OXIDATION MECHANISM OF CARCINOGENIC BIS-AZO DYESTUFF TRYPAN BLUE*

Miroslav MATRKA and Jana PÍPALOVÁ

Department of Toxicology, Research Institute of Organic Syntheses,
533 51 Pardubice — Rosice nad Labem

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Oxidation of Trypan Blue with cerium(IV) ion in acid medium gives arenediazonium cation similar to the oxidation product of N,N-dimethyl-4-aminoazobenzene. Quantitative evaluation of the arenediazonium salt formed has been carried out spectrophotometrically after previous C-azo coupling with 2-naphthol.

Trypan Blue, which is used as a Reagent Bayer 205 (ref.[1]) shows both carcinogenic and teratogenic effects[2-6]. Malignant tumours in rats were brought about by subcutaneous application of 1% aqueous solution of Trypan Blue as early as after two weeks. Recently a number of cases have been published[7-12] of malignant tumours of other organs.

In our previous papers[13-16] we dealt with oxidation of carcinogenic azo dyestuffs (particularly N,N-dimethyl-4-aminoazobenzene and its derivatives) with cerium(IV) sulphate in mineral acid medium. This reaction produces similar compounds to those formed by metabolism in rats after per os application of the studied azo compounds[17]. The oxidation of N,N-dimethyl-4-aminoazobenzene gives about 50% yield of the corresponding arenediazonium ion which is very unstable thermally and, therefore, was not proved in the previous papers. The reason of its lability consists in the oxidation destruction of azo group which is facilitated by the presence of substituents forming conjugated system with the azo group[18]. Such arenediazonium ion could represent a reactive intermediate able of reaction with nucleophilic centres of biopolymers[19].

This communication deals with oxidation of a bis-azo dyestuff Trypan Blue with cerium(IV) salts. Formation of the arenediazonium ion *II* in the reaction was proved indirectly by its azo coupling with 2-naphthol. The formed dyestuff *III* was separated chromatographically and determined spectrophotometrically. The values found correspond approximately to formation of 12 to 12·5% yield of ion *II* in the oxidation of Trypan Blue (*I*). The following scheme is suggested for the oxidation mechanism of Trypan Blue (*I*) with cerium(IV) ion.

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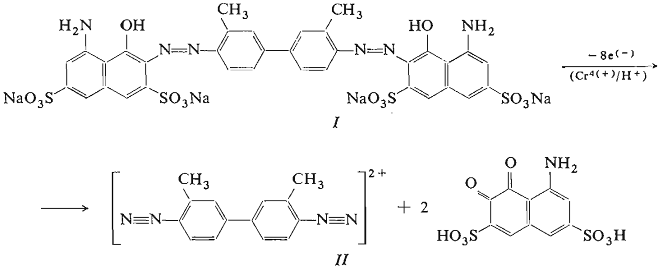

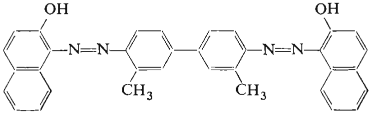

III

The arenediazonium cation *II* (if it were formed by oxidation *in vivo*) could react as an alkylation or arylation reagent with biologically important molecules in similar way as the cation formed from N,N-dimethyl-4-aminoazobenzene. In contrast to the latter case, from Trypan Blue a bis-diazonium salt is formed, and its two active groups enable theoretically more varied combinations of bonds to nucleophilic centres of a biopolymer[3]. However, reactivity of the diazonium groups in *II* will obviously be lower than that of the ion formed from N,N-dimethyl-4-amino azobenzene[20].

EXPERIMENTAL

Trypan Blue (*I*) and dyestuff *III* were synthetized in our laboratory[21] by azo coupling of bis-diazonium cation *II* with 4-hydroxy-5-aminonaphthalene-2,7-disulphonic acid and 2-naphthol, respectively. Cerium(IV) sulphate was a *p.a.* reagent Apolda.

Standard solutions of cerium(IV) sulphate and hydrochloric acid were prepared by known methods. The potentiometric titration was carried out with a Universal pH Meter type 204/1 (Radelkis) using the indication electrodes SCE OP 830 and Pt OP 600. The spectrophotometric measurements were carried out with a Specord UV–VIS apparatus (Zeiss). The thin layer chromatography was carried out with alumina layers Alufol R.

Oxidation of Trypan Blue

Solution of 48 mg (1 mmol) Trypan Blue in 10 ml water was acidified with 20 ml 1M-HCl and titrated with 0·1M-$Ce(SO_4)_2$ (potentiometric indication) with stirring and cooling with ice. The individual portions of the reagent were added at 2 min intervals. Immediately after finishing the titration, the solution was transferred in a 50 ml calibrated flask, and the volume was adjusted by addition of water. A 5 ml sample was pipetted from the solution into a mixture of 5 ml $1 . 10^{-2}$M 2-naphthol and 50 ml 0·25M-Na_2CO_3 and left to stand for 24 h. The azo dyestuff *III* formed was extracted with benzene, and the combined extracts were concentrated to about 2 ml. This solution was separated on two Alufol R plates using ascendent chromatography with chloroform–toluene (2 : 1). The bis-azo dyestuff zone was removed mechanically and extracted with benzene, the extract was diluted to 50 ml with benzene and its absorbance measured against the same solvent. The authentic standard sample of *III* was used for both chromatographic identification of the reaction product and construction of calibration straight line for its spectrophotometric determination.

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