10; N, 7.39. Found: C, 56.77; H, 6.15; N, 7.64.

**Attempted Condensation of Carbobenzoxy-DL-serine Azide with 1,3,4,6-Tetraacetyl-β-D-glucosamine.**—An excess of diazomethane in ether solution was added to a suspension of 15.3 g. of carbobenzoxy-DL-serine in 100 ml. of ethyl acetate. The acyl amino acid dissolved as the esterification proceeded. Evaporation of the solution *in vacuo* gave a sirup which was taken up in 200 ml. of absolute ethanol and 8 ml. of 85% hydrazine hydrate was added. Crystallization of the hydrazide began within an hour. After allowing it to stand overnight at room temperature some ether was added and the crystalline mass was broken up, filtered off and dried. Carbobenzoxy-DL-serine hydrazide, 14.9 g., melting at 153–154° was obtained.

A solution of 5.1 g. of this hydrazide in 50 ml. of water, 4.5 ml. of glacial acetic acid and 1.5 ml. of concentrated hydrochloric acid was cooled in an ice-bath and 2.2 g. of sodium nitrite dissolved in 15 ml. of water was added in portions with cooling and shaking over a period of ten minutes. The sirupy azide which formed was extracted with ethyl acetate and the solution was washed quickly with water, with 5% potassium bicarbonate and again with water, and dried over anhydrous sodium sulfate. The dried solution was added to 6.7 g. of 1,3,4,6-tetraacetyl-β-D-glucosamine dissolved in 100 ml. of ethyl acetate. After allowing the mixture to stand overnight, it was washed with dilute hydrochloric acid, with 5% potassium bicarbonate and with water, and evaporated to dryness *in vacuo*. The solid was taken up in ethyl acetate and precipitated by the addition of petroleum ether. A mixture of crystals was obtained which melted at 124–125°. Recrystallization yielded one fraction melting at 127–128°. The sirupy residue has not been crystallized. The analysis of the crystalline fraction indicated that it was DL-4-carbobenzoxyamidoöxazolidone-2, an isomer of which was prepared by Fruton[10] from L-serine.

*Anal.* Calcd. for $C_{11}H_{12}O_4N_2$: C, 55.92; H, 5.12. Found: C, 55.84; H, 5.21.

A few milligrams of the racemic oxazolidone was boiled with 10% hydrochloric acid for two minutes and cooled. Benzyl carbamate, melting point of 91°, in agreement with the value given by Fruton,[10] was obtained.

MADISON, WISCONSIN

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

# Chemical Interactions of Amino Compounds and Sugars. VII.[1] *p*H Dependency[2]

BY M. L. WOLFROM, DORIS K. KOLB AND A. W. LANGER, JR.

RECEIVED FEBRUARY 21, 1953

The *p*H dependency of the complex color-forming reaction between amino acids (glycine and alanine) and reducing sugars (D-xylose) has been investigated. The results indicate strong base catalysis between the initial *p*H values of 6.5 to 8.5, solvent or weak base catalysis between 3 and 5, and acid inhibition in the range 1 to 3. An evaluation of the temperature factor at *p*H 4 indicates an activation energy of 20.2 kcal.

Amino acids and reducing sugars react to form dark colored products. It is known in a qualitative sense that alkalinity favors and acidity retards the formation of these substances. Mohammad, Fraenkel-Conrat and Olcott[3] studied the rate of color formation between D-glucose and bovine serum albumin and found that the logarithm of the reaction rate rose linearly with *p*H in the range 4 to 10 with the apparent activation energy being 30.3 kcal. Frankel and Katchalsky[4] followed the *p*H drop occurring in the initial interaction between D-xylose (10.5 *M*) and glycine (0.5 *M*) at room temperature. They found that this change in *p*H passed through a maximum near *p*H 7.5 and dropped to approximately zero between the initial *p*H values 1 and 4 and also at the initial *p*H of 10.5. This *p*H change undoubtedly indicates reaction between the amino and carbonyl groups with a consequent increase in acidity due to the liberation of

(1) Previous communication in this series: M. L. Wolfrom, R. C. Schlicht, A. W. Langer, Jr., and C. S. Rooney, THIS JOURNAL, **75**, 1013 (1953).

(2) This paper reports research undertaken in part in coöperation with the Quartermaster Institute for the Armed Forces under Contract No. W11-183-qm-8145 with The Ohio State University Research Foundation, and has been assigned number 313 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

(3) A. Mohammad, H. Fraenkel-Conrat and H. S. Olcott, *Arch. Biochem.*, **24**, 157 (1949).

(4) M. Frankel and A. Katchalsky, *Biochem. J.*, **31**, 1595 (1937).

the carboxyl group from its ammonium salt combination. This narrow range of *p*H influence with its central maximum value is in harmony with the analogous situation recorded for semicarbazone formation[5] and glycosylamine hydrolysis.[6]

We report herein a quantitative study of the *p*H dependency of the complex color-forming reaction between amino acids and reducing sugars. Dilute equimolar (0.25 *M*) solutions of D-xylose and glycine were heated at 100° at an initial *p*H obtained by the addition of sodium hydroxide or hydrochloric acid. The development of the soluble colored substances formed was followed with time by measuring the light absorption at 490 mμ (see Table I for selected data). The rate of color development was characterized by plotting these data to obtain the typical curves shown in Fig. 1. These

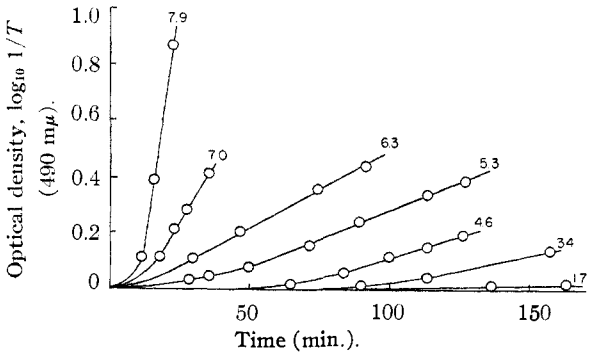


Fig. 1.—Rate of coloration of 0.25 *M* D-xylose with 0.25 *M* glycine at 100° at various *p*H (measured at the initial straight line portions of these curves) values.

curves exhibit an initial induction period[3,7] followed by an essentially straight line portion. The *p*H drifts downward with time but the value determined at the initial straight line portion of the curve was selected for comparative purposes. Since the optical density is a measure of concentration, the slope of the straight line portion of the curve was taken as a measure of the first order specific reaction constant *k* (Table I) for the rate of coloration. These specific reaction constants were then plotted as a function of the thus selected comparative *p*H to give the curve of Fig. 2. All colorations were of the yellow-brown variety save for two points where the reaction exhibited a blue-green color. These points therefore fall off the curve.

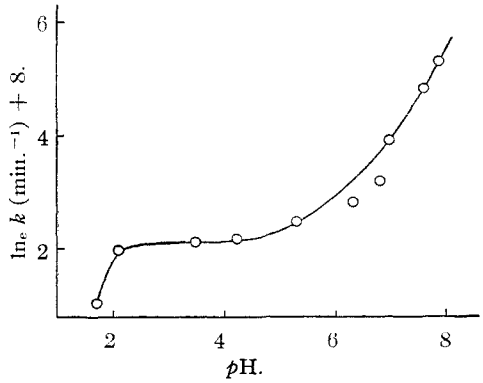


Fig. 2.—Relation between *p*H (see Fig. 1) and $\ln_e k$ for rate of coloration of solutions containing D-xylose (0.25 *M*) and glycine (0.25 *M*) measured (see Table I) at 100°. Points off the curve were measured at 490 mμ on a blue-green solution; all other colors were yellow-brown.

TABLE I

RATE OF COLOR FORMATION FROM D-XYLOSE (0.25 *M*) AND GLYCINE (0.25 *M*) (TYPICAL DATA)

| Temp., °C. | Time | *p*H[a] | Transmission (*T*), % at 490 mμ[b] | *p*H[c] | *k* (see note *d* below) |
|---|---|---|---|---|---|
| 100[e] | 0 min. | 8.45 | 100.0 | 7.9 | 0.0700 min.$^{-1}$ |
| | 10 | 8.01 | 91.7 | | |
| | 12 | 7.95 | 77.3 | | |
| | 14 | 7.89 | 59.0 | | |
| | 16 | 7.83 | 41.9 | | |
| | 20 | 7.75 | 24.7 | | |
| | 23 | 7.66 | 14.0 | | |
| | 31 | 7.50 | 6.1 | | |
| 100[e] | 0 min. | 5.40 | 100.0 | 5.3 | 0.00400 min.$^{-1}$ |
| | 28 | 5.38 | 94.0 | | |
| | 50 | 5.31 | 83.8 | | |
| | 71 | 5.10 | 70.6 | | |
| | 90 | 4.96 | 58.1 | | |
| | 102 | 4.90 | 51.4 | | |
| | 115 | 4.85 | 46.0 | | |
| | 128 | 4.84 | 41.2 | | |
| 65[f] | 0 hr. | 7.48 | 100.0 | 7.2 | 0.0270 hr.$^{-1}$ |
| | 6.5 | 7.18 | 77.4 | | |
| | 9.0 | 7.02 | 66.3 | | |
| | 10.5 | 6.85 | 60.2 | | |
| 65[f] | 0 hr. | 6.65 | 100.0 | 4.3 | 0.0104 hr.$^{-1}$ |
| | 31 | 4.90 | 89.0 | | |
| | 49 | 4.57 | 76.1 | | |
| | 55 | 4.57 | 71.4 | | |
| | 73.5 | 4.41 | 53.8 | | |
| | 80 | 4.35 | 48.5 | | |
| | 107.5 | 4.25 | 25.0 | | |
| | 147 | .. | 6.7 | | |
| 65[f] | 0 hr. | 0.70 | 100.0 | 0.7 | 0.00076 hr.$^{-1}$ |
| | 171 | 0.70 | 84.5 | | |
| | 240 | 0.70 | 73.2 | | |
| | 332 | 0.70 | 64.1 | | |
| | 432 | 0.70 | 54.3 | | |

[a] Beckman *p*H meter (model C) employing the glass electrode standardized at *p*H 7.00 with 0.1 *M* phosphate buffer; initial *p*H adjusted by addition of HCl or NaOH. [b] Lumetron (model 402E) photoelectric colorimeter. [c] Determined graphically from *p*H at beginning of straight line portion of curve of *E vs.* time (see Figs. 1 and 3). [d] First order; evaluated graphically from the slope of straight line portion of same curve. [e] Plotted in Fig. 1. [f] Plotted in Fig. 3.

Figures 3 and 4 (curve A) show similar data obtained with the same reactants but at the lower temperature of 65°. The curves of Fig. 3 fall sharply into three families corresponding to the three portions of curve A in Fig. 4. The bluish solutions obtained at *p*H 6.3 and 6.8 with glycine at 100° were not obtained at the lower temperature but mixtures initially adjusted to *p*H values in that vicinity underwent a rapid drop of several *p*H units

(5) J. B. Conant and P. D. Bartlett, THIS JOURNAL, **54**, 2881 (1932); F. H. Westheimer, *ibid.*, **56**, 1962 (1934).

(6) H. S. Isbell and Harriet L. Frush, *J. Research Natl. Bur. Standards*, **46**, 132 (1951).

(7) T.-L. Tan, M. L. Wolfrom and A. W. Langer, Jr., THIS JOURNAL, **72**, 5090 (1950).

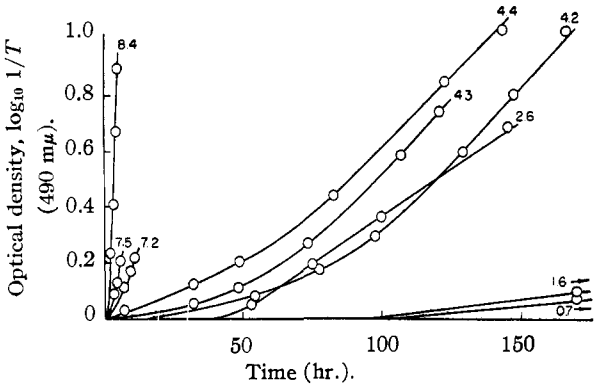


Fig. 3.—Rate of coloration of 0.25 *M* D-xylose with 0.25 *M* glycine at 65° at various *p*H (measured at the initial straight line portions of these curves) values.

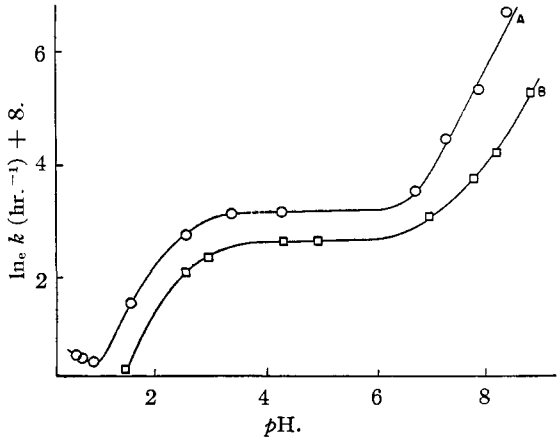


Fig. 4.—Relation between *p*H (see Fig. 3) and $\ln_e k$ for rate of coloration of 0.25 *M* (in each constituent) solutions of: A, D-xylose–glycine at 65°; B, D-xylose–DL-alanine at 65°. The lowest *p*H points on curve A were measured on brown colored solutions of an aldehydic, rather than a caramel, odor; distillation of these solutions into a solution of 2,4-dinitrophenylhydrazine yielded copious quantities of 2-furaldehyde 2,4-dinitrophenylhydrazone of m.p. 229° (dec., cor.).

before there was appreciable browning (see Table I). Curve B of Fig. 4 represents a final plot of similar data obtained for the case wherein the glycine was substituted by an equimolar amount of the closely related amino acid DL-alanine.

The curves of Fig. 2 and Fig. 4 are all of the same general type. It is probably significant that a major change in the shape of the curve occurs near *p*H 6, the isoelectric point of the amino acids. An unadjusted solution of glycine and D-xylose exhibits a *p*H near 6.5 which lowers to approximately 5 on heating.[8] Those portions of the curves between *p*H 6.5–8.5 establish strong base catalysis[9] and those between 3–5 indicate solvent or weak base catalysis. Below this a sharp change of slope may be interpreted as due to acid inhibition. In one case (curve A, Fig. 4), the reaction was followed toward still lower *p*H values and exhibited an upturn through a minimum. Here copious quantities of 2-furaldehyde were detectable, so this point represents a change in the over-all nature of the reaction and the coloration is probably due mainly to 2-furaldehyde polymerization alone. Selecting comparative values near *p*H 4 from the curves in Figs. 2 and 4 (curve A), which differ only in the temperature factor, an activation energy of 20.2 kcal. is calculable. These data then serve to show the complexity of the reaction between amino acids and reducing sugars and demonstrate that it proceeds more rapidly in the *p*H range 6.5–8.5. This is the range wherein the initial amino–carbonyl interaction is favored,[4,10] undoubtedly a significant factor.

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

(9) R. P. Bell, "Acid-Base Catalysis," Clarendon Press, Oxford, 1941, p. 8.

(10) H. v. Euler and E. Brunius, *Ber.*, **59**, 1581 (1926); **60**, 992 (1927).

COLUMBUS 10, OHIO

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[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGY, SCHOOL OF MEDICINE, UNIVERSITY OF CALIFORNIA, BERKELEY]

# Synthesis of Some O-Glucuronides and O-Glucosides of Phenolic Amino Acids[1]

BY A. TAUROG, S. ABRAHAM AND I. L. CHAIKOFF

RECEIVED FEBRUARY 16, 1953

Phenolic glucuronides of tyrosine and diiodotyrosine, and phenolic glucosides of tyrosine, diiodotyrosine and diiodothyronine were prepared by coupling the appropriately blocked amino acids with acetobromoglucuronic acid methyl ester or acetobromoglucose in the presence of finely divided silver oxide and quinoline. The carboxyl group of the amino acids was blocked by esterification. Trifluoroacetyl proved to be an excellent amino blocking group for the preparation of free glucuronides and glucosides because it is readily removed by treatment with dilute alkali at room temperature, a procedure which does not affect the glucoside or glucuronoside linkage.

While investigating the excretion products of $I^{131}$-labeled L-thyroxine in the bile of rats, we encountered an unidentified iodine-containing compound on our filter paper chromatograms.[2,3] This compound, which we named "Compound U," is the major excretion product of thyroxine in the rat. Primarily on the basis of its hydrolysis by the enzyme, β-glucuronidase, we have assumed that Compound U is a glucuronide of thyroxine.[3] Final proof of this assumption, however, must await the synthesis of thyroxine glucuronide, and the demonstration that the synthetic material is identical with Compound U.

The synthesis of glucuronides has been reported by only a few investigators. Neuberg and Nei-

(1) Aided by a grant from the U. S. Public Health Service.

(2) A. Taurog, F. N. Briggs and I. L. Chaikoff, *J. Biol. Chem.*, **191**, 29 (1951).

(3) A. Taurog, F. N. Briggs and I. L. Chaikoff, *ibid.*, **194**, 655 (1952).