Short Communications and Preliminary Notes

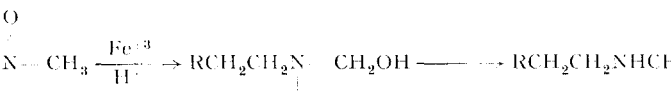
Oxidative N-dealkylation

This note describes preliminary results on the chemical and enzymic reactions of N-oxides.

When N,N-dimethyltryptamine oxide, a constituent of the seeds of *Piptadenia macrocarpa* Benth.[1], was warmed in acidic aqueous solution in the presence of ferric ions, a rearrangement leading to N-methyltryptamine occurred. A study of the reaction conditions indicated that acids such as hydrochloric, formic, and oxalic could be used, but oxalic acid was preferred, since by-products were formed in the presence of formic and hydrochloric acids. The reaction occurred relatively rapidly at temperatures in the range 60–100°; for example, at 95–100° with 3 mole equivalents of Fe^{+3} there was no apparent change in the yield of monoamine in the range 7.5–90 minutes. The amount of Fe^{+3} was critical in that more than catalytic amounts were required, while a large excess (9 mole equivalents) resulted in total destruction of the oxide. Metal ions which were ineffective (at 3 mole equivalents level) in a formate or chloride system were Co^{+2}, Ni^{+2}, Mg^{+2}, Mn^{+2}, and Cu^{+2}.

Under typical conditions (9 to 10 μM N,N-dimethyltryptamine oxide, 30 μM ferric nitrate nonahydrate, and 37 μM oxalic acid dihydrate in 1 ml of water; pH 1.3; heated at 95–100° for 30 minutes) the formation of N-methyltryptamine was accompanied by the formation of an approximately equivalent amount of N,N-dimethyltryptamine, and some N-oxide was recovered. The indole constituents were separated by paper chromatography (*n*-propyl alcohol–1 *N* ammonium hydroxide 5/1) and were identified by comparison with authentic samples using paper chromatography (four systems) and ionophoresis (borate buffer, pH 8.5). In addition, the isolated N,N-dimethyltryptamine and its N-oxide were identified by their interconversion. The amine may be oxidized on paper, before chromatography, with a drop of 3% hydrogen peroxide, and the N-oxide may be reduced to the amine with zinc dust-acetic acid in a separate reaction before chromatography. The N-methyl base is not oxidized under these conditions, and it may also be distinguished from N,N-dimethyltryptamine by ionophoresis at pH 8.5 and by chromatography in a propanol-ammonia system.

The reactions involved are evidently the following:



(A) $RCH_2CH_2N(O)(CH_3)_2 \xrightarrow[H^+]{Fe^{+3}} RCH_2CH_2N(CH_3)CH_2OH \longrightarrow RCH_2CH_2NHCH_3 + HCHO$

I II III

(B) $HCHO \xrightarrow{\text{N-oxide}} HCOOH$

An N-oxide rearrangement leading to an N-methylolamine (II) is not unexpected[2]. At the pH of the reaction mixture, the N-methylol group is hydrolyzed rapidly to the observed monomethyl compound and to formaldehyde. A subsequent oxidation-reduction reaction results in the formation of formic acid and the N,N-dimethylamine corresponding to I.

Separate experiments (by Dr. C. C. SWEELEY) indicated that a similar demethylation reaction occurred under the same conditions for hordenine oxide (prepared in the same way as synthetic N,N-dimethyltryptamine oxide[1]). In both cases the presence of formic acid in the reaction mixture was demonstrated by means of the formaldehyde-chromotropic acid color test of EEGRIWE[3]. The distillate from the reaction mixture was treated with dilute hydrochloric acid and magnesium to reduce the formic acid back to formaldehyde. Addition of chromotropic and sulfuric acids in the prescribed manner produced the violet-pink color characteristic of this test. On larger runs (50 μM of N,N-dimethyltryptamine), the color test demonstrated the presence of a small amount of formaldehyde, along with the formic acid, in the original distillate.

A related oxidative demethylation reaction was recently described by TSUYUKI, STAHMANN, AND CASIDA[4] for octamethylpyrophosphoramide ("Schradan"). An N-oxide was the first-formed product, and this underwent rearrangement, without added catalyst, to form a second substance whose hydrolysis products were the N-methyl compound and formaldehyde. The intermediate corresponding to II was formulated as an O-methylhydroxylamine, but it is more likely an N-methylolamine. The N-oxidase was obtained by both chemical and biological oxidation.

These results indicate that (A) may be a general reaction, subject to the finding of appropriate catalysts for the rearrangement step. While (A) has not yet been observed to occur in the reverse direction, it should be theoretically possible to reverse each step, and if this is correct, the sequence is a model "energy ladder" which may be useful in studying enzymic methylation and demethylation reactions. A number of observations bearing on this possibility have been made.

BLASCHKO[5] has pointed out that monoamine oxidase systems have been found in all vertebrates examined and are widely distributed throughout different tissues. Some time ago BERNHEIM AND BERNHEIM[6] reported the isolation of two monoamine oxidase fractions from rabbit liver. One was associated with the mitochondrial fraction, and one was in the supernatant; however, the same enzymic systems may have been involved in each fraction, since they were about equally active in the oxidation of tyramine and mescaline. In a recent study of the localization of amine oxidase in the rat liver cell, HAWKINS[7] found about two-thirds of the enzymic activity present in the mitochondria; the remainder was in the microsome fraction. Again there was no evidence to indicate a difference in the type of enzymic action involved in the two fractions. With regard to enzymic dealkylation, the work of BRODIE *et al.*[8] has demonstrated that N-demethylation (of methylaminoantipyrine and dimethylaminoantipyrine) can occur in liver microsomal systems and that the latter requires TPNH and oxygen to be effective.

In current experiments, using N,N-dimethyltryptamine as a substrate with mouse liver homogenate in the presence of added DPN, nicotinamide, and AMP*, the amine gave four transformation products. The major product was identified as the N-oxide (I, R = indolyl-3-), and a second product was found to be indole-3-acetic acid. The formation of the oxide was found to be DPN dependent. ATP was substituted for AMP without effect, and added Mg^{+2}, Mn^{+2}, or versene were without effect. When a mitochondrial preparation from the mouse liver homogenate was used (prepared by the method of KIELLEY AND KIELLEY[9] using isotonic sucrose solution), it was found that N,N-dimethyltryptamine was largely converted into indole-3-acetic acid. Under identical conditions the N-monomethyl base was converted at a higher rate to the same end-product, and the system was not stimulated by added DPN. When the N-oxide was used as a substrate, it was recovered after incubation, and no other indoles were formed. From this it may be concluded that the free N-oxide is not an intermediate in the monoamine oxidase reaction of mouse liver mitochondria.

The formation of the N-oxide from the parent base was found to occur by enzymic reaction in a fraction from mouse liver homogenate from which the mitochondria had been removed. Other indole products were not observed in this case. When the N-oxide was used as a substrate with homogenate and added DPN, nicotinamide, and AMP, a small amount of indole bases resulted. The quantity was too low to permit identification, and further work is concerned with raising the yield of these products.

In each case the appropriate control runs were made. These included runs with added factors but without homogenate, homogenate and added factors without substrate, and a boiled homogenate with added factors and substrate. No indole transformation products were obtained in these incubations. Separation of the indole compounds was carried out by paper chromatography in propanol-ammonia. The identifications were based on paper chromatography (four systems) and ionophoresis (borate buffer, pH 8.5).

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Received August 31st, 1955

* The quantities and conditions for these experiments were as follows: Liver-isotonic potassium chloride 1/3 homogenate from which the nuclei were removed by centrifugation (3 ml); the added factors were 6–7 μM substrate, 5 μM DPN, 20 μM AMP, 100 μM nicotinamide and 40 μM of phosphate buffer, pH 7.7. The mixture was made to a final volume of 5 ml with isotonic potassium chloride solution. Incubation was at 37° for two hours.