

## Synthesis of the hepatocarcinogen *N*-methyl-4-aminoazobenzene with tritium in the prime ring\*

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### SUMMARY

*The new compound N-methyl-4-nitrosoacetanilide was coupled with aniline-<sup>3</sup>H(ring-G) in ethanol-acetic acid and the resulting azoamide was hydrolyzed in alkali to give a high yield of N-methyl-4-aminoazobenzene labelled in the prime ring.*

### INTRODUCTION.

*N*-Methyl-4-aminoazobenzene, a hepatocarcinogen in the rat, and related dyes are converted in this species into metabolites which react with hepatic proteins and nucleic acids to form covalently bound derivatives<sup>(1-3)</sup>. These bound derivatives may play a role in the carcinogenic process induced by these dyes<sup>(1-3)</sup>. For further studies *in vivo* *N*-methyl-4-aminoazobenzene of high specific activity with label in the prime ring was required. Synthesis of this dye in high yield was achieved by coupling excess *N*-methyl-4-nitrosoacetanilide with aniline-<sup>3</sup>H(ring-G). After removal of the *N*-acetyl group with alkali the labelled aminoazo dye was purified by column chromatography. Attempts to prepare this dye directly by coupling *N*-methyl-4-nitrosoaniline<sup>(4)</sup> with aniline were unsuccessful.

### EXPERIMENTAL.

#### *N*-Methyl-4-nitroacetanilide.

*N*-Methylaniline (20 g) was added to 40 ml of acetic anhydride and the mixture allowed to stand at room temperature for 20 hours. Benzene (100 ml) was added and the mixture evaporated to dryness *in vacuo* at 50° C.

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The residue was dissolved in 150 ml of absolute ethanol and the solution taken to dryness in the same manner. This process was repeated twice more to remove acetic acid. The crystals that formed (24 g, 86% yield) were dried and recrystallized from *n*-hexane-benzene to give white plates of *N*-methylacetanilide, m.p.\* 99.7° C, lit. m.p. 100° C<sup>(5)</sup>. A mixture of 15 ml of conc. H<sub>2</sub>SO<sub>4</sub> and 25 ml of conc. HNO<sub>3</sub> (90%, d. 1.48) was cooled to 5° C and added dropwise to a stirred solution of *N*-methylacetanilide (8.5 g) in 10 ml of conc. H<sub>2</sub>SO<sub>4</sub> previously cooled to 5° C. The addition rate was adjusted to keep the reaction mixture at 30-35° C. After the addition was complete the reaction mixture was cooled to 10° C for 1 hour and then poured onto 500 g of ice. The yellow crystalline precipitate was filtered, washed with water, and dried (7.4 g, 66% yield); m.p. 152-153° C. Crystallization from ethanol yielded large brownish-white cubic crystals of *N*-methyl-4-nitroacetanilide with m.p. 154-155° C, lit. m.p. 153° C<sup>(5)</sup>.

#### *N*-Methyl-4-nitrosoacetanilide.

A mixture of 2.9 g of *N*-methyl-4-nitroacetanilide was dissolved in 160 ml of methanol and added to a solution of 3.7 g of NH<sub>4</sub>Cl in 160 ml of water. This mixture was vigorously stirred and 3.5 g of zinc dust was added in small portions over a period of ten minutes. About 3 minutes after addition of the zinc dust was started the temperature rose and when it reached 40° C an ice-bath was used to lower the temperature to 30-33° C. Fifty minutes after the additions of zinc dust were started the solution was filtered on a 12 cm Buchner funnel and the zinc oxide residues were washed with 160 ml of hot water. The filtrate and washings were cooled immediately with enough ice (300 g) to bring the temperature to 0-3° C. To this cold solution of *N*-methyl-4 hydroxyaminoacetanilide an ice-cold solution of H<sub>2</sub>SO<sub>4</sub> (18 ml conc. acid plus 50 g ice) was added with stirring. Then an ice-cold solution of 2.1 g of Na<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>·2H<sub>2</sub>O in 10 ml water was poured all at once with vigorous stirring into the previous mixture. After the reaction mixture stood in an ice-bath for 1 hour, the yellow precipitate was removed by filtration and the dark brown filtrate was extracted twice with 300 ml portions of benzene. The blue benzene extract was washed twice with 200 ml portions of water and dried over 20 g of anhydrous Na<sub>2</sub>SO<sub>4</sub>. After removal of the benzene *in vacuo* at 50° C the dark green residue was crystallized from *n*-hexane-benzene to give 0.8 g (30% yield) of green needle-like crystals, m.p. 85° C.

Anal.\*\* Calcd. for C<sub>9</sub>H<sub>10</sub>N<sub>2</sub>O<sub>2</sub> : C, 60.67; H, 5.66; N, 15.72; O, 17.96.

Found : C, 60.04; H, 5.47; N, 15.75; O, 18.30.

\* Corrected melting points were estimated to ±1° C from slopes of the heating curves obtained with the Accumelt apparatus of the American Instrument Co., Silver Spring, Maryland.

\*\* Huffman Laboratories, Wheatridge, Colorado.

The infrared spectrum of this new nitroso compound in KBr gave the following maxima and intensities : 3,400 w, 1,660 s, 1,592 s, 1,500 s, 1,455 m, 1,415 w, 1,375 m, 1,346 s, 1,310 m, 1,285 w, 1,178 m, 1,112 s, 1,076 m, 978 s, 850 s, 820 s, 730 w, 710 m, 640 w, 595 w, and 500 s  $\text{cm}^{-1}$ . The  $-\text{N}=\text{O}$  stretching frequency occurs in the 1,500 — 1,600  $\text{cm}^{-1}$  region <sup>(6)</sup>.

*N-Methyl-4-aminoazobenzene-[prime ring-<sup>3</sup>H(G)].*

Pure aniline hydrochloride was catalytically tritiated \* to give a dark brown product with tritium generally distributed in the ring of the aniline present. This product (280 mg, specific radioactivity = 12 mCi/mg) was dissolved in 28 ml of absolute ethanol and converted to the free base with the addition of 30 ml of 0.13 N NaOH in absolute ethanol. Glacial acetic acid (28 ml) was added and the aniline was coupled with an excess of *N*-methyl-4-nitrosoacetanilide (560 mg) dissolved in 42 ml of absolute ethanol. The reaction mixture was allowed to stand at room temperature for 3 days. After removal of ethanol and acetic acid *in vacuo* at 50° C a crystalline residue of azoamide was obtained. This residue was dissolved in 56 ml of ethanol and mixed with 34 ml of 11 N NaOH and 23 ml of water. This mixture was refluxed in a water bath for 1 day. After removal of the ethanol by distillation, the yellow suspension was diluted with 60 ml of water and the dye was extracted into 100 ml of benzene. The benzene extract was washed with 60 ml of water and then taken to dryness *in vacuo*. The dye residue was dissolved in 15 ml of benzene and chromatographed on a column of alumina (100-200 mesh, 2.8 cm dia.  $\times$  46 cm) with a mixture of *n*-hexane-benzene as the elution solvent. The main rapidly moving orange band was collected and the solvent removed *in vacuo*. The residue was crystallized from *n*-hexane-benzene to give 350 mg of long yellow needles. This was a yield of 77% from the impure labelled aniline hydrochloride. Yields of 90-98% were obtained in 5 similar runs with pure non-radioactive aniline hydrochloride. The crystalline dye melted at 88-89° C, lit. m.p. 88-89° C <sup>(7)</sup>; admixture with *N*-methyl-4-aminoazobenzene <sup>(7)</sup> gave no depression of melting point. The specific radioactivity of the dye was 1.2 Ci/mmmole (5.7 mCi/mg). Thin-layer chromatograms of the dye on silica gel in two solvent systems gave single visible components that were identical in  $R_F$  to those obtained with *N*-methyl-4-aminoazobenzene (benzene,  $R_F = 0.39$ ; 10% methanol in benzene,  $R_F = 0.80$ ). Analysis for radioactivity of the entire chromatogram developed with the latter solvent system showed that 99% of the radioactivity on the plate was contained in the zone occupied by *N*-methyl-4-aminoazobenzene. About 1% of the radioactivity on the plate was found in the zone ( $R_F = 0.70$ ) at which 4-aminoazobenzene chromatographs in this system. On rechromatography of the *N*-methyl-4-aminoazobenzene zone 1% of the radioactivity was again

\* Nuclear Chicago Corp., Des Plaines, Illinois.

found at  $R_F = 0.70$ . This finding suggests that the *N*-methyl dye is slowly demethylated, possibly by oxidation, during these manipulations. The infrared spectra of the labelled dye and of *N*-methyl-4-aminoazobenzene <sup>(7)</sup> were identical in minute detail and each exhibited maxima and intensities as follows : 3,320 s, 3,160 w, 3,030 w, 2,820 w, 1,605 s, 1,582 s, 1,530 s, 1,500 w, 1,460 m, 1,440 w, 1,425 w, 1,405 s, 1,305 w, 1,288 m, 1,245 m, 1,190 w, 1,145 s, 1,138 s, 1,102 m, 1,070 w, 1,018 w, 990 w, 960 w, 940 w, 928 w, 915 w, 830 s, 820 m, 765 s, 720 w, 686 s, 540 m, 508 m, 490 w, 450 w, 380 w  $\text{cm}^{-1}$ .

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