273. *The Mechanisms of N-Substitution in Glyoxaline Derivatives. Part III.* Factors determining the Orientation of N-Methylation in Substituted Glyoxalines and Benzimidazoles.*

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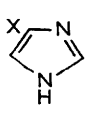
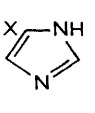
The analysis of the orientation of *N*-substitution in 4(5)-nitroglyoxaline in terms of the reaction mechanisms and the prototropic equilibria is generalised to provide an account of the expected orientation in substituted glyoxalines and benzimidazoles. Evidence on the tautomeric equilibria in these compounds is obtained from basicity measurements, and the isomer ratios in the products of methylation are estimated from the infrared spectra. Previous values for the isomer ratios in some substituted benzimidazoles are shown to be incorrect. The revised values, and the reported isomer ratios in substituted glyoxalines, are shown to be largely consistent with the expected mechanisms and the expected positions of tautomeric equilibrium.

In Part II * it was shown that the orientation of *N*-methylation in 4(5)-nitroglyoxaline in acidic and basic media could be understood from a knowledge of the prototropic equilibria involving the substrate, and from the mechanism of the reaction. The key to the problem is the apparent existence of a simple relation between the basicity of a nitrogen atom and its nucleophilic power. Some information on prototropic equilibria involving glyoxaline derivatives is available in the literature, and further results are reported in this paper. These results, when combined with some of the conclusions of Part II, lead to an account of the probable orientation of *N*-methylation in substituted glyoxalines and benzimidazoles.

(1) *Prototropic Equilibria.*—The most important influence of a substituent on the orientation of *N*-methylation comes from its effect on the tautomer ratio (I : II), for this is a measure of the relative basicity of the nitrogen atoms and hence of their relative nucleophilic power (cf. Part II). Several considerations suggest that the conjugative interaction of the substituent with the ring is relatively unimportant in determining this


(I)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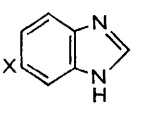
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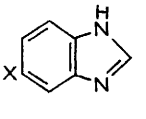
(IV)

ratio. Thus the electrons directly involved in the proton transfer are in σ-orbitals and are therefore not conjugated with the substituent. Also, any electron displacement involving the π-electrons would be expected to be relayed approximately equally to the two nitrogen atoms without the marked discrimination between different positions observed in alternant conjugated systems. Where the substituent has a π-orbital available for conjugation with the ring, one factor in the relative stability of the two isomers is the energy difference between the linear conjugated system in structure (II) and the branched conjugated system in structure (I); the former should be somewhat more stable. The tautomer ratio in 4(5)-phenylglyoxaline should give evidence on this point. The results in Table 1 show that the basicity of 1-methyl-4-phenylglyoxaline † is a little less than that of the unmethylated compound. If structure (II) were much more stable than structure (I) for 4(5)-phenylglyoxaline, then the reverse order would have been expected, because protonation of the 1-methyl-4-phenyl-isomer would involve the more basic nitrogen atom. This supports the above argument that the difference in the π-electron energy in the two structures is not an important factor in determining the tautomeric ratio.

* Part II, preceding paper.

† Throughout this paper, and, *e.g.*, in column headings of Tables, the *N*-methyl group is considered to be in position 1, independently of any other substituents.

TABLE 1. *The basicity of some substituted glyoxalines and benzimidazoles.*

Compound	pK_a	*N*-Methyl derivatives pK_a (4-isomer)	pK_a (5-isomer)
4(5)-Nitroglyoxaline [a]	−0·05	−0·53	2·13
4(5)-Phenylglyoxaline [b]	(6·00)	5·78	—
		(5-isomer)	(6-isomer)
5(6)-Nitrobenzimidazole	3·48	3·40	3·67
2-Methyl-5(6)-nitrobenzimidazole	4·37	4·40	4·20
5(6)-Chlorobenzimidazole * [c]	(3·92)	(3·88)	(3·88)
5(6)-Chloro-2-methylbenzimidazole * [c]	(4·71)	(4·75)	4·75
		(4-isomer)	(7-isomer)
4(7)-Nitrobenzimidazole	3·33	3·86	3·25

Values in parentheses are taken from the literature.

* Determined in 50% aqueous ethanol; the other results refer to solutions in water. [a] This series, Part I. [b] Kirby and Neuberger, *Biochem. J.*, 1938, **32**, 1146. [c] Davies, Mamalis, Petrow, and Sturgeon, *J. Pharm. Pharmacol.*, 1951, **3**, 420.

The basicity of the *N*-methyl derivatives of 4(5)-nitroglyoxaline (Part I) suggests that, with this substituent, structure (I) predominates by a factor of 400 over structure (II), and this can be understood from the inductive effect of the substituent on the acidity of the two protons in the conjugate acid. From the above arguments, it seems probable that other $-I$ substituents should modify the tautomeric ratio in the same way, irrespective of their conjugation with the ring.

Some results for substituted benzimidazoles, included in Table 1, provide interesting evidence for the unimportance of conjugative interaction in determining the isomer ratio. They indicate that substituents in the 5(6)-position have a negligible effect on the relative basicity of the two *N*-methyl isomers, and that the tautomer ratio (III : IV) in the unmethylated benzimidazole therefore remains near unity. If conjugative interaction were important, then, for the nitro-substituent, structure (III) would be expected to be more stable, by analogy with the greater basicity of *m*-nitroaniline than of *p*-nitroaniline. These results in the benzimidazole series are in agreement with the conclusions of Roberts *et al.*[1a] and Wepster [1b] that, where conjugative interaction is unimportant, the influence of an electron-withdrawing substituent on the basicity of amino-groups in the *meta*- and the *para*-position is almost equal. The inking CH group would also tend to equalise any differential effect of the nitro-group at the two ring-nitrogen atoms, but this cannot be the main cause of the equal tautomer ratios since this factor is also present in the substituted glyoxalines.

The basicities of 4(7)-nitrobenzimidazole and its two *N*-methyl derivatives are included in Table 1. The fact that the 1-methyl-4-nitro- is more basic than the 1-methyl-7-nitro-isomer accords with other evidence [2] that in 4(7)-nitrobenzimidazole the predominant tautomeric form has the imino-hydrogen atom hydrogen-bonded to the nitro-group.

(2) *The Expected Orientation of* N-*Methylation.*—The results obtained from the methylation of 4(5)-nitroglyoxaline (Part II) suggested that only two mechanisms need to be considered; one designated S_E2cB involving the conjugate base, and the other designated S_E2' involving the neutral molecule. The comparison of the complete set of rate coefficients with the prototropic equilibria suggested a relation of the form $k \propto K^{-a}$ ($a \sim 0{\cdot}3$) between the rate coefficient (k) for reaction at one nitrogen atom and the corresponding acid equilibrium constant (K). It seems probable that these mechanisms and this relation are generally applicable to *N*-methylation by methyl sulphate of glyoxaline derivatives.

Consider first glyoxaline derivatives with $-I$ substituents. The predominant tautomeric form should be structure (I), and the nitrogen atom more distant from the substituent

[1] (*a*) Roberts, Clement, and Drysdale, *J. Amer. Chem. Soc.*, 1951, **73**, 2181; (*b*) Wepster, *Rec. Trav. chim.*, 1956, **75**, 1473; cf. Ingold, " Structure and Mechanism in Organic Chemistry," Bell, London, 1953, p. 732.

[2] Rabinowitz and Wagner, *J. Amer. Chem. Soc.*, 1951, **73**, 3030.

should therefore be the more basic. Hence, in the reaction by the S_E2cB mechanism, the main product should be the 4-isomer but the isomer ratio should be much less than the tautomer ratio. In reaction by the S_E2' mechanism, the above relation between rates and equilibria indicates that the difference between the rate coefficients of the two tautomers should be much less than the difference between their concentrations; the predominant isomer should therefore by formed by an S_E2' reaction of the predominant tautomer, and the isomer ratio should more nearly approach the tautomer ratio. The main product should then be the 5-isomer. In glyoxaline derivatives containing a $+I$ substituent, the isomer ratios should be in the reverse order.

In 4(5)-nitroglyoxaline, the rate coefficient for the methylation of the conjugate base is greater than that for the methylation of the neutral molecule by a factor of a thousand.‡ In other substituted glyoxalines, this factor is probably similar; the transition from the S_E2cB mechanism to the S_E2' mechanism should therefore occur about 3 pH units below the acidic pK of the neutral molecule. The pK of glyoxaline [3] is about 14, and the pK of 4(5)-nitroglyoxaline is 9·3; hence for negatively substituted glyoxalines the transition between the two mechanisms should occur somewhere in the range pH 6—11. Thus it is possible to predict the main features of the orientation and the variation with pH.

The relation $k \propto K^{-a}$ leads to an interesting general conclusion. Where $0 < a < 1$, then for any substituted glyoxaline a change from the S_E2cB mechanism to the S_E2' mechanism should always change the main product of substitution. This occurs because the most basic nitrogen atom in the conjugate base is necessarily blocked by a proton in the predominant tautomer of the neutral molecule, and where $0 < a < 1$ the predominant tautomer determines the product.

In 5(6)-substituted benzimidazoles, the equal concentrations of the two tautomeric forms indicate that the basicity of the nitrogen atoms must be very similar, and therefore that the nucleophilic power is probably also similar. Thus the isomer ratios for substitution by the S_E2cB mechanism and the S_E2' mechanism should be near unity.

(3) *Comparison with the Experimental Results.*—The main results for the methylation of substituted glyoxalines and benzimidazoles by methyl sulphate under preparative conditions are set out in Table 2. Further results are available for methylation by methyl

TABLE 2. *Reported orientation of* N-*methylation by methyl sulphate under preparative conditions.*

Compound	Ratio, 4-isomer : 5-isomer Me_2SO_4	Me_2SO_4–NaOH
4(5)-Nitroglyoxaline [a b]	1 : 350	3 : 1
2,4-*Dibromo*-5-methylglyoxaline [b] *	1 : 45	1 : 1
4(5)-Bromoglyoxaline [a]	1 : 34	—
4-*Bromo*-5-methylglyoxaline [b]	only 1,5-isomer	2 : 3
4(5)-Phenylglyoxaline [a]	5 : 1	—
	Ratio,† 5-isomer : 6-isomer	
5(6)-Nitro-2-methylbenzimidazole [c]	(1 : 100)	(1 : 5)
5(6)-Bromo-2-methylbenzimidazole [c]	(1 : 50)	1 : 2
2,5(6)-Dimethylbenzimidazole [a]	1 : 10	1 : 1

* Where the designation of the methyl isomer is ambiguous, it is based upon the italicized substituent.

† Values in parentheses are criticised in section 3 of this paper.

[a] Hazeldine, Pyman, and Winchester, *J.*, 1924, **125**, 1431. [b] Forsyth and Pyman, *J.*, 1925, **127**, 573. [c] Phillips, *J.*, 1931, 1143.

sulphate in the absence of alkali, but they are consistent with those given in Table 2. The agreement of the predicted results with those obtained with 4(5)-nitroglyoxaline is to be expected; it illustrates only that the conditions of the preparative experiments give qualitatively the same orientation as that observed in the kinetic experiments involving

‡ Based on the figures in Part II, section 3, after allowance of a factor of ten for the 25° difference in temperature.

[3] Walba and Isensee, *J. Amer. Chem. Soc.*, 1955, **77**, 5488; *J. Org. Chem.*, 1956, **21**, 702.

dilute homogeneous solutions. The comparison of the other results with those predicted brings out the following discrepancies: (1) The methylation of the halogenoglyoxalines in alkali leads to almost equal isomer ratios but not to the expected slight predominance of the 4-isomer.* This may be because the medium is heterogeneous and so permits substitution to occur by the S_E2' mechanism involving the substrate dissolved in the methyl sulphate phase. (2) Substitution in 4(5)-phenylglyoxaline leads mainly to the 1-methyl-4-phenyl-, and not to the 1-methyl-5-phenyl-isomer as expected. However, with this substituent, there should be steric hindrance to reaction at the nitrogen atom nearest to the phenyl group. (3) The results for the substituted benzimidazoles are in complete disagreement with those predicted. No explanation could be found for this, and so the results were reinvestigated by infrared spectroscopy.

The *N*-methyl derivatives of 2-methyl-5(6)-nitro-, 5(6)-nitro-, and 5(6)-bromo-2-methyl-benzimidazole were synthesised unambiguously and their infrared spectra determined. These spectra were then compared with the spectrum of the mixture of two isomers obtained by methylation under the conditions used in preparative experiments. (In this work, and in the attempted chemical separation of the isomers, several new derivatives were prepared and these are described in the experimental section.)

The reaction of 2-methyl-5(6)-nitrobenzimidazole with methyl sulphate led mainly to the quaternary compound, and this was recovered from the reaction as the alcohol base. The combined yield of the two methyl isomers was about 20%. In the presence of alkali, this yield increased to 90%. The comparison of the spectrum of the mixed monomethyl product in a Nujol mull with that of the pure methyl derivatives established the following points. The spectrum of the product is that expected for a mixture of the two methyl isomers in approximately equal amounts. No other compounds are present. The addition of alkali causes a small increase in the proportion of 1,2-dimethyl-5-nitrobenzimidazole.

The infrared spectra in Nujol mulls can give only a qualitative estimate of the isomer ratio, and an attempt was made to improve this by chemical separation of the isomers, but without great success. A more accurate study of the isomer ratio was carried out for the methylation of 5(6)-nitrobenzimidazole, where the greater solubility of the methyl isomers permitted study of the infrared spectra in chloroform solution.

Methylation of 5(6)-nitrobenzimidazole was first studied as described above for the 2-methyl derivative, and with the same result, although the increase in the proportion of the 1-methyl-5-nitro-isomer in the presence of alkali was less marked. The infrared spectra were then determined for chloroform solutions at about 11·1 μ, where the 1-methyl-5-nitro-isomer has an absorption peak absent in the 1-methyl-6-nitro-isomer and the solvent. Comparison of the absorption of the reaction mixtures at this wavelength with that of the pure 1-methyl-5-nitro-isomer showed that this isomer forms 50—60% of the product. The above range includes the results of a number of experiments, some carried out in the presence and some in the absence of alkali. The experiments in the absence of alkali led mainly to the quaternary compound but the isomer ratio was not very sensitive to the extent of this subsequent reaction; the observed isomer ratios should therefore be a reliable guide to the relative reactivity of the two nitrogen atoms in the glyoxaline ring.

Methylation of 5(6)-bromo-2-methylbenzimidazole with methyl sulphate alone led to a 40% yield of the monomethyl derivatives. A comparison of the infrared spectrum of this mixture with that of the pure methyl isomers showed that the 5-bromo-1,2-dimethyl-isomer formed probably 60—70% of the product.

The above results are sufficient to show that the orientation of these reactions under preparative conditions is largely consistent with the expected values. The results in parentheses in Table 2, suggesting the almost exclusive formation of the 6-isomers, must be incorrect. It is difficult to understand how these results were obtained in the earlier

* This is on the assumption that the 4-bromine atom has a more important directive effect than the 5-methyl group. The results with methyl sulphate alone suggest that this assumption is true.

work, because no details are given of the yields of the products or of the methods of separating the isomers. It is not easy to separate completely a mixture of the two *N*-methyl derivatives of the nitrobenzimidazoles by fractional crystallisation, although the isomers derived from 5(6)-bromo-2-methylbenzimidazole can be separated by crystallisation of the picrates. It may be significant that the values listed here for the melting points of 1,2-dimethyl-6-nitro- (252°) and 6-bromo-1,2-dimethyl-benzimidazole (146°) are different from those reported in the earlier work [4] (242° and 180° respectively).

The analysis of the orientation of *N*-substitution set out in section 2 of this paper is based on a number of approximations. Thus the relation between the nucleophilic power and the basicity of the nitrogen atoms is unlikely to be exactly obeyed, particularly where the difference in basicity is slight or where proton acceptance by a nitrogen atom is stabilised by hydrogen bonding. Also, the discrepancies discussed above suggest that steric factors and the heterogeneity of the preparative conditions may influence the orientation. Nevertheless the reported isomer ratios for substitution in the glyoxalines, and the revised results for substitution in the benzimidazoles, do suggest that this analysis of the orientation in terms of mechanism and tautomeric equilibria can explain the main features of the results. This type of analysis should be of value is discussing the reactions of other compounds where *N*-substitution is complicated by prototropic equilibria.

Experimental

Materials.—Analytical data are given only for new compounds and where properties differ markedly from those previously reported; such data were obtained for almost all the other compounds mentioned and the agreement was satisfactory.

4(5)-Phenylglyoxaline (m. p. 132—133°) was prepared by Weidenhagen and Herrmann's method.[5] It was methylated by methyl sulphate,[6] and 1-methyl-4-phenylglyoxaline was separated from the product as the picrate (m. p. 243°).

5(6)-Nitrobenzimidazole was prepared by the nitration [7] of benzimidazole. The product, after recrystallisation from water, had m. p. 205°. It was treated with two equivalents of methyl iodide in methanol at 130—140°, to give 1,3-dimethyl-5(6)-nitrobenzimidazolium iodide (m. p. 261—262°). Treating a cold aqueous solution of this iodide with alkali precipitated the "carbinol base" from the quaternary salt (m. p. 128° after recrystallisation from aqueous ethanol). 1-Methyl-5-nitrobenzimidazole was prepared by the method of Davies *et al.*[8] from 2-amino-*N*-methyl-4-nitroaniline. The product, after recrystallisation from acetone, had m. p. 210°. We are indebted to Dr. Montanari of Bologna for a sample of the corresponding 6-nitro-isomer [9] (m. p. 182°).

2-Methyl-5(6)-nitrobenzimidazole, m. p. 221°, was prepared by nitration of 2-methylbenzimidazole [7] and from diacetyl-*o*-phenylenediamine by nitration and cyclisation.[10]

1,2-Dimethyl-5-nitrobenzimidazole, m. p. 229°, was prepared by the method of Fries *et al.*[11] from 2-amino-*N*-methyl-4-nitroaniline. 1,2-Dimethyl-6-nitrobenzimidazole was prepared similarly from 2-amino-*N*-methyl-5-nitroaniline and also from the products of methylation of 2-methyl-5(6)-nitrobenzimidazole by fractional precipitation and crystallisation. The products from the two preparations had the same infrared spectrum and m. p. (252°) (Found: C, 57·0; H, 5·2; N, 22·3. Calc. for $C_9H_9N_3O_2$: C, 56·5; H, 4·7; N, 22·0%). The intermediate nitroaniline derivative was given to us by Dr. Montanari.[9]

4(7)-Nitrobenzimidazole, m. p. 240° (from aqueous ethanol), was prepared from 2,6-dinitroaniline by the method of van der Want.[12] 1-Methyl-4-nitrobenzimidazole was given to us by Dr. Montanari. 1-Methyl-7-nitrobenzimidazole, m. p. 126°, was prepared from *N*-methyl-2,6-dinitroaniline by reduction with sodium sulphide and cyclisation with formic acid.[9]

[4] Phillips, *J.*, 1931, 1143.
[5] Weidenhagen and Herrmann, *Ber.*, 1935, **68**, 1953.
[6] Hazeldine, Pyman, and Winchester, *J.*, 1924, **125**, 1431.
[7] Fischer and Hess, *Ber.*, 1903, **36**, 3968.
[8] Davies, Mamalis, Petrow, and Sturgeon, *J. Pharm. Pharmacol.*, 1951, **3**, 420.
[9] Leandri, Mangini, Montanari, and Passerini, *Gazzetta*, 1955, **85**, 773.
[10] Phillips, *J.*, 1928, 175.
[11] Fries, Modrow, Raëke, and Weber, *Annalen*, 1927, **454**, 219.
[12] van der Want, *Rec. Trav. chim.*, 1948, **67**, 45.

5(6)-Bromo-2-methylbenzimidazole (m. p. 215°) was prepared from 4-bromo-2-nitroaniline by Remmers's method.[13] 5-Bromo-1,2-dimethylbenzimidazole was prepared from 4-bromo-*N*-methyl-2-nitroaniline by reduction with tin and hydrochloric acid, followed by extraction with ether from an alkaline solution and cyclisation in acetic anhydride in the presence of hydrochloric acid. The product was precipitated with ammonia and had m. p. 139° (from aqueous ethanol). 6-Bromo- (m. p. 146°; lit.,[4] 180°) and 6-chloro-1,2-dimethylbenzimidazole (m. p. 157·5°) were prepared in the same way, starting with 5-bromo- and 5-chloro-*N*-methyl-2-nitroaniline respectively (Found: C, 48·4; H, 4·3; N, 12·5; Br, 36·0. Calc. for $C_9H_9N_2Br$: C, 48·0; H, 4·0; N, 12·5; Br, 35·5%).

Picrates (see Table 3) of substituted benzimidazoles were prepared from mixtures of reactants in hot water or ethanol–water and recrystallised from aqueous ethanol. Quaternary picrates were prepared from aqueous solutions of the iodide with sodium picrate.

Nitration of 2-methyl- to give 2-methyl-5(6)-nitro-benzimidazole is complicated by the simultaneous formation of 2-methyl-5,6-dinitrobenzimidazole. This dinitro-compound was

TABLE 3. *Benzimidazole and benzimidazolium picrates.*

Substituents	M. p.	Formula	Found (%) C	Found (%) H	Found (%) N	Required (%) C	Required (%) H	Required (%) N
Benzimidazole picrates								
2-Me-5(6)-NO_2	201°	$C_{14}H_{10}N_6O_9$	41·9	2·7	19·9	41·4	2·4	20·3
1,2-Me_2-5-NO_2	248 *	$C_{15}H_{12}N_6O_9$	—	—	19·7	—	—	(19·9)
6-Cl-1,2-Me_2	240 *	$C_{15}H_{12}N_5O_7Cl$	43·8	3·1	17·7	43·9	2·9	17·2
5(6)-Br-2-Me	180—181	$C_{14}H_{10}N_5O_7Br$	38·5	2·5	15·7	38·2	2·3	15·9
5-Br-1,2-Me_2	274	$C_{15}H_{12}N_6O_7Br$	39·8	2·7	15·8	39·7	2·6	15·4
6-Br-1,2-Me_2	239—240	$C_{15}H_{12}N_5O_7Br$	39·8	2·9	15·7	39·7	2·6	15·4
Benzimidazolium picrates								
1,3-Me_2-5(6)-NO_2	199	$C_{15}H_{12}N_6O_9$	43·5	3·3	19·4	42·8	3·1	19·9
1,2,3-Me_3-5(6)-NO_2	139—140	$C_{16}H_{14}N_6O_9$	44·9	3·4	19·4	44·2	3·5	19·3
1,2,3-Me_3-5,6-$(NO_2)_2$	183	$C_{16}H_{13}N_7O_{11}$	40·7	3·1	20·7	40·2	2·9	20·3

* With decomp.

also prepared by Kym and Ratner's method,[14] and used in the preparation of 1,2,3-*trimethyl-5,6-dinitrobenzimidazolium iodide* and the corresponding " carbinol base." The iodide was prepared by heating 2-methyl-5(6)-nitrobenzimidazole with two equivs. of methyl iodide at 135° and formed yellow needles, m. p. 268° (from methanol) (Found: C, 32·3; H, 3·0; N, 14·8; I, 32·5. $C_{10}H_{11}N_4O_4I$ requires C, 31·8; H, 2·9; N, 14·8; I, 33·6%). The " carbinol base " was precipitated by the addition of alkali to an aqueous solution of the iodide and recrystallised from aqueous ethanol as yellow needles, m. p. 163—164° (Found: C, 45·4; H, 4·3; N, 19·6. Calc. for $C_{10}H_{12}N_4O_5$: C, 44·8; H, 4·5; N, 20·9%).

Measurement of Dissociation Constants.—The pK_a value of 1-methyl-4-phenylglyoxaline (5·78) was obtained by potentiometric titration of an aqueous solution of the base with hydrochloric acid. The value for 6-chloro-1,2-dimethylbenzimidazole (4·75) was obtained similarly, but in a solution containing 50% by volume of ethanol. A Cambridge pH meter was used, after standardisation with phthalate and borate buffers.

TABLE 4. *Absorption maxima* (mμ) *and extinction coefficients of 2-methyl-5(6)-nitrobenzimidazole and its N-methyl derivatives.*

Benzimidazole	In 0·1N-$HClO_4$ [a] $\lambda_{max.}$	In 0·1N-$HClO_4$ [a] $10^{-4}\varepsilon$	At pH 8 [b] $\lambda_{max.}$	At pH 8 [b] $10^{-4}\varepsilon$	In 0·1N-NaOH [c] $\lambda_{max.}$	In 0·1N-NaOH [c] $10^{-4}\varepsilon$
2-Methyl-5(6)-nitro- ...	226, 287	2·24, 0·968	228·5, 317	1·99, 0·94	250, 367	1·61, 1·14
1,2-Dimethyl-5-nitro-	229·5, 290	2·14, 0·984	240, 320	1·62, 0·923		
1,2-Dimethyl-6-nitro-	230, 287	2·12, 0·968	239, 321	1·64, 1·06		

[a] Conjugate acid. [b] Neutral molecule. [c] Conjugate base.

The other pK_a values in Table 2 were obtained spectrometrically by the methods described in Part I for measurements in buffer solutions. The spectra of most of the compounds studied have already been described,[9] and our results for the positions of the maxima and the values

[13] Remmers, *Ber.*, 1874, **7**, 348.
[14] Kym and Ratner, *Ber.*, 1912, **45**, 3245.

of the extinction coefficients agreed with the published data. Information (Table 4) on the spectra of 2-methyl-5(6)-nitrobenzimidazole and the two *N*-methyl derivatives is new. The spectra were measured in buffer solutions obtained by the partial neutralisation of acetic and chloroacetic acid. For each compound, the groups of spectra obtained at different pH values passed through isosbestic points indicating an absence of specific interaction with the buffer solutions.

Determination of Isomer Ratios.—The following experiments with 5(6)-nitrobenzimidazole illustrate the method of methylation. For methylation in the absence of alkali, the benzimidazole (~8 g.) was mixed with a slight excess of methyl sulphate. A vigorous reaction occurred on warming and a clear solution was formed. After 2—3 min., water was added and the solution was brought to pH 5. The mixture of the two methyl isomers (about 20% yield) was precipitated on storage. The precipitate was filtered off and washed with aqueous sodium hydroxide to remove unchanged starting material. Treating the filtrate with warm alkali gave a 70% yield of *NN'*-dimethyl-4-nitro-*o*-phenylenediamine (derived from the decomposition of the quaternary compound). In the presence of cold alkali, a similar yield of quaternary " carbinol base " was obtained. This was identified by comparison of the infrared spectrum with that of the " carbinol base " prepared from the quaternary iodide. The combined yields of the monomethyl isomers and the quaternary derivatives accounted for about 90% of the initial quantity of 5(6)-nitrobenzimidazole.

In the experiments in basic media, 5(6)-nitrobenzimidazole (~3 g.) was treated in aqueous 2N-sodium hydroxide (100 ml.) with a small excess of methyl sulphate. A mixture of the monomethyl compounds (yield about 70%) separated. The solids were filtered off and on further treatment of the filtrate with methyl sulphate, a 20% yield of *NN'*-dimethyl-4-nitro-*o*-phenylenediamine was obtained.

The infrared spectra of the mixtures of methyl isomers and the reference compounds were first determined in a Nujol mull by conventional techniques with a Grubb–Parsons G2A instrument. Quantitative determination of isomer ratios in chloroform solution was based on the 11·1 μ absorption band of 1-methyl-5-nitrobenzimidazole. A cell of variable length was used, set at vernier readings of 0·4 and 0·8 mm. The instrument was adjusted so that the percentage transmission was 100 at 11·1 μ when the cell contained chloroform alone and the length was 0·4 mm. The amount of the 1-methyl-5-nitro-isomer in the product was then determined from the relative absorption of a 1% solution of the reaction product and a 1% solution of the pure isomer. The following results illustrate the calculation:

Vernier	Transmission (%)			Optical density		
setting (mm.)	$CHCl_3$	1-Me-5-NO_2	Mixture	1-Me-5-NO_2	Mixture	1-Me-5-NO_2 (%)
0·4	100	72·5	83	0·141	0·081	57·5
0·8	86	46·5	60·8	0·267	0·151	56·6

The method was checked by separating about half of the 1-methyl-5-nitro-isomer from the reaction mixture by fractional crystallisation from acetone, and determining from the infrared spectrum the amount remaining. The sum of these quantities agreed with that determined directly. A set of values obtained in separate experiments for the percentage of this isomer in the product was:

Me_2SO_4 alone ..... 52, 53, 58, 56·5
Me_2SO_4 + NaOH 57, 53, 58, 61, 61

This scatter is probably mainly experimental error but may come in part from changes in the conditions since all the reaction mixtures were initially heterogeneous and the yields of monomethyl compounds varied considerably.

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