Although eq. (13) gives $v_2(\theta)$, to obtain $v_2(c)$ we need also $c(\theta)$. The steady state condition

$$v_1 = v_{-1} + v_2 \qquad (16)$$

gives

$$c(\theta) = \frac{\theta}{1-\theta}\left[\frac{2(1-\theta)}{\beta+1-2\theta}\right]^z \left(\frac{k_{-1}^\circ}{k_1^\circ} + \frac{k_2^\circ}{k_1^\circ}\left[\frac{\beta+1+2\theta\{\exp[(w_a' - w_b' - w_a)/kT] - 1\}}{\beta+1+2\theta\{\exp(-w_b/kT) - 1\}}\right]^z\right)^{-1} \qquad (17)$$

When $k_2^\circ = 0$, eq. (17) reduces to the quasi-chemical equilibrium adsorption isotherm,[3] as expected. When $w_a' - w_b' - w_a = -w_b$, the $k_2^\circ = 0$ result is again obtained except k_{-1}° is replaced by $k_{-1}^\circ + k_2^\circ$.

One complication should be mentioned. Suppose w_a/kT and θ, at steady state, have values such that the adsorbed S molecules split into two surface phases[3] (determined by equal surface pressure and chemical potential in the two phases—since equilibrium within the adsorbed phase or phases has been assumed). Let θ_1 and θ_2 refer to the two different phases. Using eq. (13), (14) and (15), let us write the left-hand side of eq. (16) as $c\varphi_L(\theta)$ and the right-hand side as $\varphi_R(\theta)$. Then it is easy to show that in the region of phase splitting $c(\theta)$ is given by

$$c(\theta) = \frac{(\theta_2 - \theta)\varphi_R(\theta_1) + (\theta - \theta_1)\varphi_R(\theta_2)}{(\theta_2 - \theta)\varphi_L(\theta_1) + (\theta - \theta_1)\varphi_L(\theta_2)} \qquad (18)$$

The present treatment applies to one, two or three dimensional lattices, the two dimensional case being the most important. The quasi-chemical method is exact in one dimension for B large, it may be recalled (Ising model).

This problem suggested itself in conversations with Drs. D. J. Botts and M. F. Morales concerning their work mentioned above.[1]

NAVAL MEDICAL RESEARCH INSTITUTE
BETHESDA, MARYLAND

Degradation of D-Glucose, D-Fructose and Invert Sugar in Carbonate-buffered Water Solutions

BY LAWRENCE J. HEIDT AND CATHERINE M. COLMAN[1]

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D-Glucose (dextrose), D-fructose (levulose) and the equimolar mixture of these sugars (invert sugar) have been found to be degraded completely into non-reducing substances under conditions commonly believed to result mainly in Lobry de Bruyn–van Ekenstein rearrangement into an equilibrium mixture of D-glucose, D-fructose and D-mannose[2] without the degradation of a large fraction of the sugars into non-reducing substances.[3] D-Fructose, for example, has been reported to establish in water at 100° and *p*H 7 to 12 an equilibrium value for the reducing power of the solution equal to about 93% of the initial value.[4]

The rate of degradation of the sugars to non-reducing substances in carbonate-buffered solutions at 100° is shown in Fig. 1. The reducing power decreases to about 10% of its initial value in 90 min. at *p*H 9.3 and in 16 min. at *p*H 10 in accord, for the most part, with the kinetics of a first order reaction with respect to the sugar and hydroxyl ion when the latter is estimated from the *p*H at 25° at constant total formal concentration of carbonate and bicarbonate. There is a slight induction period which is the longest in the case of D-glucose as is evident from Fig. 1. The small decrease in reducing power observed in earlier work[2a,4] at this temperature and over this range of *p*H may have been caused by a lowering of the *p*H by the acid produced in the reaction to the point where the rate of degradation became negligible before much of the sugar had been degraded.

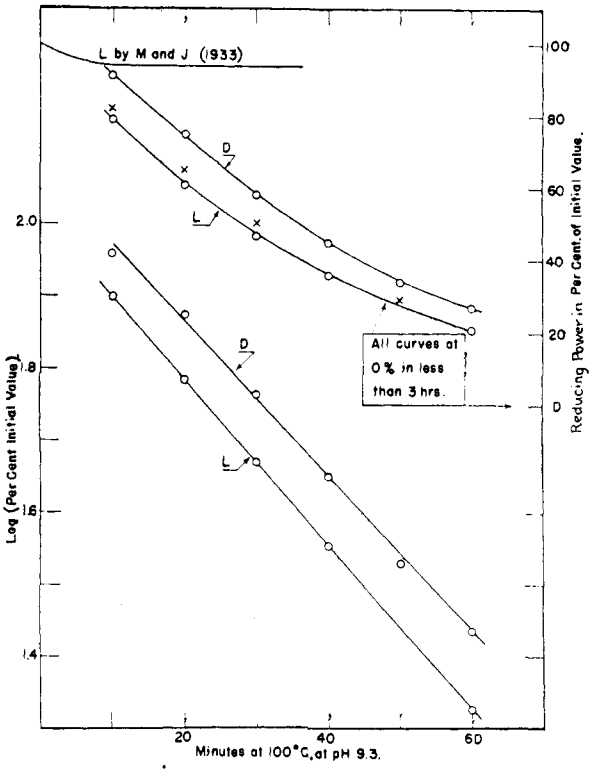

Fig. 1.—Rate of degradation at 100° of D-glucose (D), D-fructose (L) and invert sugar (X) to non-reducing material in water buffered with sodium carbonate and bicarbonate at 0.4 *M*. The solutions were initially 0.0042 *M* in hexose. The half-life of D-fructose at 100° is 6 min. at *p*H 10 and 28 min. at *p*H 9.3. Near the top of the figure is given the results reported by Mathews and Jackson[4] for D-fructose at 100° in water solution at *p*H 7 to 12.

The degradation of the sugars is accompanied by browning as is well known. The absorption spectra of the products have been found to exhibit a maximum which shifts from 2800 ± 25 Å. in the first stage of the reaction to 2650 ± 25 Å. as the reaction proceeds. This is shown in Fig. 2 which depicts the behavior of a solution of invert sugar 0.0042 *M* in hexose. Essentially the same results were obtained with D-glucose and D-fructose. The optical densities of the untreated solutions were negligible by comparison.

(1) The authors are indebted to the Sugar Research Foundation, Inc., for a grant-in-aid in support of this work.

(2) (a) C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *Rec. trav. chim.*, **14**, 203 (1895); (b) W. L. Evans, *Chem. Revs.*, **31**, 537 (1942).

(3) Some of the most convincing evidence supporting the rearrangement has been published by M. L. Wolfrom and W. L. Lewis, THIS JOURNAL, **50**, 837 (1928). Their results were obtained, however, under much milder conditions than we have employed.

(4) J. A. Mathews and R. F. Jackson, *Bur. Standards J. Research*, **11**, 619 (1933).

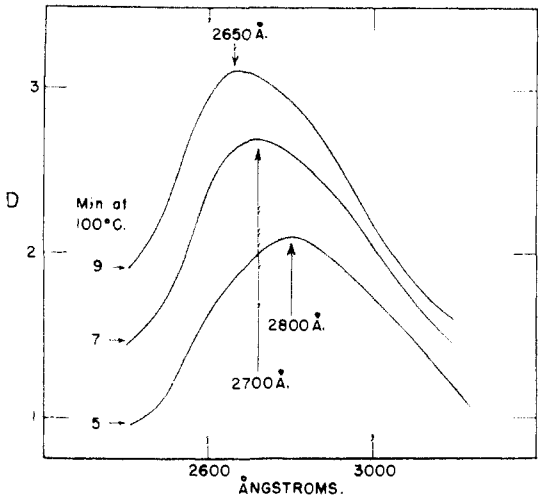

Fig. 2.—Change with time in the absorption spectrum of the products of degradation at 100° of invert sugar in water buffered with carbonate at *p*H 10. The solution was initially 0.0042 *M* in hexose. The absorbance, *D*, is based on a light path of one cm. in the solution.

Average values of the extinction coefficients have been calculated at the wave length of maximum absorption by dividing the optical density, *D*, per cm. by the moles of hexose decomposed, H_d, per liter after correcting *D* for the optical density of the untreated solution. The fractional degradation of the hexose was taken as equal to the difference between the initial and prevailing values of the reducing power of the solution divided by the initial value corrected for the cuprous oxide lost in the analytical procedure. The average values of $D/(H_d)$ calculated in this way are presented in Fig. 3; they increase rapidly as much as fivefold until about 70% of the hexose has been degraded to non-reducing material which occurs at 100° during the first ten min. at *p*H 10 and during the first 40 min. at *p*H 9.3. There is a further increase in $D/(H_d)$ of only 10 to 20%. The optical density between 2400 and 3600 Å. and the color of the solutions increase less than 5% after the reducing power has fallen below 1% of the initial value when allowance is made for loss of water by evaporation. The value reached by $D/(H_d)$ is about the same for all these sugars, but it increases with the initial concentration of the sugar nearly in proportion to the square root of this concentration.

Levulinic acid under the same conditions has an absorption curve similar to the one eventually reached in these solutions, and the value of the extinction coefficient at the peak at 2650 Å. is 27; consequently if it were responsible for the optical density in the case of Line 3, Fig. 3, 600/27 or 22 moles of it would be produced from one mole of hexose which is, of course, impossible. In a similar manner one can eliminate as products contributing largely to the optical density such compounds as lactic acid, methyl glyoxal, and the sugars and degradation products mentioned by Evans.[2b] The large value of the optical density can be attributed to compounds containing a conjugated system of alternating single and double bonds. These are not β-ketonic acids or their enolic forms because they

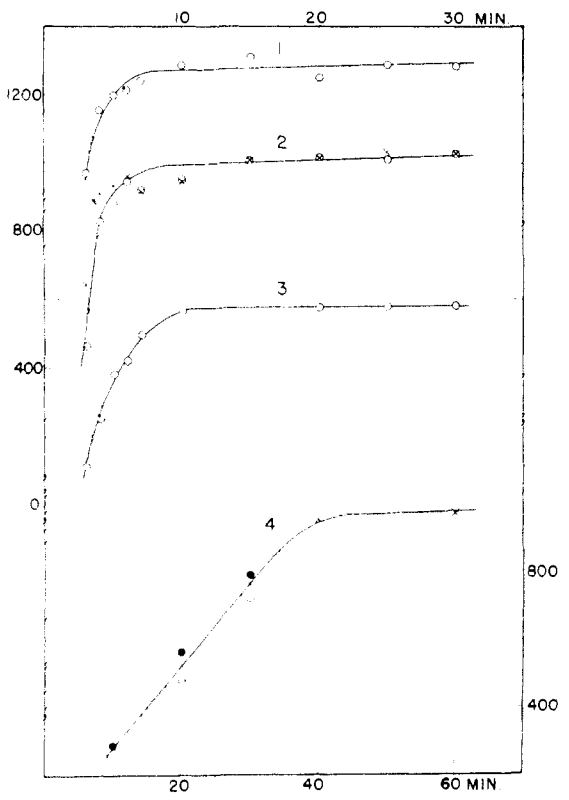

Fig. 3.—Optical density per cm. per mole of hexose degraded at 100° to non-reducing material per liter at the wave length of maximum absorbance. The value of this wave length was 2650 Å. in the case of the results represented by line 4 and by the parts of lines 1, 2 and 3 for heating periods of 10 min. or longer; the value ranged from 2830 to 2650 Å. for the other parts of lines 1, 2 and 3 in the way depicted in Fig. 2. The ordinate in every case gives the value of $D/(H_d)$. Circles, dots and crosses represent the results with D-glucose, D-fructose and invert sugar, respectively.

Line	*p*H	(Hexose)	Abscissa	Ordinate
1	10	0.0126	Upper	Left
2	10	.0042	Upper	Left
3	10	.0014	Upper	Left
4	9.3	.0042	Lower	Right

are unstable when boiled in dilute acid, whereas the optical densities of the degraded solutions obtained in this research are unaffected by this treatment.

The results presented in the figures are for aqueous solutions containing 0.0014, 0.0042 or 0.0126 mole of hexose per liter and a total concentration of sodium carbonate and sodium bicarbonate equal to 0.4 mole per liter.

The *p*H values were measured with a glass electrode at 25° both before and after the heat treatment; they were found to remain constant within 0.04 at *p*H 10 and to increase up to 0.4 unit in solutions initially at *p*H 8.9.

The reducing power was determined from measurements of the amount of cuprous oxide produced in a method[5] which employs in every analysis 5 ml. of a carbonate-buffered cupritartrate macro analytical reagent in a total volume of 10 ml.

The optical densities were measured at 20° in quartz cells by means of a Cary Record-

(5) L. J. Heath and F. W. Southam, THIS JOURNAL, **72**, 589 (1950).

ing Spectrophotometer whose wave length and optical density scales had been calibrated.

CONTRIBUTION FROM DEPARTMENT OF CHEMISTRY
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
CAMBRIDGE, MASSACHUSETTS

Paper Chromatography of Bile Acids[1]

BY DAVID KRITCHEVSKY AND MARTHA R. KIRK

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The separation of bile acids by paper chromatography has been investigated in this Laboratory and two solvent systems which give different, reproducible R_f values for several acids have been found. The two systems are *n*-propyl alcohol–ammonia–water 90:2:8 and *n*-propyl alcohol–ethanolamine–water 90:5:5. Of the two systems, the latter concentrates the moving material into a smaller area and is, therefore, preferable for identification or separation.

Using this solvent mixture, we have been able to achieve separation of various mixtures of these bile acids. Although the R_f values of dehydrocholic, cholic and norcholic acids are close together, we have been able to separate mixtures of desoxycholic, dehydrocholic and cholic acids, and of desoxycholic, dehydrocholic and norcholic acid. In these experiments we have generally observed two distinct spots of the two acids whose R_f values are close together; in some cases, however, they merge to give one spot. All experiments were carried out using 50 γ of material; mixtures contained 50 γ of each component.

For identification of the bile acids, a 15% phosphoric acid spray, slightly different from that originally proposed by Neher and Wettstein,[2] was used. The acids appeared as brown or red spots in white light, or displayed a greenish-yellow or pink fluoresence in ultraviolet light.

The results are given in Tables I and II.

TABLE I

R_f VALUES FOR BILE ACIDS

Acid	P–M–W[a] 90:5:5	P–A–W 90:2:8	E–A–W 90:2:8	P–A–W 5:2:3
Desoxycholic	0.92	0.74	0.66	0.95
Dehydrocholic	.65	.47	.65	.89
Cholic	.71	.52	.71	.94
Norcholic	.69	.51	.70	.94
Triformylnorcholic	.92	.68	.75	.94

[a] P, *n*-propyl alcohol; M, monoethanolamine; A, ammonia; W, water.

TABLE II

SEPARATIONS

Mixture	R_f values
Desoxycholic/dehydrocholic/cholic	0.95/0.65/0.72
Desoxycholic/dehydrocholic/norcholic	0.92/0.62/0.73

Experimental

The organic solvents were distilled prior to use. All mixtures are by volume as given. Whatman #1 paper was used throughout.

The material to be chromatographed was applied to a spot about 2 cm. in diameter on a 4 × 40-cm. strip of filter paper. Descending chromatography was used and after the solvent front had advanced 25–35 cm. from the origin, the strips were removed from the chromatographic chamber (a 7 × 50-cm. test-tube) and air-dried. Prior to spraying, the strips were dried at 80° for 15 minutes. The spray solution was prepared by mixing 10 parts of 85% phosphoric acid with 25 parts each of water and 95% ethanol. After the papers were sprayed, they were kept at 90° for 20 minutes. Generally, cholic and norcholic acids showed up as red or brick colored spots and occasionally one of the other acids appeared as a red spot. In ultraviolet light (Model SL Mineralight, Ultra-Violet Products, Inc., South Pasadena, California) desoxycholic acid exhibited a pink fluorescence and the other acids exhibited a greenish-yellow fluorescence. When larger quantities of these acids were used (100–200 γ) they all gave colored spots in white light as well as appearing more readily in the ultraviolet.

The R_f values were measured from the foremost point of the origin to the leading edge of the spot. The solvent mixtures which included ammonia tended to give some streaking, whereas with ethanolamine spots about 15 mm. in diameter were obtained.

All R_f values represent the average of a number of experiments.

Acknowledgment.—The authors wish to thank Dr. J. G. Buchanan for several helpful discussions and Dr. R. M. Lemmon for generous gifts of norcholic and triformylnorcholic acids.

RADIATION LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA

(1) The work described in this paper was sponsored by the United States Atomic Energy Commission.

(2) R. Neher and A. Wettstein, *Helv. Chim. Acta*, **34**, 2278 (1951).

A Redetermination of the Kinetic Constants for the System α-Chymotrypsin–Nicotinyl-L-tryptophanamide[1]

BY H. T. HUANG AND CARL NIEMANN[2]

RECEIVED MARCH 29, 1952

In previous studies of the kinetics of the α-chymotrypsin catalyzed hydrolysis of simple specific substrates[3–8] the enzyme preparations used were obtained from a single source, *i.e.*, Armour and Co., although it is true that care was taken to use preparations of different lot numbers in several of the investigations.[4,6] While it has been possible in one instance[4] to compare the K_S and k_3 values of acetyl L-tyrosinamide obtained in these laboratories with those obtained elsewhere[9–13] with different enzyme preparations the fact that differences in the reaction systems and analytical procedures were also involved in the above comparison suggested the desirability of a comparison in which the source of the enzyme preparation was the only variable.

The Armour preparation used most frequently in our previous investigations bore the lot no. 90402. This preparation had been used at three different concentrations in a total of twenty-eight separate experiments to evaluate the

(1) Supported in part by a grant from Eli Lilly and Co.

(2) To whom inquiries regarding this article should be sent.

(3) H. T. Huang and C. Niemann, THIS JOURNAL, **73**, 1541 (1951).

(4) D. W. Thomas, R. V. MacAllister and C. Niemann, *ibid.*, **73**, 1548 (1951).

(5) R. J. Foster and C. Niemann, *ibid.*, **73**, 1552 (1951).

(6) H. T. Huang, R. V. MacAllister, D. W. Thomas and C. Niemann, *ibid.*, **73**, 3231 (1951).

(7) H. J. Shine and C. Niemann, *ibid.*, **74**, 97 (1952).

(8) H. T. Huang, R. J. Foster and C. Niemann, *ibid.*, **74**, 105 (1952).

(9) S. Kaufman and H. Neurath, *Arch. Biochem.*, **21**, 245 (1949).

(10) S. Kaufman and H. Neurath, *J. Biol. Chem.*, **180**, 181 (1949).

(11) G. W. Schwert and S. Kaufman, *ibid.*, **180**, 517 (1949).

(12) S. Kaufman and H. Neurath, *ibid.*, **181**, 623 (1949).

(13) H. Neurath and J. A. Gladner, *ibid.*, **188**, 407 (1951).