tistics, The Institute of Paper Chemistry, for his counsel and assistance in the calculations. The aid of Dr. B. L. Browning, Dr. Paul F. Cundy, Mr. Laurence Bublitz, Miss Eleanor Grunwald, and Miss Marilyn Podest in making the analytical determinations is gratefully acknowledged. Permission of the Biological Laboratories of the Philadelphia Quartermaster Depot (Dr. Ralph G. H. Siu, Director) to publish is greatly appreciated.

Summary

1. A chemically reactive cellulose was prepared by regeneration from cellulose xanthate solution, replacing the water in the swollen structure successively with methanol, ethyl ether, and cyclohexane.
2. Two series of heterogeneous esterifications with tosyl chloride in pyridine were made on two such cellulose samples.
3. The products were treated with sodium iodide in acetonylacetone to replace the tosyl groups on primary alcoholic positions by iodine.
4. From the data, the reaction for both primary and secondary alcoholic groups of cellulose was proportional to the concentration of such groups, the rate constant for substitution on the primary position being 5.8 times that for the secondary position.

APPLETON, WIS. RECEIVED JULY 21, 1949

[A CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF ARKANSAS]

The Alkaline Hydrolysis of Ethyl Acetate in Acetone–Water Mixtures

BY EDWARD S. AMIS AND SAUL SIEGEL

Potts and Amis[1] studied the rate of the alkaline hydrolysis of ethyl acetate in ethyl alcohol–water mixtures. They found a normal effect for an ion–dipolar molecule reaction with respect to ionic strength effect, but with respect to the effect of dielectric constant of the media, the rate while changing in the direction expected was abnormally influenced by the dielectric constant of the media. It was hoped that a study of the reaction rate in a different medium might show whether this abnormal effect was specific to the ethyl alcohol–water solvents or whether the reaction was abnormally affected in the case of the different media by the dielectric constant. It was decided to choose a non-hydroxylic solvent and at the same time one which gave with water mixtures of known dielectric constants. Acetone was the solvent chosen and the following is a report of the results obtained.

Experimental

To obtain media of different dielectric constants, acetone and water were mixed in various proportions as given by the data of Akerlof.[2] The acetone was prepared according to the procedure of Akerlof, as follows. Acetone, J. T. Baker Analyzed, was treated in batches of about three liters with a small amount of 85% phosphoric acid and immediately distilled. Then it was dried with burnt powdered lime for a day, poured off and redistilled, rejecting all but the fraction which came over between 55.5 and 56.0°. The C. P. ethyl acetate, Eimer and Amend, is manufactured in a high state of purity and therefore, the reagent was simply dried over burnt lime for twenty-four hours and distilled; the fraction boiling between 75.9 and 76.0° was collected. The product was tested by checking a kinetic run whose reaction rate constant has been well established.

Freshly boiled distilled water was always used in preparing aqueous solutions. Carbonate-free sodium hydroxide stock solutions were prepared.[1] The concentrated solution was then diluted to approximately 0.2 *N* and stored in a paraffin-lined carboy. From this stock solution was prepared the 0.1000 and 0.0200 *N* solutions actually used in the runs.

The hydrochloric acid stock solution was prepared by diluting C. P. concentrated (37%) acid and titrating against sodium carbonate. Then, the 0.0200 *N* hydrochloric acid used in the titration was prepared as needed by dilution of the stock solution.

All volumetric apparatus and weights were calibrated for accuracy and the thermometer was calibrated against a Bureau of Standards thermometer.

The rate of ester hydrolysis was studied at 0.00, 15.87 and 26.10° and duplicate runs were performed in every case.

To test the ionic strength dependence, the dielectric constant was held constant at 75.25 and kinetic runs were made at the three temperatures mentioned above for μ-values of 0.02, 0.05, 0.10, 0.20 and 0.30. In the case of dielectric dependence μ was held at a value of 0.02 and dielectric constant varied as given in Table III.

The procedure in following the course of the reaction between ethyl acetate and sodium hydroxide was that described by Potts and Amis.[1] Samples were removed and titrated at suitable intervals during the first 80% of the reaction after which the flasks were kept at room temperature (approximately 25°) for about forty-eight hours. A final titration after two days determined the value of C_∞.

The reaction velocity constant, k', was calculated using the equation

$$k' = \frac{2.303}{tC_\infty} \log \frac{C_n(C_1 - C_\infty)}{C_1(C_n - C_\infty)} \qquad (1)$$

where

t = time elapsed in minutes
C_1 = NaOH concentration at t_1
C_n = NaOH concentration at t_n and
C_∞ = NaOH concentration at the completion of the reaction

The derivation of this equation is given by Reicher.[3] The time, t_0, of mixing was not observed since the actual concentration of reactants at an initial time, t_1, was determined after mixing by the same analytical means used

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(1) Potts, Dissertation, Louisiana State University, 1948; Potts and Amis, THIS JOURNAL, **71**, 2112 (1949).

(2) Akerlof, *ibid.*, **54**, 4125 (1932).

(3) Reicher, *Ann.*, **228**, 257 (1883).

in all subsequent analyses of samples. The equation given above does not require an extrapolation to zero time.

Data

In Table I are recorded the data for a representative run. The precision is given as the average deviation from the average.

TABLE I

REPRESENTATIVE CALCULATION OF VELOCITY CONSTANTS FROM EXPERIMENTAL DATA

Temperature = 0.00°; $D = 75.25$, 20% acetone, NaOH = 0.02 M, EtAc = 0.01 M initially

Time, min.	0.02 M HCl, ml.	0.02 M NaOH, ml.	C_n	k'
0	30	6.50	0.01880	..
15	25	4.55	.01637	1.32
30	25	6.53	.01478	1.33
45	25	7.92	.01368	1.35
60	25	8.72	.01301	1.32
∞	25	11.88	.01050	..

Average k' 1.33 ± 0.01

In Table II the data are given for the reaction velocity constant as a function of the ionic strength of the solution at 0.00, 15.87 and 26.10°.

TABLE II

EXPERIMENTAL VALUES OF VELOCITY CONSTANTS AS A FUNCTION OF IONIC STRENGTH; CORRESPONDING ENERGIES OF ACTIVATION AND FREQUENCY FACTORS

$D = 75.25$, NaOH = 0.02 N, EtAc = 0.01 M initially, ionic strength varied by adding $NaNO_3$ when necessary.

μ	k' 0.00° 20% acetone	k' 15.87° 11.60% acetone	k' 26.10° 5.60% acetone	ΔE	B
0.02	1.34	4.24	8.12	11,210	9.09
.05	1.29	4.14	8.05	11,390	9.22
.10	1.24	4.03	7.95	11,560	9.34
.20	1.16	3.89	7.81	11,870	9.55
.30	1.08	3.76	7.69	12,240	9.80

TABLE III

EXPERIMENTAL VALUES OF VELOCITY CONSTANTS AS A FUNCTION OF DIELECTRIC CONSTANT, CORRESPONDING ENERGIES OF ACTIVATION AND FREQUENCY FACTORS

$\mu = 0.02$; NaOH = 0.02 M; EtAc = 0.01 M initially

D	0.00° % Acetone	k'	15.87° % Acetone	k'	26.10° % Acetone	k'	ΔE 0.00–15.87°	B 0.00–15.87°	ΔE 15.87–26.10°	B 15.87–26.1°
75.25	20.00	1.34	11.60	4.24	5.60	8.12	11,360	9.22	10,950	8.90
69.82			20.00	3.78						
66.57	33.75	1.20	25.25	3.69	20.00	6.83	11,080	8.94	10,380	8.41
61.58	40.00	1.10								
57.21			40.00	3.15						
54.29	51.00	1.01	44.25	3.01	40.00	5.52	10,770	8.63	10,220	8.20
47.31	60.00	0.86								
45.06			60.00	2.39						
41.62					60.00	4.48				

The sodium hydroxide used was always 0.0200 N and the required ionic strength was obtained by adding sufficient sodium nitrate to the reaction mixture. The ethyl acetate was 0.01 molar initially and the dielectric constant of the medium was 75.25 in all these data. The values of the reaction velocity constant at each temperature decreases with ionic strength and the values of the energies of activation ΔE and of the Arrhenius frequency factor B increase with increasing ionic strength. Like trends were observed by Potts[1] for the same reaction in ethyl alcohol–water media. The conformity of the ionic strength dependence of the velocity constants with theory will be discussed in detail later. It should be mentioned that ΔE and B were calculated for the temperature range 0.00 to 26.10° using the equations

$$\Delta E = \frac{2.303\ RT_1T_2}{T_2 - T_1} \log \frac{k_2'}{k_1'}$$

and

$$B = \frac{T_2 \log k_2' - T_1 \log k_1'}{T_2 - T_1}$$

A plot of ΔE and of B *versus* ionic strength is given in Fig. 1. The curves are similar to comparable curves given by Potts for ethyl alcohol–water media. No theoretical attempt has yet been made to explain these trends in the case of ion–molecule reactions; however, the ionic strength dependence of ΔE and B for ion–ion reactions has been explained theoretically by LaMer and Kamner.[4]

The values of the reaction velocity constant k, the energy of activation ΔE, and the frequency factor B as functions of the dielectric constant of the media are recorded in Table III. Both ΔE and B were calculated for the temperature range 0.00 to 15.90° and 15.90 to 26.10°. The velocity constants decrease in magnitude as the dielectric constant D decreases and the same trend in general is observed in the case of both ΔE and of B. Again the discussion of the obedience of the dependence of the velocity constant of the reaction upon the dielectric constant of the media will be deferred until later. The decrease of the energy of activation with decreasing dielectric constant is to be expected from the theory of coulombic energy of activation.[5] The trend of the Arrhenius frequency factor with dielectric constant has not been explained theoreti-

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(4) LaMer and Kamner, THIS JOURNAL, **57**, 2662 (1935).

(5) Amis, *ibid.*, **63**, 1606 (1941).

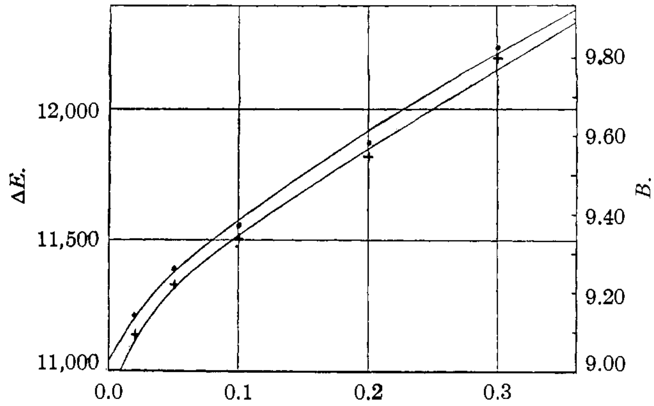

Fig. 1.—ΔE, ●; B, +.

cally in the case of ion–dipolar molecule reactions.

The ionic strength dependence of the reaction velocity constants was checked for obedience to the equation of Amis and Jaffe,[6] namely

$$\ln k' = \ln k'_{\kappa=0} - \frac{\epsilon z_B \cos \theta_0}{Dktr_0^2} (\mu_0^* - \mu e^{-\kappa r_0} (1 + \kappa r_0)) \quad (4)$$

by putting it in the form (5)

$$W = \frac{z^2}{1 + z + \frac{z^2}{2} + \frac{n^2}{2D(1 + z)}} \quad (5)$$

and plotting the theoretical curve of W *versus* z^2. A plot of the data log k' − log $k'_{\kappa=0}$ *versus* ionic strength, μ, at each temperature was fitted to the theoretical curve using the constants recorded in Table IV. The plot described is given in Fig. 2. In this figure the solid line is the theoretical curve and the symbols represent the data. The constants of Table IV are of about the magnitude expected from former applications of the equation. They are entirely comparable to those found by Potts in his study of the reaction in other media.

TABLE IV

CONSTANTS USED IN FITTING CURVES IN FIGURE 2

r_0 = 4.0 Å.

Temp., °C.	$10^{18}\mu_0^*$	n^2	$k'_{\kappa=0}$
0.00	12.79	12.21	1.38
15.9	8.20	7.11	4.27
26.1	3.96	2.40	8.17

The dielectric constant dependence of the reaction velocity constant was tested from the standpoint of equation (4) by transforming the equation into the expression

$$W' = \left(\frac{1}{D^2}\right) \frac{1}{1 + \zeta/D^{1/2} + \zeta^2/2D} \quad (7)$$

and plotting W' *versus* $1/D^2$. The data, log k' − log $k'_{\kappa=0}$ *versus* $1/D^2$, fitted the theoretical curve satisfactorily but the constants μ_0^* and n^2 used in fitting the data to the curve, while almost an order of magnitude less than those used by Potts for the same reaction in ethyl alcohol–water media, are still far too large. The value of r_0 used in this investigation was 4.0 Å. and that used by Potts was 7.0 Å., but this is reasonable since the degree of solvation of the complex in the two media may be entirely different. The large dielectric constant effect found by Potts for the alkaline hydrolysis of ethyl acetate in ethyl alcohol–water media persists to a lesser degree in acetone–water. These effects then are evidently solvent dependent and could conceivably be eliminated by proper choice of solvent. This would have to be tested by studying the reaction in other solvent media. Coulombic energy calculations[7] confirm the enhanced dielectric constant effect.

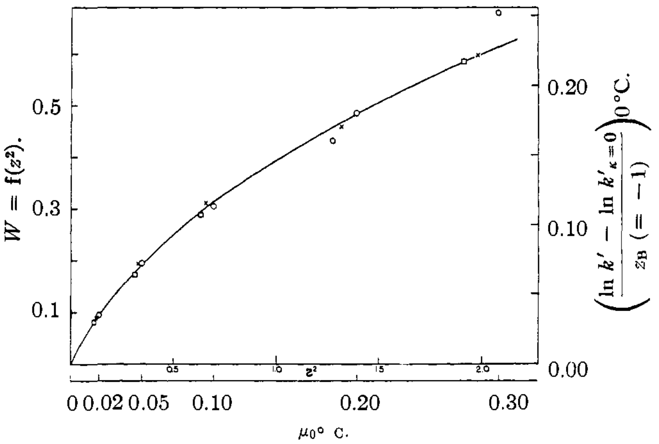

Fig. 2.—Agreement of ionic strength data with theoretical curve, $W = f(z^2)$. Data for 0.0° ○; 15.9° ×; 26.1° □. $\mu_{15.9°} = 1.058\mu_{0°}$. $\mu_{26.1°} = 1.094\mu_{0°}$. $(\ln k' - \ln k'_{\kappa=0}/z_B\ (= -1))_{15.9°} = 0.642(\ln k' - \ln k'_{\kappa=0}/z_B(= -1))_{0°}$. $(\ln k' - \ln k'_{\kappa=0}/z_B(= -1))_{26.1°} = 0.311(\ln k' - \ln k'_{\kappa=0}/z_B(= -1))_{0°}$.

Summary

The alkaline hydrolysis of ethyl acetate was studied kinetically at 0.00, 15.87 and 19.10° both in pure water and in acetone–water mixtures, in order to try to determine whether the enormous effect on the rate of hydrolysis of the dielectric constant of the solvent observed in alcohol–water mixtures was peculiar to the reaction or was partially or wholly dependent upon the solvent. The rate of reaction was studied at a constant dielectric constant of 75.25 in which the ionic strength was 0.02, 0.05, 0.10, 0.20 and 0.30 at all three temperatures. The reaction velocity constant was studied at a constant ionic strength of 0.02 at various dielectric constants including the three

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[6] Amis and Jaffe, *J. Chem. Phys.*, **10**, 598 (1942).

[7] Amis and Holmes, THIS JOURNAL, **63**, 2231 (1941).

constant dielectric constants 75.25, 66.57 and 54.29 at all three temperatures.

The equation proposed by Amis and Jaffe accounted for the ionic strength data, but revealed a dielectric constant effect which, while in the right direction, was magnified by an order of magnitude. Coulombic energy calculations confirmed the enhanced dielectric constant effect.

These enhanced dielectric constant effects upon the rate in acetone–water media were less by an order of magnitude than has been observed when ethyl alcohol–water was used as a solvent. Thus part of the enhancement is solvent dependent.

FAYETTEVILLE, ARKANSAS RECEIVED MARCH 22, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY, PURDUE UNIVERSITY]

Chromatographic Separation of Sugars on Charcoal[1]

BY ROY L. WHISTLER AND DONALD F. DURSO

Charcoal has been used industrially for the purification of sugars for many years. In 1932, Hyashi[2] reported that charcoal, stirred in an aqueous acetone–acetic acid solution of glucose and sucrose, completely adsorbed the sucrose but left the glucose in solution. Tiselius[3] proposed that charcoal might be employed in chromatographic fashion for the separation of glucose and lactose. Later,[4] he employed charcoal and 0.5% aqueous phenol solution to effect the separation of glucose from sucrose, and sucrose from maltose by the displacement development technique in which the components are added to the column and then are washed in succession into the effluent without an interval wherein only pure developer is obtained. It was also demonstrated[5] that a mixture of glucose, sucrose, raffinose and stachyose could be separated, using 0.5% aqueous ephedrin as the displacing agent. The method of Tiselius[4] has been used by Claesson[6] to resolve a mixture of glucose, sucrose and raffinose with 4% aqueous phenol; and by Montgomery, Weakley and Hilbert[7] to isolate 6-[α-D-glucopyranosyl]-D-glucose from an enzymic hydrolyzate of starch.

The Tiselius technique has been extended and modified in this Laboratory for use in the fractionation of acid hydrolyzates of guaran and xylan. These separations will be reported later. However, it may be of value to present here the pertinent facts regarding aqueous ethanol as the displacing agent, because its use leads to improved workability of the charcoal technique for the separation of sugars. Preliminary orientation experiments with known sugars indicated that the desorption characteristics of mono-, di- and trisaccharides are so different that they might be easily separated as sugar classes. The effectiveness of the separation is not affected by small variations in the composition of the developer, by the degree of dilution of the sugar solution, or by the presence of inorganic salts. An added advantage is that a mixture of substantially large quantities of sugars can be separated by the use of a single small column.

Experimental

General Procedure.—The adsorbent used was a mixture of equal parts by weight of Darco G-60[8] and Celite[9] which had been washed with water and dried. This material was packed into a glass chromatographic tube 230 mm. × 34 mm. diameter, giving a column 170 mm. in length. Before the addition of the sugar solution, the column was wet with 150 ml. of water. In studying the desorption characteristics of the individual sugars, 1 g. in the form of a 10% solution was used, although later work showed that more dilute solutions could be employed with no change in the results.

To effect the displacement of the adsorbed sugars, water and more powerful developers were used in succession. The effluent was collected in 100 ml. fractions. The course of the desorption process was followed polarimetrically, using a 2-dm. tube, to indicate the complete removal of each component before addition of the succeeding developer.

Desorption of Monosaccharides.—The method given above was used to study the desorption characteristics of glucose, galactose, mannose, xylose, arabinose, fructose and rhamnose. All of the monosaccharides are displaced at approximately the same rate by water. In all cases, complete removal of the sugar was effected by washing the column with 800 ml. of water. The rates of desorption of four of these sugars are shown in Fig. 1.

Methanol, ethanol, acetic acid and acetone, each in concentrations of 15, 30 and 70% in water, were used to study the effect of other agents upon the ease of displacement of glucose, galactose and mannose. Two hundred ml. of each of these developers, regardless of nature or concentration, caused complete removal of sugar. The increased rates of desorption of glucose with two of these agents are shown in Fig. 1.

Desorption of Disaccharides.—The displacement of maltose by various agents was studied to obtain representative data regarding this class of sugars. Maltose was not removed by 2 l. of water. It was completely desorbed by 900 ml. of 5% ethanol, 400 ml. of 15% ethanol, 800 ml. of 5% citric acid and 1.7 l. of 0.2% phenol. 1.7 liters of 3% hydrochloric acid and 1.1 l. of 7.5% hydrochloric acid gave only 30% recovery of maltose. With 5% ethanol, the displacement of melibiose, lactose, sucrose and

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[1] Journal Paper No. 404 of the Purdue University Agricultural Experiment Station, Lafayette, Indiana.

[2] F. Hyashi, *J. Biochem.* (*Japan*), **16**, 1 (1932); *C. A.*, **27**, 8 (1933).

[3] A. Tiselius, *Arkiv. Kemi, Mineral Geol.*, **14B**, No. 32, 8 pp. (1941).

[4] A. Tiselius, *Kolloid Z.*, **105**, 101 (1943).

[5] A. Tiselius and L. Hahn, *ibid.*, **105**, 177 (1943).

[6] S. Claesson, *Arkiv. Kemi, Mineral Geol.*, **24A**, No. 16, 9 pp. (1947).

[7] Edna M. Montgomery, F. B. Weakley and G. E. Hilbert, THIS JOURNAL, **71**, 1682 (1949).

[8] A product of Darco Corp., New York, N. Y.

[9] No. 535, a product of Johns-Manville Co., New York, N. Y.