# Hydrolysis of Some *N*-Alkylmaleimides

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The kinetics of the hydrolysis of maleimide (MI), *N*-methylmaleimide (MMI), *N*-ethylmaleimide (EMI), and *N*-hydroxymethylmaleimide (HMMI) were studied spectrophotometrically in pH-controlled buffer solution over the temperatue range 10—50 °C. The rate of hydrolysis is proportional to both the concentration of *N*-alkylmaleimide and that of hydroxide ion in the pH region between 7 and 9, and is independent of pH below pH 4. The catalytic rate constants for alkaline hydrolysis increase in the order: HMMI > MI > MMI > EMI. The entropies of activation for the hydrolysis at pH 7—9 are large and negative ranging from $-132$ to $-162$ J $mol^{-1}$ $K^{-1}$ and both the enthalpies and the entropies of activation increase in the order: EMI > MMI > MI > HMMI. The effect of substituent on nitrogen on the rate of hydrolysis is explained satisfactorily by Taft's $\rho^*\sigma^*$ relationship, with a value of $\rho^*$ of 0.05 at 30 °C. The experimental results can be explained by a bimolecular mechanism, in which nucleophilic attack by hydroxide ion on *N*-alkylmaleimide is the rate-determining step. The hydrolysis products are *N*-alkylmaleamic acids.

*N*-ALKYLMALEIMIDES have recently found considerable use in the determination [1,2] and modification [3,4] of mercapto groups in proteins. The reactivity of *N*-alkylmaleimides with mercapto compounds in aqueous solution has been studied by many investigators,[5-7] and is known to be influenced by hydrolysis. Gregory [8] has reported that slow hydrolysis of *N*-ethylmaleimide occurs in basic medium. However, no kinetic studies on the hydrolysis of *N*-alkylmaleimides seem to have been reported, while a number of papers on the alkaline hydrolysis of succinimide have been published. Khan [9] found that the hydrolysis of succinimide in highly alkaline medium follows an irreversible first-order consecutive reaction path of the type $A \longrightarrow B \longrightarrow C$. Tirouflet [10] showed that the pseudo-first-order rate constant for hydrolysis of succinimide is proportional to the concentration of hydroxide ion below pH 9 and independent of the concentration of hydroxide ion above pH 10. Edward [11] reported that the rate-determining step is the reaction of hydroxide ion with un-ionized succinimide. Dahlgren [12] and Kirby [13] found that the rate of the acidic hydrolysis of *N*-alkylmaleamic acids decreases with increase in pH.

As the chemical structure of *N*-alkylmaleimide, which contains the carbonyl group conjugated with a carbon–carbon double bond, is different from that of succinimide, the hydrolysis of *N*-alkylmaleimide is expected to be different from that of succinimide. In the present work, we have investigated the kinetics of the hydrolysis of maleimide (MI), *N*-methylmaleimide (MMI), *N*-ethylmaleimide (EMI), and *N*-hydroxymethylmaleimide (HMMI) to clarify the effect of nitrogen substituents on the stabilities of *N*-alkylmaleimides in aqueous solution.

## EXPERIMENTAL

*Materials.*—MI and HMMI were prepared from maleic anhydride by the method of Tawney *et al.*[14,15] and purified by recrystallization from ethyl acetate. MMI and EMI were prepared from maleic anhydride by the method of

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[1] J. Leslie, D. L. Williams, and G. Gorin, *Analyt. Biochem.*, 1962, **3**, 257.

[2] J. Leslie, *Analyt. Biochem.*, 1965, **10**, 162.

[3] G. Guidotti and W. Konigsberg, *J. Biol. Chem.*, 1964, **239**, 1474.

[4] T. Sekine and W. W. Kielley, *Biochem. Biophys. Acta*, 1964, **81**, 336.

[5] C. C. Lee and E. R. Samuels, *Canad. J. Chem.*, 1964, **42**, 168.

[6] N. M. Alexander, *Analyt. Chem.*, 1958, **30**, 1292.

[7] E. Roberts and G. Rouser, *Analyt. Chem.*, 1958, **30**, 1291.

[8] J. D. Gregory, *J. Amer. Chem. Soc.*, 1955, **77**, 3922.

[9] M. Niyaz Khan and A. Aziz Khan, *J. Org. Chem.*, 1975, **40**, 1793.

[10] J. Tirouflet and E. Trouit, *Compt. rend.*, 1955, **241**, 1053.

[11] J. T. Edward and K. A. Terry, *J. Chem. Soc.*, 1957, 3527.

[12] G. Dahlgren and N. L. Simmerman, *J. Phys. Chem.*, 1965, **69**, 3926.

[13] A. J. Kirby and P. W. Lancaster, *J. Chem. Soc. (B)*, 1971, 1206.

[14] P. O. Tawney, R. H. Snyder, and C. H. Stiteler, *J. Org. Chem.*, 1960, **25**, 56.

[15] P. O. Tawney, R. H. Snyder, and A. R. Williams, *J. Org. Chem.*, 1961, **26**, 15.

Mehta *et al.*[16] MMI was purified by recrystallization from ethyl ether, and EMI by sublimation under reduced pressure. The properties of *N*-substituted maleimides are given in Table 1.

*Kinetic Methods.*—All reactions were run in buffer solutions controlled at pH 2.08—9.26 ± 0.05, and kept at 10—50 ± 0.5 °C. The reaction was started by adding aqueous *N*-alkylmaleimide (10 cm$^3$, $10^{-2}$ and $0.5 \times 10^{-3}$ mol dm$^{-3}$) to buffer solution (90 cm$^3$). The final concentration of *N*-alkylmaleimide was $10^{-3}$ and $0.5 \times 10^{-4}$ mol dm$^{-3}$. The reaction time was counted from the moment of liquid–liquid contact. The time required for complete mixing was negligible when compared with the reaction time. At appropriate intervals, a portion of the solution was removed, and the rate of hydrolysis was measured spectrophotometrically by following the disappearance of the absorption maxima I (MI, 277; MMI, 300; EMI, 300; HMMI, 283 nm) and II (MI, 217; MMI, 219; EMI, 219; HMMI, 218 nm). Potassium chloride–hydrogen chloride was used as buffer for pH 2—4, potassium dihydrogenphosphate–sodium hydroxide for pH 6—7, and boric acid–sodium hydroxide for pH 8—9. The pH was measured by use of Hitachi–Horiba F-7 pH meter. The pH of the initial and final solution differed by no more than 0.1 pH unit even in extreme cases. Distilled water was prepared by use of Toyo-AQUEOUS GS-15R. The ionic strength was maintained at 0.5 with added sodium chloride.

*Dissociation Constants.*—The dissociation constants and the degree of ionization were determined by use of the spectrophotometric method of Edward.[11]

*Alkalimetry and Hydrolysis Products.*—*N*-Alkylmaleimide corresponding to a concentration of $10^{-3}$ mol dm$^{-3}$ was dissolved in distilled water (100 cm$^3$) and alkalimetry with carbonate-free sodium hydroxide was carried out by means of a pH meter. The pH was measured after remaining constant for 30 min. When the pH values were plotted against the volume of alkali, a clear inflection point appeared at pH *ca.* 8.5 for all *N*-alkylmaleimides examined. The colour of the solution including phenolphthalein as an indicator changed from colourless to red at pH 8.5, which is the equivalent point. Below pH 10, the colour of the solution remained reddish after standing for 30 min.

Hydrochloric acid equivalent to sodium hydroxide required for neutralization was added to the solution titrated with alkali, and the solution was evaporated to dryness at 50 °C under reduced pressure for 7 days. The acetone-soluble fraction of the residue was twice recrystallized from ethanol.

*Degree of Hydrolysis.*—The absorbance of bands I and II decreased with time and the absorption maxima shifted to shorter wavelength with time. Isosbestic points appeared regardless of pH. The u.v. spectrum at infinite time was identical with that of authentic *N*-alkylmaleamic acid prepared by the method of Gardner.[17] As the system consists of *N*-alkylmaleimide and *N*-alkylmaleamic acid, the degree of hydrolysis was determined from the absorbance of bands I and II by use of a calibration curve.

*Analytical Procedure.*—I.r. spectra were measured for KBr pellets by a JASCO-IR-E spectrophotometer, n.m.r. spectra by a JEOL-MH-100 spectrometer, and u.v. spectra by use of a Hitachi-124 double beam spectrophotometer.

## RESULTS AND DISCUSSION

*Dissociation Constants.*—Edward[11] reported that the ionized succinimide is resistant to alkaline hydrolysis and affects the reaction kinetics. Since MI and HMMI are also considered to ionize in alkaline medium, the degree of ionization was determined. The percentages of ionization of MI and HMMI obtained are 0.006 at pH 5.94, 0.09 at pH 7.16, 0.83 at pH 8.12, 6.61 at pH 9.02, and 11.48 at pH 9.26. Thus it is reasonable to assume that MI and HMMI scarcely ionize below pH 8. On the other hand, the ionization constants of MMI and EMI were not obtained. It seems that MMI and EMI do not ionize easily in alkaline medium.

*Hydrolysis Products.*—The acid values obtained from alkalimetry are in good agreement with those calculated from *N*-alkylmaleamic acids. The i.r. spectra of the isolated hydrolysis products show the amide I (1 680—1 690 cm$^{-1}$) and amide II bands (1 520—1 620 cm$^{-1}$). The n.m.r. spectra of the hydrolysis products show two doublets characteristic of an AB system at δ 5.88—6.40 and 6.34—6.62. These signals are in good agreement with those for authentic *N*-alkylmaleamic acids. These data are further supported by elemental analysis. On the other hand, amines such as ammonia, and *N*-methyl-, *N*-ethyl-, and *N*-hydroxymethyl-amine were not detected by Nessler's reagent[18] for hydrolysis at 30 °C below pH 9.26. These results indicate that the hydrolysis products are *N*-alkylmaleamic acids, and that *N*-alkylmaleamic acids are scarcely hydrolysed below 50 °C and pH 9.26.

TABLE 1

Properties of *N*-substituted maleimides

| | | Elemental analysis | | | U.v. spectra | | | |
|---|---|---|---|---|---|---|---|---|
| Compound | M.p. (°C) | C(%) | H(%) | N(%) | $\lambda_{max.,1}$/nm | $\varepsilon_1$ [b] | $\lambda_{max.,2}$/nm | $\varepsilon_2$ [b] |
| MI | 94.0 | 49.2 | 3.1 | 14.5 | 277 | 603 | 217 | 16 500 |
| | (lit.,[16] 92.0—94.0) | 49.5 [a] | 3.1 [a] | 14.4 [a] | | | | |
| MMI | 94.0 | 54.3 | 4.6 | 12.65 | 300 | 602 | 219 | 16 160 |
| | (lit.,[18] 94.0—95.0) | 54.5 [a] | 4.55 [a] | 12.6 [a] | | | | |
| EMI | 46.0 | 57.5 | 5.7 | 11.3 | 300 | 604 | 219 | 14 600 |
| | (lit.,[18] 46.0) | 57.6 [a] | 5.6 [a] | 11.2 [a] | | | | |
| HMMI | 98.0 | 47.05 | 3.95 | 11.0 | 283 | 500 | 218 | 15 040 |
| | (lit.,[17] 99.0) | 47.25 [a] | 3.95 [a] | 11.0 [a] | | | | |

[a] Calc. [b] dm$^3$ mol$^{-1}$ cm$^{-1}$.

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[16] N. B. Mehta, A. P. Phillips, and R. E. Brooks, *J. Org. Chem.*, 1960, **25**, 1012.

[17] F. Gardner, 'Maleic Anhydride Derivatives,' Wiley, New York, 1952, p. 105.

[18] D. F. Boltz, 'Colorimetric Determination of Nonmetals,' Interscience, New York, 1958, p. 91.

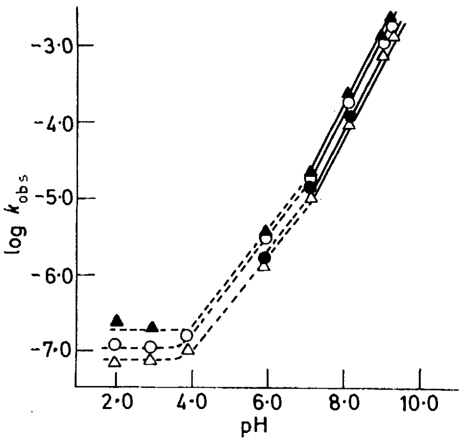


FIGURE 1 Rate–pH profile for hydrolysis at 30 °C and ionic strength 0.5: ○, MI; ●, MMI; △, EMI; ▲, HMMI

*Rate of Hydrolysis.*—Since both the concentrations of hydroxide ion and that of hydrogen ion are constant during the reaction in the pH-controlled solution, it is reasonable to assume that the reaction follows the pseudo-first-order equation (1). $k_0$, $k_{H^+}$, and $k_{OH^-}$ are

$$-d[N\text{-alkylmaleimide}]/dt = k_{obs.}[N\text{-alkylmaleimide}] \quad (1)$$

$$k_{obs.} = k_0[H_2O] + k_{H^+}[H^+] + k_{OH^-}[OH^-] \quad (2)$$

the specific rate constants for spontaneous, acidic, and alkaline hydrolysis, respectively, and $[H^+]$ and $[OH^-]$ are the initial concentrations of hydrogen ion and hydroxide ion, respectively. The rate constant ($k_{obs.}$) was calculated from the integrated form of equation (1), which is satisfied for the hydrolysis of *N*-alkylmaleimides. As the values of $k_{obs.}$ obtained for band I are nearly equal to those obtained for band II, it is suggested that the variation of band I assigned to the $n \longrightarrow \pi^*$ transition of the carbonyl group is closely related to that of band II assigned to the $\pi \longrightarrow \pi^*$ transition of the carbon–nitrogen double bond. The values of $k_0$ were calculated from equation (3), for which $k_{obs.}$ was obtained from hydrolysis in distilled water free from acid and base.

$$k_0 = k_{obs.}/[H_2O] = k_{obs.}/55.5 \quad (3)$$

Since the contribution of $k_0$ and $k_{H^+}$ to $k_{obs.}$ is small in the pH region 7.02—9.26, equation (2) approximates to (4), where $K_w$ is the ionic product of water. The rate–pH

$$\log k_{obs.} = \log k_{OH^-}[OH^-] = \log k_{OH^-}K_w + pH \quad (4)$$

profile (Figure 1) shows that equation (4) is satisfied for alkaline hydrolysis of the *N*-alkylmaleimides over the temperature range 10—50 °C. Deviations from equation (4) in the case of MI and HMMI above pH 9 seem to be caused by the ionization described previously. The catalytic rate constants for alkaline hydrolysis ($k_{OH^-}$) given in Table 2 increase in the order: MHMI > MI >MMI > EMI.

Below pH 7 equation (2) approximates to (5). The curves in Figure 1 suggest that the contribution of $k_0$ to $k_{obs.}$ increases with lowering the pH. Below pH 4, the values of $k_{obs.}$ depend only on $k_0$. These results are in

$$k_{obs.} = k_0[H_2O] + k_{H^+}[H^+] \quad (5)$$

agreement with hydroxide ion catalysing the hydrolysis of the *N*-alkylmaleimides, whereas hydrogen ion does not.

TABLE 2

Rate constants, $k_{OH^-}$/dm$^3$ mol$^{-1}$ s$^{-1}$, for hydrolysis at ionic strength 0.5

| | $t$/°C | | | | |
|---|---|---|---|---|---|
| Imide | 10 | 20 | 30 | 40 | 50 |
| MI [a] | 55.0 | 74.1 | 95.7 | 120 | 162 |
| MMI [b] | 29.5 | 40.7 | 58.9 | 81.3 | 115 |
| EMI [b] | 21.4 | 34.7 | 46.8 | 70.8 | 91.2 |
| HMMI [c] | 75.9 | 93.3 | 110 | 151 | 182 |

[a] 277 nm. [b] 300 nm. [c] 283 nm.

*Thermodynamic Parameters of Activation.*—Energies of activation ($E_a$) were calculated from $k_{OH^-}$ by use of the Arrhenius equation. Enthalpies ($\Delta H^\ddagger$), entropies ($\Delta S^\ddagger$), and free energies of activation ($\Delta G^\ddagger$) calculated from the activation energies and the frequency factors are summarized in Table 3. The values of $\Delta H^\ddagger$ increase in the order: EMI > MMI > MI > HMI. This is the reverse of that for $k_{OH^-}$, and is in accord with the order expected from the electron-releasing effect of the nitrogen substituents discussed later. The large negative values of $\Delta S^\ddagger$, ranging from −131 to −162 J mol$^{-1}$ K$^{-1}$, suggest that the reaction path from the initial to the transition state is the rate-determining step. The values of $\Delta S^\ddagger$ increase in the same order as that of $\Delta H^\ddagger$: EMI > MMI

TABLE 3

Thermodynamic parameters of activation for hydrolysis at ionic strength 0.5

| Imide | $\Delta H^\ddagger$/ kJ mol$^{-1}$ | $\Delta S^\ddagger$ [a]/ J mol$^{-1}$ K$^{-1}$ | $\Delta G^\ddagger$/ kJ mol$^{-1}$ |
|---|---|---|---|
| MI | 17.20 | −151.0 | 62.94 |
| MMI | 21.02 | −142.4 | 64.17 |
| EMI | 24.85 | −131.8 | 64.79 |
| HMMI | 13.37 | −161.8 | 62.39 |

[a] 30 °C.

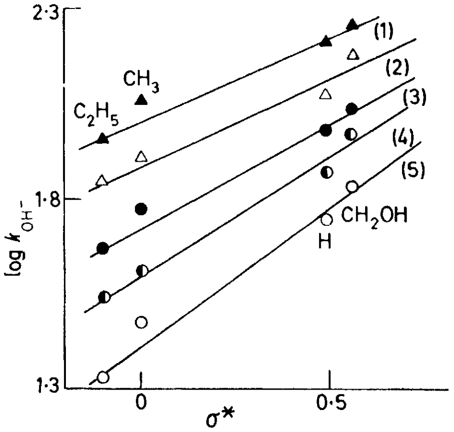
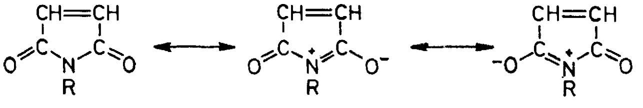


FIGURE 2 Taft's plots: log $_{OH^-}$ *versus* σ* for hydrolysis at ionic strength 0.5: (1), 10; (2), 20; (3), 30; (4), 40; (5), 50 °C

$>$MI $>$ HMMI. As the values of $\Delta H^\ddagger$ are larger than those of $T\Delta S^\ddagger$, the substituent effects depend primarily on the enthalpy change.

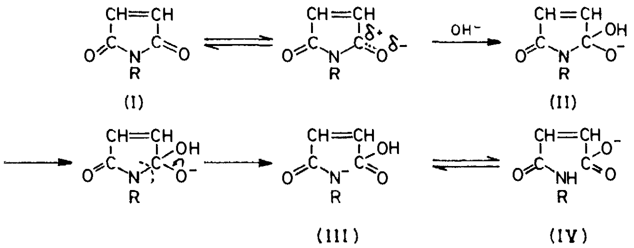
*Substituent Effects.*—log $k_{OH^-}'$ is plotted against Taft's $\sigma^*$ values in Figure 2. The graphs are approximately linear at every temperature studied. Introduction of an electron-withdrawing nitrogen substituent accelerates the rate of hydrolysis. The value of $\rho^*$ is 0.72 at 10, 0.63 at 20, 0.55 at 30, 0.46 at 40, and 0.44 at 50 °C. This suggests that the rate-determining step is nucleophilic attack by hydroxide ion on $N$-alkylmaleimide, and that the $N$-alkylmaleimides in this work have the same reaction mechanism.

Matsuo[19] suggested that an electron-releasing substituent is preferred in stabilizing the ionic structure of an imide ring. This stabilizing effect strengthens the C–N–C bond of the imide, and lowers the potential energy. Furthermore, electron-releasing substituents accelerate enolization of the carbonyl group increasing the electron density of the carbonyl oxygen. This effect retards approach by a hydroxide ion to the $N$-alkylmaleimide molecule. On the other hand, it is presumed that the electron density of the carbonyl carbon, which is polarized positively, remains unchanged or decreases with acceleration of enolization of the carbonyl group.



(I) (Ia) (Ib)

*Mechanism of Hydrolysis.*—The results can be explained by a bimolecular reaction mechanism, in which formation of intermediate (II) by nucleophilic attack of hydroxide ion on carbonyl carbon of the imide ring is the rate-determining step. $N$-Alkylmaleamic acid (IV) is formed from intermediate (II) by cleavage of a C–N–C bond and by transfer of a proton as in the Scheme.



Scheme

Under the experimental conditions, the hydrolysis of $N$-alkylmaleamic acid to maleic acid with elimination of ammonia or $N$-alkylamine hardly occurs. This result is consistent with those of Dahlgren[12] and Kirby[13] for $N$-alkylmaleamic acids, phthalamic acids, and substituted maleamic acids. The $N$-alkylmaleimides in this work are stable in aqueous acid, in which amides, esters, and $N$-substituted maleamic acids are hydrolysed. This is consistent with the fact that acid catalyses the ring closure of $N$-alkylmaleamic acid.

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[19] T. Matsuo, *Bull. Chem. Soc. Japan*, 1964, **37**, 1844.