[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CINCINNATI]

THE EFFECT OF ORGANIC BASES UPON THE EXTENT AND MECHANISM OF THE REDUCING ACTION OF SODIUM METHYLATE ON NITROBENZENE AND AZOXYBENZENE[1]

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Introduction

In a recent study of the reducing action of sodium methylate upon nitrobenzene in methyl alcohol solution, Fry and Cameron[2] have shown quantitatively that the extent to which Klinger's[3] reaction

$$4C_6H_5NO_2 + 3CH_3ONa \longrightarrow 2(C_6H_5N)_2O + 3HCOONa + 3H_2O \qquad (A)$$

proceeds, as measured by the formate yield, was progressively decreased by the addition of increasing quantities of water, but when increasing quantities of pyridine, an organic base, were added, the extent of the reduction was progressively increased. Furthermore, it was found that in the presence of pyridine, azoxybenzene was partially reduced to azobenzene, according to the equation

$$2C_6H_5NO_2 + 2CH_3ONa \longrightarrow C_6H_5N{=}NC_6H_5 + 2HCOONa + 2H_2O \qquad (A')$$

In order to interpret these results, the following equations were proposed to represent the mechanism of the reducing action of sodium methylate.

$$\begin{array}{llll} & 3CH_3ONa + 3HOH & \longrightarrow 3CH_3OH + 3HONa & (x) \\ & 3CH_3OH \;\; + 3NaOH & \longrightarrow 3HCOONa + 12H & (y) \\ & 4C_6H_5NO_2 + 12H & \longrightarrow 2(C_6H_5N)_2O + 6H_2O & (z) \\ \Sigma(x),\ (y),\ (z) & 4C_6H_5NO_2 + 3CH_3ONa & \longrightarrow 2(C_6H_5N)_2O + 3HCOONa + 3H_2O & (A) \end{array}$$

The summation of Equations x, y and z is Klinger's equation (A). It should be noted in passing that the intermediate equation (y) involving the acidic dissociation of sodium hydroxide is an example of a general type equation, $R(H)_n + n\mathrm{HONa} \longrightarrow R(ONa)_n + nH_2$, evidence for which has been given in previous papers.[4]

Consequently, any factor that hinders the acidic dissociation of sodium hydroxide, such as the addition of water, which promotes basic dissociation, limits the extent to which the intermediate equation (y) progresses, and hence limits the yield of formate. This was found to be true. On the other hand, the addition of basic pyridine would favor acidic dissociation and increase the extent of the reaction, as was also observed.

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[1] A synopsis of a thesis presented to the Faculty of the Graduate School, University of Cincinnati, by Paul E. Bowman, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] Fry and Cameron, THIS JOURNAL, **49**, 864 (1927).

[3] Klinger, *Ber.*, **15**, 866 (1882).

[4] Fry, Schulze and Weitkamp, THIS JOURNAL, **46**, 2268 (1924); Fry and Schulze, *ibid.*, **48**, 958 (1925); Fry and Otto, *ibid.*, **50**, 1120 (1928); Fry and Schulze, *ibid.*, **50**, 1131 (1928); Fry and Otto, *ibid.*, **50**, 1138 (1928).

To test further the validity of this proposed explanation of the reducing action of sodium methylate, based upon the apparent acidic dissociation of sodium hydroxide, experiments with five other bases were conducted.

Experimental

The previously described standardized methods of procedure and analysis,[2] in effecting the reduction of nitrobenzene and azoxybenzene with sodium methylate, in methyl alcohol solution, were again employed. In the first place, three independently repeated reductions of nitrobenzene, each in duplicate and with no organic base present, gave checks varying by not more than 1% and averaging 88.45% of the theoretical yield of sodium formate according to Klinger's equation (A). This figure is used as a standard for comparison in all subsequent experiments in measuring the extent of the reduction, calculated from yields of sodium formate.

Reductions of (a) nitrobenzene and (b) azoxybenzene were conducted in a reaction mixture of 250-cc. volume prepared in duplicate and containing sodium methylate, made by dissolving 17.3 g. of sodium in methyl alcohol, together with either 0.2 mole of nitrobenzene or 0.1 mole of azoxybenzene incorporated with $^1/_8$, $^1/_4$, $^1/_2$ or $^3/_4$ mole of each one of the five organic bases used, namely, (A) aniline, (B) monomethylaniline, (C) dimethylaniline, (D) quinoline and (E) iso-amylamine. This entailed a total of eighty separately conducted reductions.

The reaction mixtures were heated in 500-cc. round-bottomed flasks, with reflux condensers, on an actively boiling water-bath for five hours. The reaction mixtures were then treated as described in a previous paper.[2] The extents of the reduction of both nitrobenzene and azoxybenzene were

TABLE I

SUMMARY OF DATA: EFFECTS OF ORGANIC BASES UPON THE EXTENT OF REDUCTION OF NITROBENZENE

Moles of organic base	A	B	C	D	E
	Extent of reduction, %				
	Aniline	Monomethyl-aniline	Dimethyl-aniline	Quinoline	Iso-amylamine
$^1/_8$	96.85	91.70	93.93	95.62	63.45
$^1/_4$	99.26	98.61	98.95	102.66	40.70
$^1/_2$	98.28	103.06	105.56	107.62	38.22
$^3/_4$	94.26	104.44	107.12	113.02	51.75

TABLE II

SUMMARY OF DATA: EFFECTS OF ORGANIC BASES UPON THE EXTENT OF REDUCTION OF AZOXYBENZENE

Moles of organic base	A	B	C	D	E
	Extent of reduction, %				
	Aniline	Monomethyl-aniline	Dimethyl-aniline	Quinoline	Iso-amylamine
$^1/_8$	3.00	3.52	3.43	3.53	3.00
$^1/_4$	3.38	4.00	3.47	4.80	2.61
$^1/_2$	4.18	4.77	7.30	6.33	2.76
$^3/_4$	4.93	5.46	8.72	7.43	2.52

calculated in terms of the yield of sodium formate, using Equation A above for the nitrobenzene reduction and Equation A′ above for the azoxybenzene reduction.

Since the checks obtained in every reduction conducted in duplicate differed less than 1%, the yields of sodium formate are averaged for the duplicate reductions. These averages are recorded in Tables I and II.

Discussion of Data

In every reduction of nitrobenzene, Table I, the presence of an organic base, with the exception of iso-amylamine, increased markedly the extent of the reduction beyond the normal 88.45% when no organic base was present. With the exception of iso-amylamine and aniline, the increase in the extent of the reduction follows the order of the increasing molar concentrations of the bases present and their respectively increasing basic properties.

The decrease in the yields of sodium formate when aniline was present (Column A, Table I) is probably due to the fact, observed by Baeyer,[5] that nitrosobenzene, an intermediately formed reduction product of nitrobenzene, reacts with aniline according to the equation $C_6H_5NO + C_6H_5NH_2 \longrightarrow C_6H_5N{=}NC_6H_5 + H_2O$, and produces no sodium formate.

The peculiar behavior with iso-amylamine will be considered in detail later.

The data of Table II show that azoxybenzene is somewhat susceptible to reduction to azobenzene by sodium methylate, which is oxidized to sodium formate provided an organic base is present. This serves to explain the partly increased yields of sodium formate and is in harmony with the assumption that organic bases favor acidic dissociation of sodium hydroxide, thereby effecting reduction of azoxybenzene to azobenzene—*a reduction which does not occur in the absence of an organic base.*

Here also, with the exception of iso-amylamine, the increase in the extent of the reduction follows the order of increasing molar concentrations of bases present, but quinoline, which is more basic than dimethylaniline, effected less reduction than the latter when $^1/_2$ and $^3/_4$ moles were present. These anomalies are reserved for future investigation.

The Exceptional Effect of Iso-amylamine

Iso-amylamine being more basic than the other four bases used should have greatly increased the yield of sodium formate, but the low yields of formate (Column E, Table I) were entirely out of harmony with the expected results and, furthermore, the amount of azoxybenzene was greatly in excess of that corresponding to the sodium formate obtained.

The key to these anomalies was the finding of *formaldehyde* in the reaction mixture *in combination with iso-amylamine.* When the azoxybenzene–

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[5] Baeyer, *Ber.*, 7, 1639 (1874).

benzene extract of the reaction mixture was treated with dilute sulfuric acid, the presence of formaldehyde was shown by its odor and characteristic reactions. When the acid extract was neutralized, the presence of iso-amylamine was attested by its odor. This is in line with the work of Henry,[6] who has shown that when a primary amine reacts with formaldehyde, a compound is formed (in this case, iso-amylmethylene-imide) which, upon addition of dilute acids, is resolved into the amine and formaldehyde.

The finding of formaldehyde in the reaction mixture is confirmation of the intermediate type reaction equation previously postulated[4] and according to which the equation for the reaction can be developed as follows:

$$\begin{array}{rllll}
 & 3CH_3OH & + 3NaOH & \longrightarrow 3CH_3ONa + 3H_2O & (x) \\
 & 3CH_3ONa & + 3HONa & \longrightarrow 3CH_2(ONa)_2 + 6H & (y) \\
 & 3CH_2(ONa)_2 & + 3H_2O & \longrightarrow 3CH_2O + 6NaOH & (z) \\
 & 2C_6H_5NO_2 & + 6H & \longrightarrow (C_6H_5N)_2O + 3H_2O & (z') \\
\Sigma(x), (y), (z), (z') & 2C_6H_5NO_2 & + 3CH_3OH & \longrightarrow 3CH_2O + (C_6H_5N)_2O + 3H_2O & (B)
\end{array}$$

This equation (B) accounts for the presence of formaldehyde in the reaction mixture and also, since no sodium formate is formed as in the Klinger equation (A), for the yield of azoxybenzene in excess of that indicated by the yield of sodium formate. The determination of the amount of formaldehyde combined with iso-amylamine in the reaction mixture would serve to measure the extent of the concurrence of Reaction B.

Quantitative Determination of Formaldehyde Combined with Iso-amylamine.—The following method was devised for estimating quantitatively the amounts of formaldehyde combined with the iso-amylamine in the reaction mixtures, depending upon the fact that iso-amylamine, analogously to ammonia, reacts with mercuric chloride to give a white precipitate of mercuric iso-amylamidochloride. The azoxybenzene–benzene extracts of the reaction mixtures were treated with dilute sulfuric acid, the separated acid solution was diluted to 500 cc., and 10-cc. aliquot portions of this solution, containing iso-amylamine sulfate and free formaldehyde, were saturated with mercuric chloride. Then, as dilute sodium hydroxide solution was added, drop by drop, the iso-amylamine was set free and combined with mercuric chloride, precipitating mercuric iso-amylamidochloride. Further addition of sodium hydroxide was continued until all the excess mercuric chloride was converted to mercuric oxide, but care must be taken to avoid an excess of sodium hydroxide since mercuric oxide is reduced by formaldehyde in a too alkaline solution. After filtration, the filtrate was neutralized and its formaldehyde content determined by the standard hydrogen peroxide method.[7]

Solutions of known content of the iso-amylamine–formaldehyde compound gave check results by this new method.

Determination of the Distribution of the Reduction of Nitrobenzene between Two Concurrent Reactions.—Since nitrobenzene in the presence of iso-amylamine is reduced according to the following equations

$$\begin{array}{ll}
4C_6H_5NO_2 + 3CH_3ONa \longrightarrow 2(C_6H_5N)_2O + 3HCOONa + 3H_2O & (A) \\
2C_6H_5NO_2 + 3CH_3ONa \longrightarrow (C_6H_5N)_2O + 3CH_2O + 3NaOH & (B)
\end{array}$$

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[6] Henry, *Ber.* (Ref.), **26**, 934 (1893).

[7] "J. Assoc. Official Agr. Chemists," **1916**, p. 75.

and since the quantities of sodium formate and formaldehyde, respectively, produced have been determined, it is possible to calculate therefrom the extent to which nitrobenzene is reduced in conformity with each of these respective reactions.

In Table III the determined and the calculated yields in their relationships to the concurrent reactions (A) and (B) are fully tabulated.

TABLE III

DATA ON REDUCTION OF NITROBENZENE ACCORDING TO CONCURRENT EQUATIONS (A) AND (B)

A Isoamylamine, moles	B Distribution Equations, A and B	C HCOONa Eq. A found, g.	D HCHO Eq. B found, g.	E $(C_6H_5N)_2O$ calcd., g.	F $C_6H_5NO_2$ calcd., g.	G $C_6H_5NO_2$ reduced, %	H Total reduction, Eq. A and Eq. B, %
$^1/_8$	A	6.4747	....	12.5685	15.6213	63.50	88.74
	B	....	2.2714	4.9917	6.2085	25.24	
$^1/_4$	A	4.1517	....	8.0591	10.0129	40.70	87.22
	B	....	4.1868	9.2109	11.4438	46.52	
$^1/_2$	A	3.8992	....	7.5680	9.4039	38.22	93.43
	B	....	4.9690	10.9318	13.5812	55.21	
$^3/_4$	A	5.2786	....	10.2467	12.7310	51.75	86.61
	B	....	3.1379	6.9033	8.5678	34.86	

The data in Column H show that the nitrobenzene is more extensively reduced in the presence of iso-amylamine than is indicated by the corresponding data in Table I, Column E, which, it should be recalled, relate only to the yield calculated as formate on the basis of the occurrence of Reaction A. In Column F, Table III, a marked anomaly is noted when $^3/_4$ mole of iso-amylamine was present in that Reaction A is more extensive than Reaction B. This unexpected result has not yet been explained and is reserved for further investigation as a special study.

To check and establish further the concurrence of Reactions A and B, the actual amounts of azoxybenzene found in reaction mixtures with $^1/_8$ and $^1/_2$ mole of iso-amylamine present, were recovered, purified and weighed. The *actual yields* of azoxybenzene were found to be practically equal to the sum of the *calculated yields* of azoxybenzene formed in conformity with the concurrent reactions (A) and (B). Table IV embodies the data.

TABLE IV

DATA CONFIRMING CONCURRENT REACTIONS (A) AND (B)

Iso-amylamine, moles	HCOONa (Eq. A) found, g.	HCHO (Eq. B) found, g.	$(C_6H_5N)_2O \Leftrightarrow$ HCOONa (Eq. A) calcd., g.	$(C_6H_5N)_2O \Leftrightarrow$ HCHO (Eq. B) calcd., g.	$(C_6H_5N)_2O \Leftrightarrow$ (Eq. A) + (Eq. B) calcd., g.	$(C_6H_5N)_2O$ found, g.	$\frac{\text{Calcd., g.}}{\text{Found, g.}}$ %
$^1/_8$	6.4747	2.2714	12.5685	4.9971	17.5656	17.53	100.20
$^1/_2$	3.8992	4.9690	7.5680	10.9318	18.4998	18.79	98.45

General Conclusions

1. The presence of an organic base markedly increases the extent of the reduction of nitrobenzene to azoxybenzene by sodium methylate.
2. The presence of an organic base causes some further reduction of azoxybenzene to azobenzene.
3. Increasing molar concentrations of organic bases present in the reaction mixtures caused increased percentage reductions of nitrobenzene and of azoxybenzene.
4. Formaldehyde, one of the predicted intermediate products of the reaction mechanism scheme, is formed in the reaction mixtures containing iso-amylamine, with which it combines to form iso-amylmethylene-imide.
5. The formaldehyde so combined with the iso-amylamine can be isolated and quantitatively determined according to a newly proposed method.
6. The yield of formaldehyde conforms to the equation for a new reaction, $2C_6H_5NO_2 + 3CH_3ONa \longrightarrow (C_6H_5N)_2O + 3CH_2O + 3NaOH$, which is concurrent with Klinger's reaction, $4C_6H_5NO_2 + 3CH_3ONa \longrightarrow 2(C_6H_5N)_2O + 3HCOONa + 3H_2O$.
7. The distribution of the extent of the reduction of nitrobenzene in the presence of iso-amylamine has been calculated and found to conform to the equation for the concurrent reactions noted in (6).
8. The observations of this study afford additional evidence for the previously proposed reaction mechanism schemes involving the apparent acidic dissociation of sodium hydroxide.

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THE ACTION OF DIAZOMETHANE ON THE PYRIMIDINE CONSTITUENTS OF NUCLEIC ACIDS

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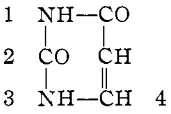
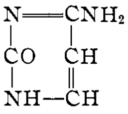
Notwithstanding the large amount of work which has been devoted to the study of nucleic acids, the position of attachment of the carbohydrate nucleus to the pyrimidine constituents (uracil, I, cytosine, II and thymine, III) still awaits definite solution, although the burden of evidence[1] appears to be increasingly in favor of linkage in position 3 rather than 4.



I



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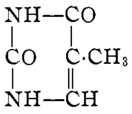

III

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[1] Burian, *Ber.*, **37**, 708 (1904); Johnson and Clapp, *J. Biol. Chem.*, **5**, 163 (1908); Wheeler and Johnson, *ibid.*, **3**, 183 (1907); *cf.* Ref. 2.