Detoxication Mechanisms. II. The Iron-Catalyzed Dealkylation of Trimethylamine Oxide[1-3]

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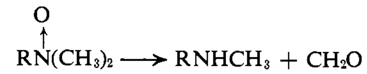
Abstract: The catalytic role of iron salts in the decomposition of tertiary amine oxides to a secondary amine and an aldehyde has been reinvestigated and clarified. In contrast to previous reports iron(II) rather than iron(III) has been found to be the initiator for the reaction. The reaction has been shown to proceed by a free-radical path, and trapping experiments with butadiene have demonstrated the presence of the amminium radical ion. Mechanisms 4 and 6 are proposed in acid solution. Tertiary amine is also a product of the reaction. It is produced as shown in eq 5. The $(CH_3)_3N$ to CH_2O ratio and the reaction rate vary with the anion used and increase in the order ClO_4^-, SO_4^{2-}, Cl^-, PO_4^{3-}. The stability constants of the corresponding iron(II) complexes also increase in this order. The enhanced rate and increased yield of $(CH_3)_3N$ can be explained by chelation accelerating eq 4 and 5 and decelerating 6.

The N-oxides of aliphatic amines occur extensively in biological systems but little is known about the function of these compounds. In plants amine oxides have been postulated as intermediates in the synthesis of alkaloids.[4,5] Tracer studies have demonstrated that the "berberine bridge" is formed from a tertiary amine, possibly *via* the N-oxide.[5]

In mammalian systems there has been a considerable controversy concerning the role of the amine oxide function in the oxidative dealkylation of tertiary amines. It has been demonstrated that the microsomal fraction of the liver cells (derived from the endoplasmic reticulum) is the site of this oxidative dealkylation of amines. The oxidative enzyme systems require molecular oxygen and NADH and are distinguished by their ability to oxidize almost any organic compound regardless of structure. For example, aromatic compounds are oxidized to phenols, alkyl chains to acids, and amines are oxidized to aldehydes and acids. The enzymes contain iron in both the II and III oxidation states.[6]

Horning and co-workers[7] were the first to observe that liver homogenates oxidize tertiary amines to the amine oxides and cleave the N-oxides to secondary amines and aldehydes.



$$R\overset{\overset{\displaystyle O}{\uparrow}}{N}(CH_3)_2 \longrightarrow RNHCH_3 + CH_2O$$

These workers postulated that N-oxide was an intermediate in the detoxication of tertiary amines. They were also able to carry out this rearrangement by using simple iron chelates and tertiary amine oxides. There have been a number of additional reports of the oxidation of tertiary amines to N-oxides by liver homogenates.[8] However, studies using liver microsomal fractions have not been able to clearly establish the intermediacy of the amine oxide group in the cleavage reaction.[9] An alternative hypothesis involving direct cleavage of the tertiary amine without the intermediacy of the N-oxide has also been proposed.[10]

It appears unlikely that it will be possible to determine if the amine oxide is an intermediate in the detoxication of amines because of the heterogeneous nature of the liver microsomal fraction. Therefore, we undertook a study of the simpler reactions between iron chelates, amines, and amine oxides with the goal of achieving a better understanding of the biological system.[11] Our first paper will be concerned with the reaction of iron chelates with amine oxides.

Horning and co-workers first discovered that iron(III) chelates react with tertiary amine oxides.[7]

$$R\overset{\overset{\displaystyle O}{\uparrow}}{N}(CH_3)_2 \longrightarrow RNHCH_3 + CH_2O + RN(CH_3)_2$$

In a collaborative study Craig, Dwyer, Glazer, and Horning investigated the mechanism of the reaction of

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[1] Direct inquiries to J. P. F., Department of Chemistry, Rensselaer Polytechnic Institute, Troy, N. Y. 12180. For Part I, see J. P. Ferris and R. D. Gerwe, *Tetrahedron Letters*, 1613 (1964).

[2] This research was supported by U. S. Public Health Service, Grant 13572 from the Institute of General Medical Sciences of the Public Health Service.

[3] Abstracted in part from the Ph.D. Thesis of R. D. G., Florida State University, 1965, U. S. Public Health Service Predoctoral Fellow, 1962–1965.

[4] E. Wenkert, *Experientia*, 10, 346 (1954).

[5] D. H. R. Barton, R. H. Hesse, and G. W. Kirby, *Proc. Chem. Soc.*, 267 (1963); A. R. Battersby, R. J. Francis, M. Hirst, and J. Staunton, *ibid.*, 268 (1963).

[6] R. T. Williams, "Detoxication Mechanisms," John Wiley and Sons, Inc., New York, N. Y., 1959; T. E. King, H. S. Mason, and M. Morrison, "Oxidases and Related Redox Systems," John Wiley and Sons, Inc., New York, N. Y., 1965; K. Bloch and O. Hayaishi, "Biological and Chemical Aspects of Oxygenases," Maruzen Company, Ltd., Tokyo, 1966.

[7] M. S. Fish, N. M. Johnson, E. P. Lawrence, and E. C. Horning, *Biochim. Biophys. Acta*, **18**, 564 (1955); M. S. Fish, C. C. Sweeley, N. M. Johnson, E. P. Lawrence, and E. C. Horning, *ibid.*, 21, 196 (1956); M. S. Fish, C. C. Sweeley, and E. C. Horning, *Chem. Ind.* (London), 24 (1956); M. S. Fish, N. M. Johnson, and E. C. Horning, *J. Am. Chem. Soc.*, **78**, 3668 (1956); C. C. Sweeley and E. C. Horning, *ibid.*, **79**, 2620 (1957).

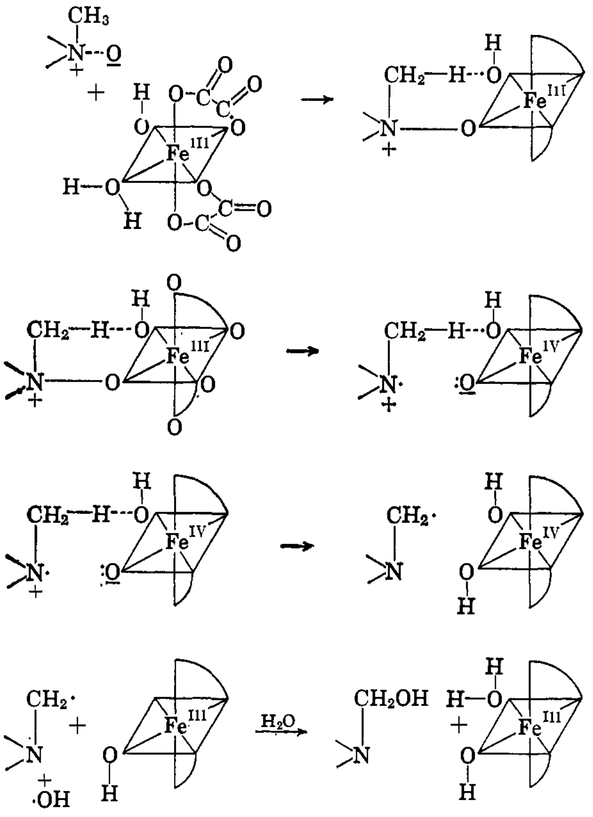
[8] J. Baker and S. Chaykin, *Biochim. Biophys., Acta*, **41**, 548 (1960); S. Chaykin and K. Bloch, *ibid.*, 31, 213 (1959); A. May, *Enzymologia*, **18**, 142 (1957).

[9] D. M. Ziegler and F. H. Pettit, *Biochem. Biophys. Res. Commun.*, **15**, 188 (1964); F. H. Pettit, W. Orme-Johnson, and D. M. Ziegler, *ibid.*, **16**, 444 (1964); F. H. Pettit and D. M. Ziegler, *ibid.*, **13**, 193 (1963).

[10] H. Keberle, W. Riess, W. Schmid, and K. Hoffmann, *Arch. Intern. Pharmacodyn.*, **142**, 125 (1963); R. E. McMahon and H. R. Sullivan, *Life Sci.*, **3**, 1167 (1964); R. E. McMahon and N. R. Easton, *J. Pharmacol. Exptl. Therap.*, **135**, 128 (1962); R. E. McMahon and N. R. Easton, *J. Med. Pharm. Chem.*, **4**, 437 (1961); R. E. McMahon, *Tetrahedron Letters*, 2307 (1966); R. E. McMahon, *J. Pharm. Sci.*, **55**, 457 (1966).

[11] The rationale for this approach has been stated succinctly by G. L. Eichorn, "Metal Ion Catalysis in Biological Systems," Advances in Chemistry Series, No. 37, American Chemical Society, Washington, D. C., 1963, pp 45–47.

trimethylamine oxide with iron(III) chelates.[12] These workers postulated the following mechanism.



The most efficient dealkylating catalyst proved to be one iron(III) ion chelated with two molecules of oxalic acid. Thus, two adjacent nonchelated sites on the iron were considered to be essential for the reaction. The tertiary amine products were assumed to originate from the reduction of the amine oxide with formaldehyde.

$$(CH_3)_3NO + CH_2O \longrightarrow (CH_3)_3N + HCO_2H$$

Radical intermediates were postulated by analogy in the reaction of amine oxides with anhydrides (Polonovski reaction[13]) which yields free radicals. Literature precedent for the iron(IV) oxidation state was cited.

Since certain aspects of the mechanism of Craig, *et al.*,[12] were not proved and because we felt that the iron(IV) oxidation state seemed unlikely under the reaction conditions, we reexamined this dealkylation reaction.[14]

Results and Discussion

We confirmed that heating a mixture of iron(III) nitrate, oxalic acid, and trimethylamine oxide yielded formaldehyde.[12] Gas chromatographic analysis proved that dimethylamine and trimethylamine are also products of the reaction.

Our initial mechanism studies were directed to the postulate that iron(III) was the catalyst in the reaction.[12] Iron(II) had been reported to catalyze the reaction,[12,15] and we felt this was a more likely possibility. In contrast to the previous report[12] we found that iron(III) did not catalyze the rearrangement. However, iron(II) is an efficient catalyst. These data suggest that iron(II) is produced when iron(III) is heated in the presence of reducing acids. The reduction of iron(III) to iron(II) by citric and tartaric acids has been reported by Clark.[16] We found that when mixtures of iron(III) and oxalic acid are heated together iron(II) is formed. When a nonreducing acid (sulfuric, succinic) is used, no iron(II) is produced (Table I). No formaldehyde is obtained from trimethylamine oxide by reaction with iron(III) and these acids. These data demonstrate the central role of iron(II) in the reaction but do not eliminate the possibility that iron(III) is essential to the reaction also.

Table I. Reduction of Iron(III) to Iron(II)[a]

Acid	Fe(II), $M \times 10^{-2}$ Before heating	After 40 min at 100°	Fe(III) converted to Fe(II), $M \times 10^{-2}$	% Fe(III) converted to Fe(II)
Oxalic acid	0.06	9.1	9.0	45
Succinic acid	0.77	0.94	0.17	~1
Sulfuric acid	0.79	0.81	0.02	<1

[a] Initial concentration of $Fe_2(SO_4)_3$ was 0.10 *M*.

Table II. Formation of Iron(III) during Iron(II)-Catalyzed Demethylation of Trimethylamine Oxide

Heating period at 100°, hr	Iron(III),[a] moles × 10^{-3}	Iron(II),[b] moles × 10^{-3}
None	0.2	14
1	2.0	9.6
2	3.4	8.8
4.18	4.2	5.6
25.1[b]	4.0	5.0

[a] As a result of mechanical difficulties, the total amount of iron(II) plus iron(III) detected after each heating period was not constant. The data do demonstrate the gradual oxidation of iron(II) to iron(III) throughout the N-oxide dealkylation. [b] Reaction of trimethylamine oxide with iron(II) sulfate is complete in 6 hr, hence the small change between 4.18 and 25.1 hr.

The previous mechanisms suggested for this rearrangement did not account for the formation of tertiary amines. These were suggested to have been formed in a side reaction by the reduction of the amine oxide with formaldehyde.[12] This postulate was disproven by heating formaldehyde and trimethylamine oxide under the reaction conditions. Formaldehyde was recovered quantitatively. Furthermore, if trimethylamine is produced in this way the yield of formaldehyde should be equal to that of dimethylamine minus the yield of trimethylamine. Direct analysis showed that the yields of dimethylamine and formaldehyde were equal.

It seemed likely that trimethylamine was produced by an iron(II) reduction of the amine oxide. The yield of formaldehyde is maximum when the molar ratio of Fe(II) to amine oxide is 2.2 but decreases as the ratio increases. Furthermore, it was demonstrated that iron(II) is converted to iron(III) during the course of the re-

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(12) J. C. Craig, F. P. Dwyer, A. N. Glazer, and E. C. Horning, *J. Am. Chem. Soc.*, **83**, 1871 (1961).

(13) M. Polonovski and M. Polonovski, *Bull. Soc. Chim. France*, **41**, 1190 (1927).

(14) Oxidation of iron(II) by H_2O_2 has been shown not to yield iron(IV) [T. J. Conocchioli, E. J. Hamilton, Jr., and N. Sutin, *J. Am. Chem. Soc.*, 87, 926 (1965)]. On the basis of the close similarity between the protonated amine oxide and hydrogen peroxide, it is unlikely that iron(IV) is produced from the amine oxide.

(15) M. Ishidate and A. Hanaki, *Nature*, **191**, 1198 (1961); A. Hanaki and M. Ishidate, *Biochim. Biophys. Acta*, **57**, 180 (1962). The authors postulate an intermediate N-oxide but never prove its presence.

(16) L. J. Clark, *Anal. Chem.*, **34**, 348 (1962).

Table III. Variations of Product Yields and Reaction Rates with Anion

	Catalyst			
	$Fe(ClO_4)_2$	$FeSO_4$	$FeSO_4 + KH_2PO_4$	$FeCl_2$
Total volatile amine, %	92	96,98	98	96
HCHO, %	79	65	13	10
Me_3N, %	13[a]	33,37[b]	85[a]	86[a]
Fe(III),[c] %	21,21	65	175	188
Half-time for formaldehyde production, min	105	52	10	40 (estimated)

[a] Obtained from the difference in yield of total amine and formaldehyde. [b] Obtained by an independent titration (see Experimental Section). [c] Yield of iron(III) equals (no. of moles iron(III) formed/no. of moles Me_3NO reacted) × 100. The amount of iron(III) was measured by titration of unreacted iron(II) with Ce(IV).

Table IV. Reaction of Trimethylamine Oxide with Iron(II) in the Presence of Added Alcohols

Added alcohol	Iron salt	Time	Product	Yield,[a] %	Yield of HCHO in absence of added alcohol, %
Cyclohexanol	$Fe(ClO_4)_2$	40 min	Cyclohexanone	10	13
Benzyl alcohol	$Fe(ClO_4)_2$	24 hr	Benzaldehyde	70	79

[a] Based on trimethylamine oxide; 5 g of the alcohol was added to the standard reaction mixture. The product was identified by the infrared spectrum, melting point, and tlc mobility of its 2,4-DNP derivative. Controls with the iron salt and amine oxide omitted yielded no carbonyl products.

arrangement (Table II). Finally, analysis of several reactions revealed that two iron(III) ions are produced per molecule of trimethylamine (Table III). These data clearly show trimethylamine is produced by reduction of the amine oxide by iron(II).

Craig, *et al.*,[12] postulated free-radical intermediates without experimental verification. The above implication of iron(II), a one-electron reducing agent, is consistent with a radical reaction. More direct evidence is our observation that ethyl methacrylate polymerizes when added to the reaction mixture.

It was necessary to get a direct measure of the number of radicals produced in the reaction since such a polymer could result from a small number of radicals which were not involved in the main course of the reaction. This measurement was made possible by adding alcohols to the reaction mixture. Because alcohols are oxidized to carbonyl compounds by radicals (eq 1–3),[17] the number of these radicals can be estimated from the observed yield of aldehyde or ketone.

$$R_3\overset{+}{N}\cdot + R_2\overset{H}{C}OH \longrightarrow R_3\overset{+}{N}H + R_2COH \qquad (1)$$

$$R_2\dot{C}OH + Fe(III) \longrightarrow R_2CO + Fe(II) + H^+ \qquad (2)$$

or

$$R_2\dot{C}OH + R_3\overset{+}{N}OH \longrightarrow R_2C(OH)_2 + R_3\overset{+}{N}\cdot \qquad (3)$$
$$\searrow R_2CO + H_2O$$

When excess cyclohexanol or benzyl alcohol was added to the reaction mixture, no formaldehyde was produced (Table IV). Furthermore, the yield of carbonyl derivative was equal to that of the formaldehyde usually produced in the absence of added alcohol. These results clearly show that the main course of the rearrangement is by a free-radical pathway.

The experimental data to date suggested the following reaction steps.

$$(CH_3)\overset{+}{N}OH + Fe(II) \xrightarrow{H^+} (CH_3)_3\overset{+}{N}\cdot + Fe(III) + H_2O \qquad (4)$$

$$(CH_3)_3\overset{+}{N}\cdot + Fe(II) \xrightarrow{H^+} (CH_3)_3\overset{+}{N}H + Fe(III) \qquad (5)$$

$$(CH_3)_3\overset{+}{N}\cdot + Fe(III) \longrightarrow (CH_3)_2\overset{+}{N}{=}CH_2 + H^+ + Fe(II) \qquad (6)$$
$$\xrightarrow{H_2O} (CH_3)_2\overset{+}{N}H_2 + CH_2O$$

or

$$(CH_3)_3\overset{+}{N}\cdot \longrightarrow (CH_3)_2\underset{H}{\overset{+}{N}}CH_2\cdot \qquad (7)$$

$$(CH_3)_2\underset{H}{\overset{+}{N}}CH_2\cdot + (CH_3)_3\overset{+}{N}OH \longrightarrow$$
$$(CH_3)_2\underset{H}{\overset{+}{N}}CH_2OH + (CH_3)_3\overset{+}{N}\cdot \qquad (8)$$
$$\longrightarrow (CH_3)_2\overset{+}{N}H_2 + CH_2O$$

The formation of the amminium radical ion (4) is undoubtedly the first step in the reaction. This step finds precedence in the closely related reactions of iron(II) with peroxides[18] and chloramines.[19] The reduction of such an intermediate to trimethylamine (5) has ample precedent[19] as well and is consistent with our experimental data. We considered two possible ways for formaldehyde formation, either oxidation of the amminium radical ion with iron(III) (6) or radical-induced decomposition of the amine oxide by the tautomer of the amminium radical ion (7 and 8). Step 6 was suggested by similar postulates for the reaction of iron(III) and copper(II) with peroxide systems.[18] The nitrogen to carbon radical conversion suggested in

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(17) B. R. Cowley and W. A. Waters, *J. Chem. Soc.*, 1228 (1961); B. G. Gowenlock and D. R. Snelling, "The Reactions of Alkyl-Substituted Amino Radicals," Advances in Chemistry Series, No. 36, American Chemical Society, Washington, D. C., 1962, p 154; I. M. Kolthoff and A. I. Medalia, *J. Am. Chem. Soc.*, 71, 3777 (1949); I. M. Kolthoff and A. I. Medalia, *ibid.*, 71, 3784 (1949); see also papers by N. Uri, *Chem. Rev.* 50, 404 (1952); E. S. Huyser and C. J. Bredeweg, *J. Am. Chem. Soc.*, **86**, 2401 (1964).

(18) For recent reviews see L. S. Boguslavakaya, *Russ. Chem. Rev.*, 34, 503 (1965); J. K. Kochi, *Science*, **155**, 415 (1967).

(19) For a recent review see M. E. Wolff, *Chem. Rev.*, **63**, 55 (1963). See also F. Minisci and R. Galli, *Tetrahedron Letters*, 167 (1964), and other papers in this series.

(7) has also been postulated in the Polonovski and other reactions,[20] whereas (8) is similar to steps in the mechanisms postulated for the oxidation of alcohols to carbonyl compounds[17] and the radical-induced decomposition of peroxides.[21] Finally, one cannot eliminate the possibility that some of the product results from radical disproportionation (9).[22]

$$2(CH_3)_3\overset{+}{N}\cdot \longrightarrow (CH_3)_2\overset{+}{N}{=}CH_2 + (CH_3)_3\overset{+}{N}H \qquad (9)$$

However, disproportionation is probably not a major pathway as it would place an upper limit of 50% on the formaldehyde yield. Normally the yields are in the 60–80% range. In addition, it is difficult to postulate a mechanism for the interaction of two positive amminium radical ions.

We next attempted to trap the free-radical intermediate[23] with the aim of distinguishing between the quarternary ammonium radical ion of step 6 and the tautomeric carbon radical postulated in steps 7 and 8. When the amine oxide rearrangement was conducted in the presence of butadiene an 83% yield of a quarternary amine adduct was isolated. This material may be a mixture of dimers resulting from radicals I and II. No butadiene adducts that are tertiary amines were obtained from this reaction. The formation of a quarternary product suggests that the amminium radical ion is the principal species in solution and not the tautomeric carbon radical (see steps 7 and 8). Furthermore the presence of oxygen does not affect the yield of formaldehyde. Carbon radicals should be readily attacked by oxygen while nitrogen radicals should not be affected.

$$(CH_3)_3\overset{+}{N}CH_2CH{=}CHCH_2\cdot$$

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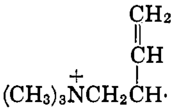

$$(CH_3)_3\overset{+}{N}CH_2\underset{}{\overset{\overset{\displaystyle CH_2}{\|}}{\overset{\displaystyle CH}{|}}}\!\!\!\!CH\cdot$$

II

Finally, if eq 7 were correct, it should be possible to induce amine oxide decomposition with other radicals. No formaldehyde was observed when the amine oxide was heated with benzoyl peroxide and di-*t*-butyl peroxide. Decomposition of chloramines (to yield amminium radical ions) in the presence of trimethylamine oxide also gave no formaldehyde. These data suggest that eq 7 and 8 are not the important pathways for the formation of formaldehyde from trimethylamine oxide.

Since the radical-induced decomposition of the amine oxide seemed unlikely, the possibility of iron(III) oxidation of the amminium radical ion was tested (eq 6). If (6) is correct then there should be a competition between iron(II) and iron(III) for the ammonium radical ion to give trimethylamine and formaldehyde, respectively. We have already shown that a large excess of iron(II) depresses the yield of formaldehyde. Experiments in which equimolar quantities of iron(III) were added to the decomposition have led to enhanced yields of formaldehyde (Table V), a result consistent with (6).

Table V. Effect of Added Salts on the Formaldehyde Yield

Catalyst[a]	Added salt[b] (moles)	Reflux time, min	Formaldehyde yield, %
$Fe(ClO_4)_2$		100	28, 30
$Fe(ClO_4)_2$	$NaClO_4$ (0.08)	100	28, 29
$Fe(ClO_4)_2$	$Fe(ClO_4)_3$ (0.0133)	100	33, 36, 37, 38
$FeSO_4$		40	28, 31
$FeSO_4$	Na_2SO_4 (0.1)	40	48, 50
$FeSO_4$	$Fe_2(SO_4)_3$ (0.02)	40	54

[a] 0.0133 mole of iron(II) salt and 0.009 mole of trimethylamine oxide used in 200 ml of 0.49 *N* acid. [b] Same ionic strength used throughout.

An essential feature of the previous mechanism was the presence of two vicinal nonchelated sites on the iron(III) for chelation with the amine oxide.[12] A similar hypothesis was suggested by Wang[24] to explain the extraordinary efficiency of the triethylenetetramine (TETA) complex of iron(III) for the conversion of hydrogen peroxide to oxygen. Although our results so far indicated iron(III) could not initiate the amine oxide dealkylation, there was the possibility that this pseudoenzymatic system might catalyze the reaction. Low and variable yields of formaldehyde were obtained. However, these were due to the reduction of some of the iron(III) to iron(II) (Table VI). Adding a small amount of iron(II) to the iron(III) TETA mixture [1 part of iron(II) to 30 parts of iron(III)] gives a greatly enhanced yield of formaldehyde. This result shows that only a small amount of iron(II) is necessary to initiate the reaction and demonstrates the central role of both iron(II) and iron(III) in the reaction providing further support for the mechanism proposed in steps 4–6.

Table VI. Decomposition of Trimethylamine Oxide with Iron Complexes of TETA

Iron salt	pH	% yield of HCHO[a]
$Fe(NO_3)_3$	6.3	None
$Fe(NO_3)_3$	10.6	2–10
$Fe(NO_3)_3$[b]	10.6	0.5
$Fe(NO_3)_3$–$FeSO_4$[c]	10.6	20
$Fe(NO_3)_3$–$FeSO_4$[d]	10.5	11
$Fe(NO_3)_3$[e]	10.3	None
$Fe(NO_3)_3$[f]	10.8	None
g	10.6	None

[a] Determined spectrophotometrically on the 2,4-dinitrophenylhydrazone derivative after tlc purification. [b] Reaction conducted in a nitrogen atmosphere. [c] Equimolar amounts. [d] Salts in molar ratio 31:1. [e] TETA omitted and solution made basic with ammonium hydroxide. [f] Amine oxide excluded. [g] Iron salt excluded.

Variable yields of glyoxal were also obtained from the TETA reactions. This interesting product apparently results from the oxidation of the TETA but its

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(20) (a) V. Boekelheide and D. L. Harrington, *Chem. Ind.* (London), 1423 (1955); (b) L. Horner and W. Kirmse, *Ann. Chem.*, **597**, 48 (1955); (c) L. Horner, H. Bruggemann, and K. H. Knapp, *ibid.*, **626**, 1 (1959); (d) L. Horner and K. H. Knapp, *Makromol. Chem.*, **93**, 69 (1966).

(21) E. S. Huyser and C. J. Bredeweg, *J. Am. Chem. Soc.*, **86**, 2401 (1964). For a related reaction with peroxy acids see K. Tokumaru and O. Simamura, *Bull. Chem. Soc. Japan*, **36**, 333 (1963); K. Tokumaru, O. Simamura, and M. Fukuyama, *ibid.*, **35**, 1673 (1962).

(22) S. Wawzonek, M. F. Nelson, Jr., and P. J. Thelen, *J. Am. Chem. Soc.*, **73**, 2806 (1951).

(23) For trapping experiments in similar systems see F. Minisci and R. Galli, *Tetrahedron Letters*, 167 (1964); F. Minisci and R. Galli, *ibid.*, 3197 (1964); R. S. Neale, *J. Am. Chem. Soc.*, **86**, 5340 (1964); R. S. Neale and R. L. Hinman, *ibid.*, **85**, 2666 (1963); H. T. Clarke, H. B. Gillespie, and S. Z. Weisshaus, *ibid.*, **55**, 4571 (1933).

(24) J. H. Wang, *ibid.*, **77**, 822, 4715 (1955).

mode of formation is not yet clear. Further investigation of this point is now in progress.

An attempted kinetic study of amine oxide rearrangement by nmr was unsuccessful, although the signal for the methyl group of the trimethylamine oxide (3.62 ppm) in acid solution is greater than that of the protonated trimethylamine (2.92 ppm, doublet, $J = 5.0$ cps) and dimethylamine (2.78 ppm, triplet, $J = 5.6$ cps). However, it was not possible to follow the course of the reaction using these peaks because iron(III) generated during the reaction gave rise to strong paramagnetic broadening.

Finally, some initially surprising anion effects were found to be consistent with the mechanism given in eq 4–6. We observed that the reaction rate and formaldehyde yield varied with the anion used. The reaction rate *increases* in the order ClO_4^-, SO_4^{2-}, Cl^-, PO_4^{3-}, and the formaldehyde yield *decreases* in the same order (Table III). The stability constants of iron(III) complexes of these ions also increase in the order ClO_4^-, SO_4^{2-}, Cl^-, PO_4^{3-}.[25] There can be several explanations of these phenomena based on the proposed mechanism (4–6). Anions which complex more strongly with iron(III) enhance the rate of (4) by accelerating the production of amminium radical ions.[26] The same chelation effect will accelerate the reduction step 5 resulting in an enhanced yield of trimethylamine and a correspondingly lower yield of formaldehyde. Finally this chelation effect will deaccelerate (6), thus decreasing the rate of formaldehyde synthesis. It is not clear which process predominates.

The liver enzymic system responsible for amine detoxication appears to possess all the properties that we found essential for our simple model system. Both contain iron in the II and III oxidation states and both processes yield the same products. Furthermore in our model system we observed that the reaction rate varies with the ligand attached to the iron so that it should be possible to construct (or evolve) an iron enzyme that would catalyze the dealkylation at 37°. What has not been determined is whether the N-oxide is a necessary intermediate in amine dealkylation. Further investigation of this point is in progress.

Experimental Section

Reagents and Procedures. Trimethylamine oxide dihydrate was prepared by oxidation of trimethylamine with hydrogen peroxide and recrystallization from ethanol–ether, mp 95.5–96.5°. Analytical reagent iron salts were used as received. Iron(II) perchlorate solutions were standardized by ceric titration.[27] Thin layer chromatography of the 2,4-DNP's was by the procedures of Rosmus and Deyl and Craig, Mary, and Wolf.[28] The volatile amines were determined by making the reaction mixture strongly basic and distilling into excess standard acid. The acid was then titrated to the methyl red end point. The yield of tertiary amine was determined by distillation of the volatile amines into acetic acid, addition of acetic anhydride, and, after 24-hr, titration of the trimethylamine to the methyl violet end point. Vpc analyses of the amines were carried out on an F & M Model 500 gas chromatograph using a 6-ft Dowfax 9N9 column.[29] Polaragraphic analyses were made on a Sargent Model 21 instrument equipped with a dropping mercury and saturated calomel electrodes. The amounts of iron(II) and iron(III) were calculated by comparison with standard curves. Nmr spectra were determined in acid solution on a Varian A-60 spectrometer with tetramethylsilane as internal standard.

Standard Trimethylamine Oxide Rearrangement. Trimethylamine oxide dihydrate (1 g, 0.009 mole) was heated to reflux in a nitrogen atmosphere with 0.0133 mole of the iron(II) salt in 200 ml of 0.49 *N* acid. Except where noted, the acid used corresponded to that of the anion of the iron salt. The reaction mixture was poured into 2,4-dinitrophenylhydrazine solution, and the precipitate was filtered after standing 20 min at room temperature. The precipitate was dried *in vacuo* to constant weight.

When iron(III) and a chelating acid was used the procedure of Craig, *et al.*,[12] was followed.

Reaction of Butadiene, Iron(II), and Trimethylamine Oxide. A mixture of 3.0 g (27.0 mmoles) of trimethylamine oxide and 11.3 g (40.5 mmoles) of iron(II) sulfate in 600 ml of 0.49 *N* sulfuric acid was purged with nitrogen and then heated to reflux for 5 hr with butadiene bubbling through the solution. A portion of the reaction was added to a 2,4-DNP solution, and an 11% yield of formaldehyde 2,4-DNP was obtained. (In the absence of butadiene the formaldehyde yield is 60%.) A portion of the reaction mixture was made basic. The iron hydroxides were filtered, and the filtrate was extracted with chloroform. No material was isolated in this fraction, indicating the absence of tertiary amines. A portion of the reaction mixture (equivalent to 13.1 mmoles of amine oxide) was neutralized with base, and the iron salts were filtered. The filtrate was acidified and 100 ml of a solution of 4.48 g (13.1 mmoles) of sodium tetraphenylboron was added dropwise. A yield of 4.69 g (83%) of product was obtained. After two recrystallizations from acetone-water, the material melted at 240–243°.

Anal. Calcd for $C_{62}H_{70}B_2N_2$: C, 86.10; H, 8.16; N, 3.24. Found: C, 85.88; H, 8.67; N, 3.64.

When the same reaction mixture was treated with $HgI_2 \cdot 2KI$ (Mayer's reagent) a yellow, gummy precipitate was obtained. This precipitate in ethanol suspension was treated with H_2S and yielded, after filtration and crystallization from ethanol, 22 mg of a methiodide, mp 225° dec.[30] Treatment of an aqueous solution of this methiodide with sodium tetraphenylboron yielded a white precipitate, mp 235–240°, which had an infrared spectrum identical with that obtained by adding sodium tetraphenylboron directly from the reaction mixture.

Hydrogenation of the Quaternary Butadiene Adduct. A solution of 263 mg of the tetraphenylboron salt in 10 ml of dimethylformamide was hydrogenated using 50 mg of PtO_2 at 50 psi for 3.5 hr. A yield of 87 mg of product was obtained by addition of water to the dimethylformamide solution. The product melted at 180–181° after recrystallization from acetone–water and acetone–ethanol.[31]

Anal. Calcd for $C_{62}H_{74}B_2N_2$: C, 85.70; H, 8.58. Found: C, 85.69, 85.74; H, 9.35, 9.47.

Reaction of Trimethylamine Oxide with TETA Complexes of Iron(II) and Iron(III). The general procedure consisted of heating a mixture of trimethylamine oxide, the iron salt, and TETA (molar ratio 1:3:30, respectively) at 80° for 40 min and precipitating the carbonyl products with 2,4-DNP. In a typical experiment to a 50 ml aqueous solution of ferric nitrate nonahydrate (5.45 g, 13.5 mmoles) was added 21.5 ml (135 mmoles) of TETA and trimethylamine oxide dihydrate (500 mg, 4.50 mmoles), and the mixture was heated at 80° for 40 min. After centrifugation, the super-

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[25] L. G. Sillen and A. E. Martell, Special Publication No. 17, The Chemical Society, London, 1964.

[26] For a discussion of similar effects in other systems see W. Lattimer, "Oxidation Potentials," Prentice-Hall, Inc., New York, N. Y., 1952, pp 220–228; W. M. Clark, "Oxidation-Reduction Potentials of Organic Systems," Williams & Wilkens Co., Baltimore, Md., 1960, pp 458–464; R. E. Huffman and N. Davidson, *J. Am. Chem. Soc.*, **78**, 4836 (1956); D. A. House, *Chem. Rev.*, **62**, 185 (1962); R. Bock and M. Herrmann, *Z. Anorg. Allgem. Chem.*, **273**, 1 (1953); C. F. Wells and M. A. Salam, *Nature*, **203**, 751 (1964); **205**, 690 (1965). For discussion of this effect with other anions see H. Taube, *J. Am. Chem Soc.*, **77**, 4481 (1955).

[27] W. C. Pierce and E. L. Haenisch, "Quantitative Analysis," John Wiley and Sons, Inc., New York, N. Y., 1956, p 257.

[28] J. Rosmus and Z. Deyl, *J. Chromatog.*, **6**, 187 (1961); J. C. Craig, N. Y. Mary, and L. Wolf, *J. Org. Chem.*, **29**, 2868 (1964).

[29] J. F. O'Donnell and C. K. Mann, *Anal. Chem.*, **36**, 2097 (1964).

[30] The *cis*- and *trans*-methiodides of $(CH_3)_2NCH_2CH{=}CHCH_3$ melt at 169 and 164°, respectively. The *cis*- and *trans*-methiodides of $(CH_3)_2NCH_2CH{=}CHCH_2OH$ melt at 98 and 85–86°, respectively [M. Olomucki, *Ann. Chim.* (Paris), **5**, 845 (1960)]. Therefore, the reaction product is none of the above structures.

[31] The dimethiodide of 1,8-bis(dimethylamino)octane was prepared, mp 260–261° [lit. mp 254–255° (V. M. Solov'ev and A. P. Skoldinov, *J. Gen. Chem. USSR*, **33**, 1773 (1963)) and 260–261° (E. J. Zaimis, *Brit. J. Pharmacol.*, **5**, 424 (1950))]. Treatment of this with sodium tetraphenylboron gave the bistetraphenylboron salt, mp 285–296°, after recrystallization from acetone. This material had a very similar infrared spectrum to that of the reduction product.

natant was filtered through Celite, and the filtrate was made acidic with 6 *N* hydrochloric acid and added to the 2,4-DNP reagent to yield 122 mg of product which was a mixture of the 2,4-DNP's of glyoxal and formaldehyde. The yield of formaldehyde was determined spectrophotometrically by the method of Craig, Mary, and Wolf[28] and the results are listed in Table VI.

The glyoxal was identified by washing the 2,4-DNP precipitate with chloroform and then crystallizing the chloroform-insoluble material from benzene. This material had the same melting point (330–332°) and infrared spectrum as an authentic sample of the 2,4-DNP of glyoxal.

Reduction of Iron(III) to Iron(II) by TETA. A mixture of 892 mg (2.21 mmoles) of ferric nitrate nonahydrate and 4.4 ml (28 mmoles) of TETA in 10 ml of water was heated at 80° for 40 min. The mixture was acidified to pH 3, filtered through Celite, and analyzed for iron(II) spectrophotometrically using *o*-phenanthroline.[32] A 3.2% yield of iron(II) was obtained.

Attempted Radical-Induced Decomposition of Trimethylamine Oxide. a. With N-Chloropiperidine. To a solution of trimethylamine oxide (1.00 g, 0.009 mmole) in 200 ml of deoxygenated concentrated sulfuric acid placed in a quartz irradiation tube was added 1.07 g (0.009 mole) of N-chloropiperidine.[33] The resulting solution was stoppered under a nitrogen atmosphere and irradiated 2.25 hr with an ultraviolet source having maximum intensity at 253 mμ. The irradiated solution was then added to a 2,4-DNP solution. No precipitate of formaldehyde 2,4-DNP derivative was observed. A similar mixture was heated 11 hr at 95° under a nitrogen atmosphere. Again no formaldehyde was obtained.

b. With Di-*n*-butylchloramine. The procedures outlined above were repeated, except with 1.4 g (0.009 mole) of di-*n*-butylchloramine.[33] No formaldehyde could be detected at the end of a 72-hr irradiation period or after heating the reaction mixture at 95° for the same length of time.

Di-*n*-butylchloramine-induced trimethylamine oxide dealkylations were also attempted by heating or irradiating the N-oxide (1.00 g, 0.009 mole) with the chloramine (1.47 g, 0.009 mole) in 20 ml of deoxygenated aqueous solutions in which the sulfuric acid content was lowered to 29% and finally to 1% of solution. In all cases no formaldehyde could be detected at the end of the reaction period.

To determine whether or not formaldehyde was being decomposed under the reaction conditions employed above, 0.0045 mole of formaldehyde was heated at 95° with 0.009 mole of di-*n*-butylchloramine in 200 ml of 29% aqueous sulfuric acid. At the end of 3 hr, 82% of the formaldehyde was recovered as the 2,4-dinitrophenylhydrazone.

c. With Dibenzoyl Peroxide and Di-*t*-butyl Peroxide. Trimethylamine oxide (1.00 g, 0.009 mole) was refluxed 6.5 hr in a nitrogen atmosphere with 0.0045 mole of the peroxide in 200 ml of 0.49 *N* sulfuric acid solution. No precipitate of formaldehyde was observed when the reaction mixture was added to 2,4-DNP.

To determine whether or not formaldehyde was being decomposed under the reaction conditions employed, 0.0040 mole of formaldehyde was refluxed with 0.0045 mole of dibenzoyl peroxide in 200 ml of 0.49 *N* sulfuric acid solution. A 90% recovery of formaldehyde was obtained after a 4.3-hr heating period.

Polymerization of Ethyl Methacrylate. Trimethylamine oxide (1.00 g, 0.009 mole) was refluxed 40 min in a nitrogen atmosphere with 0.0133 mole of iron(II) sulfate and 10 g of ethyl methacrylate[34] in 200 ml of deoxygenated 0.49 *N* sulfuric acid solution. Polymerization of the ethyl methacrylate began within 5 min of reflux. At the end of the heating period, the reaction mixture was cooled and the polymeric material collected by filtration, washed with water, and dried overnight in a desiccator. The poly(ethyl methacrylate) (8.5-g yield) thus obtained showed a carbonyl absorption band in the infrared at 1745 cm^{-1}.

The above procedure was repeated except that the N-oxide and monomer were refluxed in 0.49 *N* sulfuric acid in the absence of the iron(II) salt. No polymerization could be detected throughout the heating period. The cooled reaction mixture was then extracted with ether, and the combined ether extracts were washed with water, dried over sodium sulfate, and concentrated at atmospheric pressure. Ethyl methacrylate (7.4 g) was thus recovered and identified by the superimposability of its infrared spectrum with that of authentic monomer.

A similar blank experiment was run in which trimethylamine oxide was the reagent excluded. Again no polymerization could be detected and 3.4 ml of monomer could be recovered.

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(32) I. M. Kolthoff and P. J. Elving, "Treatise on Analytical Chemistry," Vol. 2, Part II, Interscience Publishers, Inc., New York, N. Y., 1962, p 295.

(33) C. Schopf, A. Komzak, F. Braun, E. Jacobi, M. L. Bormuth, M. Bullnheimer, and I. Hagel, *Ann. Chem.*, **559**, 1 (1948).

(34) The inhibitor was removed by washing with 5% NaOH and then with water.