272. *The Mechanisms of* N-*Substitution in Glyoxaline Derivatives. Part II.* The Methylation of* 4(5)-*Nitroglyoxaline by Methyl Sulphate.*

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A kinetic study of the methylation of 4(5)-nitroglyoxaline by methyl sulphate in dilute homogeneous solutions indicates two reaction paths: one involves the conjugate base of 4(5)-nitroglyoxaline, and the other, studied with formic acid as solvent, involves the neutral molecule. The orientation of the methylation by these two reaction paths is related to the prototropic equilibria studied in Part I of this series.*

In heterogeneous systems the orientation of the *N*-methylation of 4(5)-nitroglyoxaline is very sensitive to the pH of the medium.[1] Similar orientational changes occur for methylation in dilute homogeneous solution, and their interpretation requires a knowledge of the prototropic equilibria and reaction mechanisms involved. The equilibria were discussed in Part I; * the present paper is concerned with the kinetic form of the methylation by methyl sulphate under various conditions.

(1) *Methylation in Alkaline Media.*—The methylation of 4(5)-nitroglyoxaline by methyl sulphate occurs at a convenient rate in dilute aqueous sodium hydroxide containing 10% of ethanol at 25°. Under these conditions, 4(5)-nitroglyoxaline is almost completely dissociated to the conjugate base (cf. Part I) and the mechanism should therefore be of the type designated S_E2cB. Some kinetic studies were carried out on the reaction to check the kinetic form and to determine the rate coefficients for reaction at the two nitrogen atoms.

These kinetic studies were complicated by the simultaneous hydrolysis of the methyl sulphate to methyl hydrogen sulphate and methanol. Now methyl hydrogen sulphate is a strong acid, so reaction modifies both the methyl sulphate concentration and the pH. Interpretation of the kinetics was simplified by using a large excess of methyl sulphate over 4(5)-nitroglyoxaline; the corrections to be applied to take account of the hydrolysis of methyl sulphate are then rendered more certain. A brief study was made of this hydrolysis since the earlier work [2] on it was not applicable to our conditions. The kinetic form in aqueous sodium hydroxide containing 10% of ethanol follows equation 1; at 25°, $k_1 = 1{\cdot}7 \times 10^{-4}$ sec.$^{-1}$, $k_2 = 1{\cdot}6 \times 10^{-2}$ mole^{-1} sec.$^{-1}$ l.

$$\text{Rate} = k_1[Me_2SO_4] + k_2[Me_2SO_4][OH^-] \quad . \quad . \quad . \quad . \quad . \quad (1)$$

The concurrent methylation of 4(5)-nitroglyoxaline was followed from the spectrum of the solutions. Details of a typical kinetic run are given in Table 1. The second column gives the observed nitroglyoxaline concentrations, and the third gives the instantaneous first-order rate coefficients (k_1), calculated by dividing the instantaneous rate of methylation by the nitroglyoxaline concentration. The values of k_1 fall steadily during a kinetic run, but when they are divided by the instantaneous concentration of methyl sulphate (column 4), the resulting second-order rate coefficients (k_2) are effectively constant. This suggests that the reaction is of the first order with respect to both nitroglyoxaline and methyl sulphate.

The run cited covers an appreciable change in pH, arising from the concurrent hydrolysis, and the constancy of the second-order rate coefficients is an indication that there is no pH-dependent term. To check this, kinetic runs were carried out with lower hydroxide-ion concentrations. The second-order rate coefficients were constant during

* Part I, preceding paper.

[1] Forsyth and Pyman, *J.*, 1925, **127**, 573.
[2] Klemenc, *Monatsh.*, 1917, **38**, 353; Pollak and Baar, *ibid.*, p. 501.

TABLE 1. *Instantaneous first-order and second-order rate coefficients for the methylation of 4(5)-nitroglyoxaline* (GH) *in 0·09M-sodium hydroxide at 25°.*

Time (min.)	10^4[GH]	10^4k_1 ($sec.^{-1}$)	10^2[Me_2SO_4]	10^2k_2 ($mole^{-1}$ $sec.^{-1}$ l.)	Time (min.)	10^4[GH]	10^4k_1 ($sec.^{-1}$)	10^2[Me_2SO_4]	10^2k_2 ($mole^{-1}$ $sec.^{-1}$ l.)
0	0·529	—	3·61		25	0·305	1·82	1·00	1·82
5	0·459	4·24	2·65	1·60	30	0·294	1·50	0·83	1·76
10	0·390	3·56	2·00	1·78	35	0·283	1·15	0·63	1·68
15	0·349	2·60	1·61	1·61	45	0·266	0·78	0·50	1·57
20	0·327	1·91	1·27	1·51					

a single run but there was a slight increase in the mean values as shown by the following figures:

[NaOH]	0·09	0·06	0·03
10^2k_2 ($mole^{-1}$ $sec.^{-1}$ l.)	1·67	1·90	2·01

This may be an ionic-strength effect, since the ionic strength was constant during a single run but varied with the initial concentration of sodium hydroxide. The absence of a significant pH-dependent term shows that the kinetic form is as equation 2. The reaction can be considered as a nucleophilic attack of the conjugate base of 4(5)-nitroglyoxaline (G^-) on a neutral methyl sulphate molecule.

$$\text{Rate} = k_2[Me_2SO_4][G^-] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

The analysis of the isomeric ratio in the product was also carried out spectrometrically since, although the spectra of the two *N*-methyl isomers are similar, their basicities are not (Part I), and protonation causes a marked change in the spectrum. Thus in 0·1M-perchloric acid, 1-methyl-5-nitroglyoxaline is effectively present as the conjugate acid, but 1-methyl-4-nitroglyoxaline is effectively present as the neutral molecule. The results showed that about 11% of the product was 1-methyl-5-nitroglyoxaline, and the remainder 1-methyl-4-nitroglyoxaline. From this ratio, and from the value of k_2 in equation 2, it is possible to derive the rate coefficients for substitution at the two nitrogen atoms and these values are given in section 3. The value of k_2 in equation 2 is almost equal to that in equation 1; this shows that the hydroxide ion and the conjugate base of 4(5)-nitroglyoxaline have almost identical nucleophilic power towards the carbon atoms in methyl sulphate.

(2) *Methylation in Acidic Media.*—Experiments were carried out in aqueous acids in an attempt to observe a reaction path involving substitution in the neutral molecule of 4(5)-nitroglyoxaline. Attempts to methylate 4(5)-nitroglyoxaline by methyl sulphate in aqueous hydrochloric acid were unsuccessful, presumably because the rate of hydrolysis of methyl sulphate greatly exceeded the rate of methylation. Some methylation could be observed in 50% aqueous formic acid, but the kinetics of this reaction were complicated by concurrent hydrolysis of the methyl sulphate. Finally, anhydrous formic acid was found to be suitable, for the reaction of methyl sulphate with the medium was then greatly decreased.

The methylation of 4(5)-nitroglyoxaline with 0·1M-methyl sulphate in anhydrous formic acid containing sodium formate was studied at 50°. The reaction is slow, the main initial product being 1-methyl-5-nitroglyoxaline, but, in contrast to the methylation in alkaline solution, the formation of the 1,3-dimethyl quaternary compound occurs significantly. Kinetic complications arising from the formation of the quaternary compound were avoided by studying the initial rates of reaction. The extent of methylation was determined from the spectrum of samples in alkaline solution, the measurements being limited to about 10% reaction. The sum of the calculated concentrations of 4(5)-nitroglyoxaline and 1-methyl-5-nitroglyoxaline then agreed well with the initial concentration of 4(5)-nitroglyoxaline.

Details of a kinetic run are given in the Experimental section. With a constant concentration of sodium formate, the reaction is of the first order both with respect to the

stoicheiometric concentration of 4(5)-nitroglyoxaline (GH) and with respect to methyl sulphate. The kinetic form is therefore as shown in equation 3. Evidence for the constancy of k_2 is given in Table 2.

$$\text{Rate} = k_2[\text{GH}][\text{Me}_2\text{SO}_4] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

TABLE 2. *Methylation in anhydrous formic acid containing* 1·0M-*sodium formate. Variation of the initial rate with the reactant concentration at* 50°.

10^3[GH]	$10[Me_2SO_4]$	10^8 × Initial rate (mole sec.$^{-1}$ l.$^{-1}$)	10^4k_2 (mole^{-1} sec.$^{-1}$ l.)	10^3[GH]	$10[Me_2SO_4]$	10^8 × Initial rate (mole sec.$^{-1}$ l.$^{-1}$)	10^4k_2 (mole^{-1} sec.$^{-1}$ l.)
8·52	1·06	11·0	1·22	2·35	1·06	3·21	1·29
6·85	,,	10·1	1·39	8·48	0·78	9·60	1·45
5·25	,,	7·58	1·36	,,	0·52	6·21	1·41
4·09	,,	6·22	1·43	,,	0·23	2·65	1·36

The variation of the reaction rate with the concentration of sodium formate gives further information on the reaction mechanism. The concept of acidity functions can be applied to formic acid media,[3] and the value of the H_0 function appears to vary linearly with $-\log_{10} c_{H^+}$ where c_{H^+} stands for the concentration of solvated hydrogen ions $[H{\cdot}CO_2H_2^+]$. Approximate H_0 values for formic acid solutions containing formate ions can therefore be calculated from the autoprotolysis constant of formic acid (10^{-6}) and the value of H_0 in the pure acid [4] (−2·21). These values are included in Table 3; they overlap with those determined directly from indicator measurements. From the thermodynamic pK_a of 4(5)-nitroglyoxaline in water (−0·05) and from the H_0 values in formic acid containing sodium formate, it is possible to calculate the fraction of unprotonated nitroglyoxaline molecules in the solution. The deviation of the protonation of 4(5)-nitroglyoxaline from the conventional H_0 scale in water is presumably unimportant here because of the proportionality of h_0 to c_{H^+}.

TABLE 3. *Methylation in anhydrous formic acid at* 50°. *Variation of the initial rate with the concentration of sodium formate.*

$[GH]_{stoich.} = 8{\cdot}52 \times 10^{-3}$M				$[Me_2SO_4] = 0{\cdot}106$M	
[H·CO_2Na]	H_0	10^8 × Initial rate (mole sec.$^{-1}$ l.$^{-1}$)	10^4k_2 (mole^{-1} sec.$^{-1}$ l.)	10^3[GH]$_{mol.}$	$10^4k_2'$ (mole^{-1} sec.$^{-1}$ l.)
1·00	+0·81	11·0	1·22	7·27	1·43
0·50	+0·48	10·5	1·16	6·21	1·59
0·25	−0·19	6·8	0·75	4·93	1·30
0·10	−0·28	5·5	0·61	3·09	1·68
0·05	−0·48	3·2	0·35	1·93	1·56

In Table 3, the variation in k_2 (eqn. 3) with the concentration of sodium formate is compared with the related variation in the concentration of molecular 4(5)-nitroglyoxaline (column 5). In the final column, values are given for a second-order rate coefficient k_2' calculated from equation 3 but in terms of the concentration of molecular 4(5)-nitroglyoxaline. The constancy of k_2' shows that only the unprotonated nitroglyoxaline molecule is involved in the reaction, and, by the argument set out in Part I, section 1, the mechanism is therefore considered to be a S_E2' substitution. The results in Table 3 also show that the only effect of the formate ions on the reaction is through an alteration in the acidity of the medium; no base-catalysed proton-transfer appears to be involved.

The formation of the 1,3-dimethyl quaternary compound was first suspected because the spectra of the product solutions did not vary with pH in the way expected for a mixture containing only 4(5)-nitroglyoxaline and its *N*-methyl derivatives. This was particularly

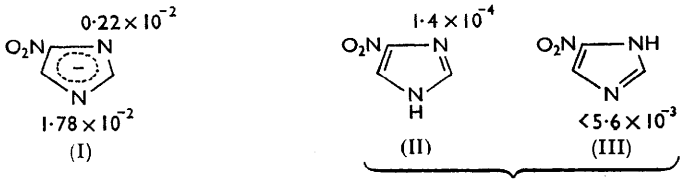
[3] Hammett and Dietz, *J. Amer. Chem. Soc.*, 1930, **52**, 4795; Hammett and Deyrup, *J. Amer. Chem. Soc.*, 1932, **54**, 4239.

[4] Long and Paul, *Chem. Rev.*, 1957, **57**, 1.

marked at temperatures above 50°. In the standard preparation of 1-methyl-5-nitroglyoxaline,[5] 25% of the starting material is not accounted for, and it seems likely that the methosulphate can be formed in significant quantities. Some of the methosulphate was prepared by this reaction and a preliminary study was made of its properties. It is not stable in aqueous solutions for, when freshly prepared, the solution has a main absorption maximum at 2670 Å, but this changes slowly and after six days the main maximum is at 3500 Å. The change occurs much more rapidly in the presence of hydroxide ions, and presumably equilibria similar to those suggested for the quaternary benzimidazole compounds are involved.[6]

Because of the ready formation of the quaternary compound, the products of the initial methylation were isolated chemically instead of being determined spectrometrically. By using excess of 4(5)-nitroglyoxaline and by recovering the unchanged material it was shown that at least 86% of the initial product was 1-methyl-5-nitroglyoxaline, and the percentage is probably higher because the only other material recovered was a yellow oil believed to be a decomposition product of the quaternary compound. No trace of 1-methyl-4-nitroglyoxaline was observed.

(3) *Correlation of Rate Coefficients and Equilibrium Constants.*—From the overall rate



S_E2cB (aq. soln., 25°) S_E2' (formic acid, 50°)

Rate coefficients (mole⁻¹ sec.⁻¹ l.) for methylation by methyl sulphate.

coefficient for reaction through the conjugate base and from the corresponding product analysis it is possible to assign values to the rate coefficients for reaction at the two nitrogen atoms in the conjugate base and these are given in (I). The neutral molecule of 4(5)-nitroglyoxaline exists mainly as structure (II) (cf. Part I), and from the value of k_2 in equation 3 (when calculated for molecular nitroglyoxaline) and from the product analysis it is possible to calculate that the rate coefficient for the S_E2' reaction of structure (II) is approximately as shown above. It is more difficult to obtain a value for the S_E2' reaction of structure (III), but from the fact that less than 10% of the 1,5-methyl derivative is formed in acidic media, and from the relative concentrations of (II) and (III) (400 : 1) we find that the rate coefficient cannot be greater than $5{\cdot}6 \times 10^{-3}$ mole^{-1} sec.$^{-1}$ l. If, under the above conditions, the reactivity of both nitrogen atoms were reduced by the same factor in the transition from the S_E2cB to the S_E2' mechanism, the value would be $1{\cdot}1 \times 10^{-3}$. This would be consistent with the apparent absence of 1-methyl-4-nitroglyoxaline in the product.

The two tautomeric forms of the neutral molecule are in equilibrium with the common conjugate base and conjugate acid. Since the tautomer ratio is about 400 : 1 in favour of structure (II), the basicity of the two nitrogen atoms in the conjugate base must differ by this factor, and the basicity of the two forms of the neutral molecule must differ by the same factor. Comparison of the rate coefficients for the reaction of the two nitrogen atoms by both the S_E2cB mechanism and the S_E2' mechanism shows that the ratio of their nucleophilic reactivities is much less than the ratio of their basicities; thus, for the S_E2cB mechanism, the two ratios are 8 and 400 respectively. If there is a linear free-energy relation between the reaction rates and the relevant equilibria, an equation of the form

[5] Hazeldine, Pyman, and Winchester, *J.*, 1924, **125**, 1431.

[6] Hofmann, "Imidazole and its Derivatives, Part I," Interscience Publ. Inc., New York, 1953, p. 281.

$k \propto K^{-a}$ should hold, where k is a rate constant and K is the acidic equilibrium constant for protonation of the same nitrogen atom. The above values for the relative reactivity of the two nitrogen atoms in both the S_E2cB and the S_E2' mechanisms are then consistent with $a = 0{\cdot}3$.

The rate coefficients for the reaction of the same nitrogen atom in the conjugate base and the neutral molecule are also consistent with this equation. Thus the basicity of the nitrogen atom nearer to the nitro-group varies by a factor * of 5×10^6 when the other nitrogen atom is protonated. The exact comparison of the rate coefficients is complicated by the 25° difference in temperature, but if this is considered to be equivalent to a power of ten then the rate coefficients differ by a factor of $1{\cdot}5 \times 10^2$.

The above calculation is only intended as a useful guide in understanding the reaction. However it does show that the observed orientations and the relative reactivity of the neutral molecule and the conjugate base can be considered to be derived from a simple relationship between the basicity of a nitrogen atom and its nucleophilic power.[7] Some general consequences of this relationship in the methylation of glyoxaline derivatives are discussed in Part III (following paper).

Experimental

The general experimental technique and the preparation of 4(5)-nitroglyoxaline and its derivatives were as described in Part I. Formic acid (" AnalaR ") was dried over boric oxide and fractionated. The fraction boiling at 101°/760 mm. was collected and stored in the dark. The purity was checked by freezing-point measurements (f. p. 8·39°). Methyl sulphate (reagent grade) was fractionated under reduced pressure and the main fraction (b. p. 76°/15 mm.) was collected.

Spectrometric Analysis of the Reaction Mixture.—This was limited to conditions where the spectrum of the product solutions showed that the concentration of the 1,3-dimethyl quaternary compound was negligible; only the absorption of 4(5)-nitroglyoxaline and its *N*-methyl derivatives need then be considered. The spectra of the two *N*-methyl compounds are similar, and where one predominated the extent of reaction was determined from the spectrum in alkaline solution, where the remaining 4(5)-nitroglyoxaline showed the characteristic spectrum of the conjugate base. The extinction coefficients of the pure compounds at the wavelengths used in analysing the reaction mixtures are given in Table 4. From the values there listed for the compounds in 0·1M-sodium hydroxide, and from the optical density of the reaction mixture at 3000 and 3500 Å, it is possible to calculate independently the concentration of the remaining 4(5)-nitroglyoxaline and that of the *N*-methyl derivative. In all the kinetic runs reported here, the sum of these two concentrations agreed well with the initial concentration of 4(5)-nitroglyoxaline.

Table 4. *Extinction coefficients* ($\times 10^{-3}$) *used in the analysis of reaction mixtures.*

	Medium			
	0·1M-NaOH *		0·1M-$HClO_4$	
Compound	at 3000 Å	at 3500 Å	at 2650 Å	at 3000 Å
4(5)-Nitroglyoxaline	2·64	10·19	2·92	6·02
1-Methyl-4-nitroglyoxaline	7·27	0·851	2·76	7·08
1-Methyl-5-nitroglyoxaline	8·37	1·34	6·17	1·93

* These values are not very sensitive to the pH when this is above 12.

To determine the ratio of substitution at the two nitrogen atoms, as used in the analysis of the product from alkaline methylation, the spectrum of the reaction mixture in 0·1M-perchloric acid was also studied. This medium is sufficiently acidic effectively to protonate 1-methyl-5-nitroglyoxaline, but not 1-methyl-4-nitro- or 4(5)-nitro-glyoxaline. The appropriate extinction coefficients are listed in Table 4. From these values and from the optical density of the reaction

* The equilibrium constants for the addition of the first and the second proton to the conjugate base differ by a factor of 2×10^9 (Part I), but the first proton goes on the other nitrogen atom and so this value has to be divided by the tautomer ratio to obtain the factor above.

[7] Cf. Bruice and Schmir, *J. Amer. Chem. Soc.*, 1958, **80**, 148.

mixture at the wavelengths and under the conditions shown in Table 4, four simultaneous equations can be set up and solved to give the three unknown concentrations. The sum of these again agreed with the initial concentration of 4(5)-nitroglyoxaline.

Chemical Analysis of the Reaction Mixture.—This was applied to the product from methylation in anhydrous formic acid. 4(5)-Nitroglyoxaline (1·65 g.) was heated with methyl sulphate (1 ml.) in anhydrous formic acid (50 ml.) for several hours at 35° in a sealed tube. The formic acid was then removed under reduced pressure, and the residue treated with water. The remaining nitroglyoxaline (1·0 g.) did not dissolve and was filtered off. The filtrate was just neutralised with solid sodium carbonate, and the solution was then evaporated to dryness under reduced pressure. The residue was extracted with ether, and the ether was then removed, leaving 1-methyl-5-nitroglyoxaline (0·62 g.), m. p. 53·4° (Found: C, 38·0; H, 4·0; N, 32·8. Calc. for $C_4H_5N_3O_2$: C, 37·8; H, 4·0; N, 33·0%). A small quantity of the residue was insoluble in ether, and on recrystallisation from ethanol gave a yellow compound (~0·05 g.), presumably a breakdown product of the quaternary compound. There was no evidence for 1-methyl-4-nitroglyoxaline; if present, this compound would also have been in the ether-insoluble residue.

Kinetic Runs.—The kinetic runs involving methylation in alkali were followed directly from the change in the spectrum of the reaction mixture when contained in an optical cell maintained at 25°. Experiments on the methylation of the monomethyl derivatives indicated that no significant dimethylation should occur during a kinetic run. The kinetic runs with anhydrous formic acid as solvent were carried out by the conventional technique using sealed glass tubes containing aliquot parts of the reaction mixture thermostatically maintained at 50°. Tubes were transferred to a mixture of solid carbon dioxide and ethanol at suitable times; after warming to room temperature samples (0·98 ml.) were run into aqueous solutions containing sufficient sodium hydroxide to bring the pH to 13. The volume was made up to 100 ml. and the absorption spectrum was measured against an equivalent solution containing sodium formate. Examples of the observed optical densities and the calculated concentrations are given in Table 5. The kinetic study was based on the initial rates of methylation and limited to the first 10% reaction to avoid difficulties arising from the formation of the quaternary compound.

TABLE 5. *Determination of initial rate of methylation in anhydrous formic acid containing* M-*sodium formate at* 50°.

Initial concentrations: [GH] = 8·52 × 10^{-3}M; [Me_2SO_4] = 0·106M.

	Optical density[a]		Calc. concn. (× 10^3)	
Time (min.)	300 mμ	350 mμ	GH	GMe
0 [b]	0·213	0·847	8·55	(0)
30	0·229	0·830	8·35	0·18
60	0·237	0·815	8·19	0·33
108	0·244	0·790	7·92	0·52

Calc. initial rate of methylation = 11 × 10^{-8} mole sec.$^{-1}$ l.$^{-1}$.

[a] In alkali, after dilution of the reaction mixture by a factor of 103. [b] An arbitrary zero, taken about 5 min. after immersion in the thermostat bath.

The ancillary investigation into the hydrolysis of methyl sulphate in aqueous solutions containing 10% ethanol was carried out by extracting samples, diluting them with acetone, and titrating the acid formed. Fuller details are given elsewhere.[8]

Preparation of 1,3-*Dimethyl-4-nitroglyoxalinium Methyl Sulphate.*—4(5)-Nitroglyoxaline (10 g.) was heated with dimethyl sulphate (22 g.) in a sealed tube at 100° for 3 hr. The mixture was cooled and dissolved in ethanol; ether was then slowly added to precipitate 1,3-*dimethyl-4-nitroglyoxalinium methyl sulphate.* Recrystallised from ethanol–ether, this had m. p. 165° (Found: C, 28·6; H, 4·4; N, 16·6; S, 12·5. $C_6H_{11}N_3O_6S$ requires C, 28·5; H, 4·4; N, 16·6; S, 12·6%). The *picrate* was prepared by heating a concentrated aqueous solution of the methosulphate and sodium picrate; it recrystallised from water as yellow needles, m. p. 138—139° (Found: C, 36·1; H, 3·5; N, 23·0. $C_{11}H_{10}N_6O_9$ requires C, 35·6; H, 3·0; N, 22·7%).

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[8] Grimison, M.Sc. Thesis, London, 1956.