FORMATION OF ARENEDIAZONIUM ION IN OXIDATION OF N,N-DIMETHYL-4-AMINOBENZENE AND SOME *meta*-SUBSTITUTED DERIVATIVES WITH CERIUM(IV) ION IN ACID MEDIUM*

Miroslav MATRKA and Jitka PÍPALOVÁ

Department of Toxicology,
Research Institute of Organic Syntheses, 532 18 Pardubice - Rybitví

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Oxidation has been studied of an experimental hepatocarcinogene N,N-dimethyl-4-aminoazobenzene and some its *meta*-substituted derivatives with cerium(IV) ion in 1M hydrochloric acid medium with potentiometric indication. It has been possible to prove formation of more than 50 per cent arenediazonium ion and N,N-dimethylbenzoquinonimine besides the known metabolites. Furthermore, five *meta*-substituted derivatives of N,N-dimethyl-4-aminoazobenzene have been studied, and the amount of the arenediazonium ion formed has been found to depend critically on character of the 3'-substituent.

The previous communications of this series dealt with oxidation of N,N-dimethyl--4-aminoazobenzene and some its derivatives with ceric ion or other oxidants. Among the oxidation products we could identify almost all current metabolites, which was simultaneously proved by testing on animals[1-10].

When evaluating a number of oxidation products we found that there was a relatively great difference between amount of the consumed oxidant and that of the identified products. Therefore, we had to focus our attention on the rest which had not been identified by then. It was presumed that the formed compounds are decomposed during isolation. Desai & Giles[11] found that some azo compounds of the type of Sudans (the dyestuffs used for dyeing of fats) undergo oxidative splitting to form arenediazonium ion and the corresponding quinonimine, which led us to reexamine this possibility. The authors[11] stated that the said oxidation mechanism only applies to the dyestuffs in which azo group is conjugated with a suitable substituent (OH, NH_2, OCH_3 *etc.*) in the aromatic ring. N,N-Dimethyl-4-aminoazobenzene behaves similarly, since the group in conjugation is $N(CH_3)_2$ which allows to form the respective quinonimine compound after splitting off of azo group. It was proved that the rest of the molecule gives the respective arenediazonium ion in 50

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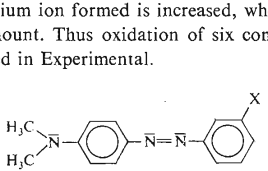
per cent yield. If the other benzene ring carries an electrogenative *meta*-substituent, the amount of the diazonium ion formed is increased, whereas electropositive substituents decrease this amount. Thus oxidation of six compounds was carried out by the procedure described in Experimental.



I, X = H
II, X = CH_3
III, X = Br
IV, X = COOH
V, X = OC_2H_5
VI, X = OCH_3

EXPERIMENTAL

Reagents and Solutions

N,N-Dimethyl-4-aminoazobenzene (*I*) was commercial sample of *p.a.* purity grade (Schering, BRD); 3′-methyl-N,N-dimethyl-4-aminoazobenzene (*II*), 3′-bromo-N,N-dimethyl-4-aminoazobenzene (*III*), N,N-dimethyl-4-aminoazobenzene-3′-carboxylic acid (*IV*), 3′-ethoxy-N,N-dimethyl-4-aminoazobenzene (*V*), and 3′-methoxy-N,N-dimethyl-4-aminoazobenzene (*VI*) were prepared in our laboratory by known method of C-azo coupling of N,N-dimethylaniline with the corresponding derivative of benzenediazonium chloride. Purity of the products was checked by elemental analyses, electronic spectra, melting points, and thin-layer chromatography.

Model compounds of the presumed products of the oxidation splitting of the azo compounds were obtained by azo coupling of the corresponding *meta*-substituted benzenediazonium chlorides with 2-naphthol. Their purity was checked in the same way as that of the compounds *I*—*VI*. The other reagents used were commercial chemicals of *p.a.* or *c.p.* purity grades (Lachema, ČSSR, or Apolda DDR).

For the titrations we used 0·1M-$Ce(SO_4)_2$ solution prepared by usual way[12].

Apparatus

For the potentiometric titrations we used a Radelkis (Hungary) apparatus Universal pH-Meter OP 204/1 with platinum (OP 600) and calomel (OP 030) electrodes. The spectrophotometric measurements were carried out with a Specord UV-VIS (Zeiss, Jena). The chromatographic separation was carried out on commercial plates with hardened silica gel (Silufol R; Kavalier — ČSSR).

Procedure of the Potentiometrically Indicated Oxidimetric Titrations

A sample of $5 . 10^{-4}$ mol N,N-dimethyl-4-aminoazobenzene derivative was dissolved in 5 ml acetone, and 1M-HCl was added to make the total volume 50 ml. The titrated solution was cooled with ice bath, and the reagent was added by 0·2 ml portions in one minute intervals. For the derivatives *IV*, *V*, and *VI* the solution of the azo dyestuff ($1 . 10^{-2}$M) in acetone was pipetted (5 ml) and diluted with 25 ml 1M-HCl, the subsequent procedure being similar.

Procedure of the Spectrophotometric Measurements

After the end of the above-described titration, a 5 ml sample was withdrawn from the reaction solution and added to 50 ml 0·25M-Na_2CO_3 containing 5 ml 0·01M 2-naphthol. After 15 to 30 min, the azo dyestuff formed was extracted from the reaction solution with 5 × 10 ml benzene (in the cases of *IV*, *V*, and *VI*, pH of the solution was adjusted by addition of 1M-HCl). The combined extracts were concentrated to about 2 ml and transferred on the start line of the hardened silica gel thin layer. The chromatogram was developed with n-hexane/ethyl acetate with the ratios 2 : 1 for (*I*), 3 : 1 (for *II* and *III*) and 1 : 1 (for *IV*), and with n-hexane/dioxane/ethyl acetate mixture 8 : 1 : 1 (for *V* and *VI*).

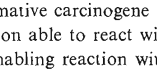
The azo dyestuff zone was separated mechanically and dissolved in benzene or (for *IV*) benzene/methanol mixture 1 : 1. The obtained mixtures were separated with a centrifuge, the clear solution was diluted with benzene (or benzene/methanol 1 : 1) to the final volume of 50 ml and used for spectrophotometry in visible region (Table I)

RESULTS AND DISCUSSION

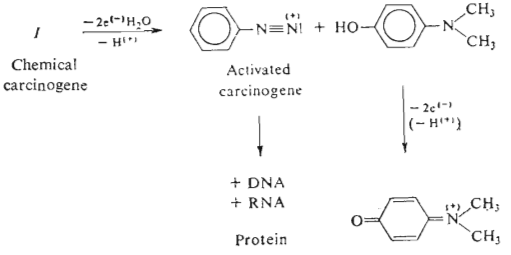
From the results obtained it follows that even N,N-dimethyl-4-aminoazobenzene itself is oxidized with ceric ion in 1M-HCl medium to give predominantly the respective benzenediazonium cation and quinonimine derivative of N,N-dimethyl-4-aminophenol. The currently accepted theory about mechanism of chemical carcinogenesis[13] presumes that a chemical carcinogene (predominantly organic compounds) must be, first of all, enzymatically metabolized to a so called "activated carcinogene" (or critical carcinogene, ultimative carcinogene *etc.*, which means always the last phase of the metabolic activation able to react with DNA or RNA) which has distinctly electrophilic character enabling reaction with nucleophilic centres of genetic material of cell nucleus (*e.g.* DNA, RNA). This category does not involve the carcino-

TABLE I

Amount of the 3-X-substituted benzenediazonium ion formed by oxidation of N,N-dimethyl--4-amino-3′-X-azobenzene

	The amount formed, %
H	50·0
CH_3	49·0
Br	47·0
COOH	61·0
OC_2H_5	11·0
OCH_3	16·0

gens of the type of alkylating or arylating agents *etc.* The latter react without necessary metabolic activation. In the sense of this hypothesis, the oxidation of N,N--dimethyl-4-aminoazobenzene with ceric ions to benzene diazonium cation can be taken as a model transformation of the initial carcinogene to the "activated" one:



The mechanism is supported by reactions of arenediazonium salts with DNA, RNA (and also their bases), proteins *etc.*[14–16] and, last but not least, also by the finding[17] that arenediazonium ions are carcinogenic even without metabolic activation. Preussmann and coworkers[18] solved a similar problem in a study of mechanism of carcinogenic action of dialkylaryltriazene compounds (analogs of commercial Rapidogen dyestuffs), presuming an easy protolytic decomposition (*e.g.* in stomach) to arenediazonium cation (the activated carcinogens) and respective dialkylamine. However, no positive results were obtained, since the protolysis was accompanied by competing oxidative N-dealkylation giving monoalkylaryltriazenes which themselves are strong alkylation reagents and react with DNA, RNA *etc.*[19] The only positive results were obtained in few cases where the protolysis was faster than the enzymatic oxidative N-dealkylation to monoalkylaryltriazenes which would subsequently alkylate DNA or RNA (ref.[19]).

At present we have finished investigation of kinetics of reaction of arenediazonium ion with the bases of nucleic acids, and the results seem to support our hypothesis[20]. The final evidence will be obtained from investigation of enzymatic oxidation of N,N-dimethyl-4-aminoazobenzene by means of microsomal fractions *in vitro* and from proved formation of arenediazonium ion by metabolic activation *in vivo*.

REFERENCES

1. Matrka M., Marhold J.: This Journal *33*, 4273 (1968).
2. Matrka M., Marhold J., Ságner Z.: Pípalová J.: This Journal *33*, 3761 (1968).
3. Marhold J., Rambousek V., Matrka M., Pípalová J.: Neoplasma *16*, 1 (1969).
4. Matrka M., Marhold J., Chmátal V., Pípalová J.: This Journal *34*, 1348 (1969).

5. Matrka M., Marhold J., Ságner Z.: This Journal *34*, 1615 (1969).
6. Marhold J., Rambousek V., Chmátal V., Štěrba V., Matrka M.: Neoplasma *16*, 191 (1969).
7. Matrka M., Marhold J., Chmátal V., Štěrba V.: This Journal *34*, 3952 (1969).
8. Matrka M., Pípalová J., Chmátal V., Štěrba V., Kroupa J., Marhold J.: This Journal *35*, 2944 (1970).
9. Matrka M., Rambousek V., Diviš J., Zvěřina V., Marhold J.: This Journal *36*, 2725 (1971).
10. Marhold J., Matrka M.: Fluoride Quarterly Reports *2*, 85 (1969).
11. Desai N. F., Giles C. H.: J. Soc. Dyers Colour. *1949*, 639.
12. Tomíček O.: *Odměrná analýza*, p. 198. Přírodovědecké nakladatelství, Prague 1949.
13. Miller J. A., Miller E. C.: J. Nat. Cancer Inst. *47*, V–XIII (1971).
14. Moudrianakis E., Beer M.: Biochim. Biophys. Acta *95*, 23 (1965).
15. Bergmann D. E., Pullman B. (Eds): *Physico-Chemical Mechanisms of Carcinogenesis*, p. 183. Academic Press, London 1969.
16. Kössel H.: Hoppe-Seyler's Z. Physiol. Chem. *340*, 210 (1965).
17. Herman R. C., DuBois K. P.: J. Pharmacol. Exp. Ther. *95*, 262 (1949).
18. Preussmann R., Ivankovic S., Landchütz C., Gimmy J., Flohr E., Griesbach U.: Z. Krebsforsch. *81*, 285 (1974).
19. Preussmann R.: *Fortschritte der Krebsforschung*, p. 163. Schattauer, Stuttgart 1969.
20. Matrka A., Mejstřík V., Květinová L., Matrka M.: Neoplasma, in press.

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