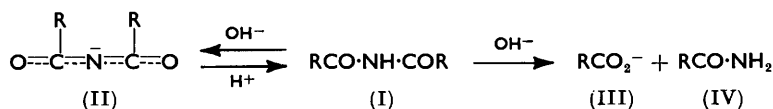


698. *The Hydrolysis of Amides and Related Compounds. Part IV.\* Diacetylamine and Succinimide in Aqueous Alkali.*

By J. T. EDWARD and K. A. TERRY.

The rates of hydrolysis of diacetylamine and succinimide in alkaline solution are proportional to the fraction of the imide ionized, and not to the concentration of hydroxide ion. These results are explicable by a mechanism in which the rate-determining step is the reaction of a hydroxide ion with an un-ionized molecule of imide. Some misinterpretations in the literature are indicated.

THE alkaline hydrolysis of the imide grouping (AH; I) is of interest in connection with recent procedures for peptide degradation.<sup>1</sup> While kinetic studies have been made of the hydrolysis ( $I \longrightarrow III + IV$ ) of some linear<sup>2</sup> and cyclic imides,<sup>3,4</sup> no attention has been paid to the effect of ionization:  $AH \longrightarrow A^- (II) + H^+$ , on the rate of hydrolysis. From analogy with the alkaline cleavage of several other ionizable compounds,<sup>5,6</sup> the ionized imide (II) should be resistant to alkaline hydrolysis, and this should affect the kinetics of the reaction.



Accordingly, we have measured the ionization constants and rates of hydrolysis of diacetylamine (I; R = Me) and succinimide (I; RR = [CH<sub>2</sub>]<sub>2</sub>), by spectrophotometric methods. Diacetylamine<sup>7</sup> and succinimide<sup>8</sup> show weak absorption in neutral solution, but greatly enhanced absorption in alkali,<sup>9</sup> with  $\lambda_{\text{max}}$  at 240 m $\mu$  and at <222 m $\mu$ , respectively. Since solutions of both materials at low concentrations obey Beer's law,<sup>7,8</sup> we have used the variation in absorption with pH to determine the ionization constants of the compounds,<sup>10</sup> and the progressive decrease in absorption on storage to follow their hydrolysis.

#### EXPERIMENTAL

*Materials and Apparatus.*—Diacetylamine,<sup>11</sup> acetamide, succinimide, and succinamic acid<sup>12</sup> were recrystallized to constant m. p. Aqueous sodium hydroxide of different concentrations was used for obtaining the pH range 11.5–14; and phosphate or borate buffer solutions for lower pH's. The pH values of all solutions were determined potentiometrically, a Radiometer pH meter 23 being used, with glass and calomel electrodes, before and after hydrolysis; since in most cases the ionic strength of the solution was small ( $\sim 0.02M$ ), it may be assumed that  $\text{pH} \approx -\log[\text{H}^+] \approx -\log K_w/[\text{OH}^-]$ ,  $K_w$  being the ionic product of water.<sup>10</sup> Absorption measurements were made with a Beckman DU spectrophotometer on solutions in 1-cm. quartz cells, kept at  $25.00^\circ \pm 0.02^\circ$  by circulating water in the cell carrier.<sup>13</sup>

*Rate Measurements.*—(a) *Diacetylamine.* Aqueous solutions of diacetylamine ( $10^{-4}$ – $5 \times 10^{-3}M$ )

\* Part III, *J.*, 1957, 2009.

<sup>1</sup> Battersby and Robinson, *J.*, 1955, 259.

<sup>2</sup> Titherley and Stubbs, *J.*, 1914, **105**, 299; Desai and Desai, *J. Indian Chem. Soc.*, 1951, **28**, 99.

<sup>3</sup> Ingold, Sako, and Thorpe, *J.*, 1922, **121**, 1177.

<sup>4</sup> Sircar, *J.*, 1927, 600, 1252.

<sup>5</sup> Goldschmidt and Oslan, *Ber.*, 1899, **32**, 3390; 1900, **33**, 1140; Hauser, Swamer, and Ringler, *J. Amer. Chem. Soc.*, 1948, **70**, 4023; Gustafsson and Johanson, *Acta Chem. Scand.*, 1948, **2**, 42.

<sup>6</sup> Pearson and Mayerle, *J. Amer. Chem. Soc.*, 1951, **73**, 926; Pearson and Sandy, *ibid.*, p. 931.

<sup>7</sup> Polya and Spotswood, *Rec. Trav. chim.*, 1949, **68**, 573.

<sup>8</sup> Saidel, *Nature*, 1953, **172**, 955.

<sup>9</sup> Cf. Stuckey, *J.*, 1947, 331.

<sup>10</sup> Gold, "pH Measurements," Methuen, London, 1956, p. 90.

<sup>11</sup> Dehn, *J. Amer. Chem. Soc.*, 1912, **34**, 1399.

<sup>12</sup> Geoffrey and Vogel, *J.*, 1934, 1103.

<sup>13</sup> Campbell and Simpson, *Chem. and Ind.*, 1953, 887.

and of sodium hydroxide or buffer solution (2.00 ml.), each at  $25.00^\circ \pm 0.02^\circ$ , were mixed in a quartz cell, and the absorption at 240  $m\mu$  measured as soon as possible, and at intervals thereafter; from these values the molar extinction coefficients  $\epsilon_t$  at different times  $t$  were calculated. In strongly alkaline solution the absorption quickly approached zero, as would be expected for the reaction (I; R = Me)  $\longrightarrow$  (III; R = Me) + (IV; R = Me), since an aqueous solution of acetamide and sodium acetate has a total extinction  $\epsilon = 5$  at 240  $m\mu$ . Plots of  $\log \epsilon_t$  against  $t$  gave straight-line curves (e.g., Fig. 1), from the slopes of which the apparent first-order rate constants  $k_e$  for the hydrolysis of the imide were calculated (see Table). From the graphical data of Titherley and Stubbs,<sup>2</sup> diacetylamine has a half-life of about 25 sec., or an apparent first-order rate constant  $k_e \approx 0.028 \text{ sec.}^{-1}$ , in 2 equivalents of 0.1N-sodium hydroxide at room temperature, i.e., in a solution of pH 12.9 changing to pH 12.7 during the reaction (since for diacetylamine  $pK'_a = 12.9$ : see below). This is in rough agreement  $\pm$  with our values for this pH range.

(b) *Succinimide*. Hydrolysis was followed by the change in  $\epsilon_t$  of 0.01–0.001M-solutions at 235  $m\mu$ , the molar extinction coefficient (= 35) of succinamic acid being taken as  $\epsilon_\infty$ . The hydrolysis of succinamic acid in alkaline solution was found to lead to a further drop in absorption,

FIG. 1. Hydrolysis of diacetylamine at pH 11.94 (curve a) and 13.52 (curve b).

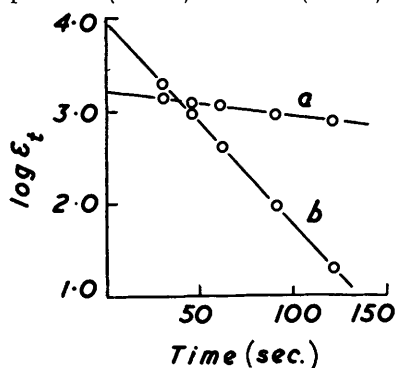
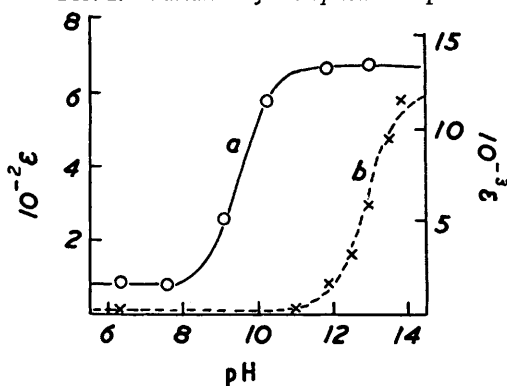


FIG. 2. Variation of absorption with pH.



	Theory	Exptl.
Left-hand scale: succinimide at 235 $m\mu$ ...	a	o
Right-hand scale: diacetylamine at 240 $m\mu$ ...	b	x

but was so slow in comparison with the hydrolysis of succinimide that it could be ignored. Plots of  $\log(\epsilon_t - \epsilon_\infty)$  against time gave satisfactory straight-line curves, from which values of  $k_e$  (see Table) were obtained. Sircar<sup>4</sup> followed by titration the hydrolysis of succinimide by hydroxide ion (both initially 0.005M) but failed to take account of the neutralization reaction. The pH of this solution should change from about 10.6 to 10.2 in going from 0 to 80% hydrolysis, and  $k_e$  should drop from 93 to 83% of the maximum value (see below), attained in more alkaline solutions. A replotting of Sircar's results gives  $k_e = 3.73 \times 10^{-5} \text{ sec.}^{-1}$ , in poor agreement with our results for this pH range.

*Dissociation Constants*.—The values of the extinction coefficients  $\epsilon$  of the imides in solutions of different pH, before any hydrolysis, were obtained from the intercepts of their rate curves (e.g., Fig. 1) with the abscissæ, and are given as points in Fig. 2. It is evident that the points fit reasonably well the theoretical curves for the equation<sup>10</sup>

$$\text{pH} = \text{p}K'_a + \log(\epsilon - \epsilon_u)/(\epsilon_i - \epsilon)$$

where  $\epsilon_u$  is the molar extinction coefficient of the un-ionized imide,  $\epsilon_i$  of the ionized imide,  $\epsilon$  of a mixture of un-ionized and ionized molecules at the indicated pH, and  $\text{p}K'_a$  is defined by  $\text{p}K'_a = \text{pH} - \log\{[A^-]/[AH]\}$ . In plotting the theoretical curves,  $\text{p}K'_a$ 's of 9.5 and 12.9 have been assumed for succinimide and diacetylamine, and  $\epsilon_i = 12,100$  at 240  $m\mu$  for diacetylamine. Thermodynamic-dissociation index values  $\text{p}K_a [= \text{pH} - \log\{[A^-]/[AH]\}]$ , the quantities in parentheses being activities] of 9.560 and 9.656 have been reported<sup>14, 15</sup> for succinimide.

<sup>14</sup> Simms, *J. Phys. Chem.*, 1928, **32**, 1121.

<sup>15</sup> Schwarzenbach and Lutz, *Helv. Chim. Acta*, 1940, **23**, 1162.

## RESULTS AND DISCUSSION

The apparent first-order rate constant  $k_e$  for the hydrolysis of a simple amide in an excess of alkali is proportional to the hydroxide-ion concentration.<sup>16</sup> However, the rate constants for the hydrolysis of diacetylamine and succinimide are not proportional to the concentration of hydroxide ion, but, within the limits of experimental error, to the fraction  $\alpha$  of imide ionized, where  $\alpha = [A^-]/([AH] + [A^-]) = K'_a/([H^+] + K'_a)$  (see Table).

First-order rate constants ( $k_e$ ) for hydrolysis at different pH's.

Diacetylamine					
pH .....	11.94	12.52	12.95	13.52	13.83
$10^2 k_e$ (sec. <sup>-1</sup> ) .....	0.64	2.03	4.10	5.07	7.50
$10^2 k_e/\alpha^a$ .....	6.0	6.9	7.7	6.3	8.4
$k_e/[OH^-]$ .....	0.68	0.61	0.46	0.15	0.11
Succinimide					
pH .....	9.11	10.24	11.90	13.00	
$10^4 k_e$ (sec. <sup>-1</sup> ) .....	0.31	0.81	1.02	0.95	
$10^4 k_e/\alpha^b$ .....	1.09	0.96	1.02	0.95	
$k_e/[OH^-]$ .....	2.4	0.47	0.013	0.001	

<sup>a</sup> Assuming  $pK'_a = 12.9$ . <sup>b</sup> Assuming  $pK'_a = 9.5$ .

The kinetic results are consistent with the assumption that the rate-determining step in the hydrolysis is the attack of the un-ionized imide by a hydroxide ion. The rate of hydrolysis  $v$  will then be given by

$$v = k_2[AH][OH^-] = k_2K_w[A^-]/K'_a \quad (1)$$

where  $k_2$  is a second-order rate constant. Since  $k_e$  is based on the change in stoichiometric concentration of imide, ionized and unionized,

$$v = k_e([A^-] + [AH])$$

$$\text{or } k_e = k_2K_w[A^-]/K'_a([A^-] + [AH]) \quad (2a)$$

$$= k_2K_w\alpha/K'_a \quad (2b)$$

On the basis of this mechanism, succinimide is more stable than diacetylamine to alkaline hydrolysis chiefly because of its greater acidity. Thus it is hydrolysed 710 times more slowly than diacetylamine at pH 11.9; at this pH it is 99.6% ionized to the unreactive form, while diacetylamine is only 12.1% ionized. The stabilities, if given in terms of the  $k_2$  values in equations (1) and (2) rather than the  $k_e$  values, are of the same order of magnitude. Similar considerations apply to the alkaline hydrolysis of glutarimide, reported by Sircar<sup>4</sup> to be more rapid than that of succinimide, and explained on theoretical grounds.<sup>17</sup> In fact, analysis of Sircar's data in terms of equation (2), the reported<sup>15</sup> dissociation constant ( $pK_a = 11.43$ ) of glutarimide being used, indicates the un-ionized molecule to be hydrolyzed about five times more slowly than the un-ionized molecule of succinimide, although the drift in pH during the hydrolysis makes the calculation only very approximate. Other kinetic work<sup>2,3</sup> on imide hydrolysis requires similar revision.

The differences in the acid dissociation constants of the imides are probably the result of conformational differences.<sup>18</sup> Thus with diacetylamine, the planar amide linkage<sup>19</sup> restricts the conformations of the molecule to (Va), (Vb), and (Vc). The first conformation, in which diacetylamine would have two *cis* amide linkages like succinimide, is

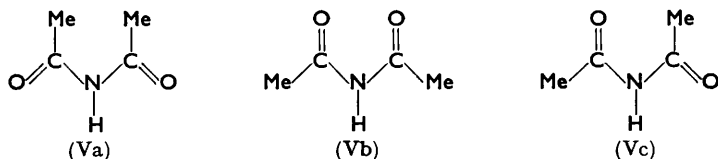
<sup>16</sup> Reid, *Amer. Chem. J.*, 1899, **21**, 284; 1900, **24**, 397; Meloche and Laidler, *J. Amer. Chem. Soc.*, 1951, **73**, 1712.

<sup>17</sup> Brown, Brewster, and Schechter, *J. Amer. Chem. Soc.*, 1954, **76**, 467.

<sup>18</sup> Cf. Hesse and Krehbiel, *Annalen*, 1955, **593**, 35.

<sup>19</sup> Pauling, Corey, and Branson, *Proc. Nat. Acad. Sci.*, 1951, **37**, 205.

improbable because of steric repulsions between the two methyl groups.<sup>20, 21</sup> These repulsions would be at a minimum in the extended form (Vb), which seems most probable. However, the formation of the anion (II; R = Me) corresponding to conformation (Vb) would create large electrostatic repulsions between the neighbouring carbonyl oxygens,<sup>20</sup> on which much of the negative charge would be localized, and hence formation of this ion would be expected to take place less readily than the formation of the ion (II; R = [CH<sub>2</sub>]<sub>2</sub>) from succinimide. The weak acidity of glutarimide<sup>15</sup> may be caused by a lack of planarity in one of the amide linkages due to ring buckling; this will hinder the charge delocalization necessary in forming the ion (II; RR = [CH<sub>2</sub>]<sub>2</sub>).



In solutions 2 or more pH units above  $\text{pH} = \text{p}K'_a$ ,  $\alpha$  approaches unity, and  $k_e$  approaches a maximum value. Hence the most selective fission of a succinimide ring in the presence of peptide linkages (*e.g.*, ref. 1) will be achieved at pH's below about 9.

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<sup>20</sup> Calvin and Woods, *J. Amer. Chem. Soc.*, 1940, **62**, 3152.

<sup>21</sup> Forbes and Mueller, *Canad. J. Chem.*, 1955, **33**, 1145.