SYNTHESIS OF ^{14}C-LABELLED BIS-AZO BIPHENYL DYES

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SUMMARY

Two ^{14}C-labelled bis-azo biphenyl dyes have been prepared from uniformly ring-labelled ^{14}C-benzidine and ^{14}C-3,3'-dimethylbenzidine. The amines were tetra-azotized with sodium nitrite and HCL. Direct Blue 6 was then prepared from tetra-azo benzidine by allowing it to react with two equivalents of H acid (8-amino-1-naphthol-3,6-disulfonic acid mono sodium salt) under basic conditions. Acid Red 114 was prepared from tetra-azo 3,3'-dimethylbenzidine by sequential reaction with one equivalent of G acid (2-naphthol-6,8-disulfonic acid dipotassium salt) followed by one equivalent of phenol. This product was then esterified with p-toluenesulfonyl chloride to give acid Red 114.

Key words: bis-azo biphenyl dyes, ^{14}C-benzidine, ^{14}C-3,3'-dimethylbenzidine, Acid Red 114, Direct Blue 6.

INTRODUCTION

Bis-azo biphenyl dyes have recently received widespread attention as possible human carcinogens (1-4). In the course of our research into the biological transformation of these dyes, it became necessary to prepare radiolabelled Direct Blue 6 and Acid Red 114 for distribution studies in animals (5). Although these dyes are widely known commercially (6), their syntheses are not available in the chemical literature and were consequently redeveloped from first

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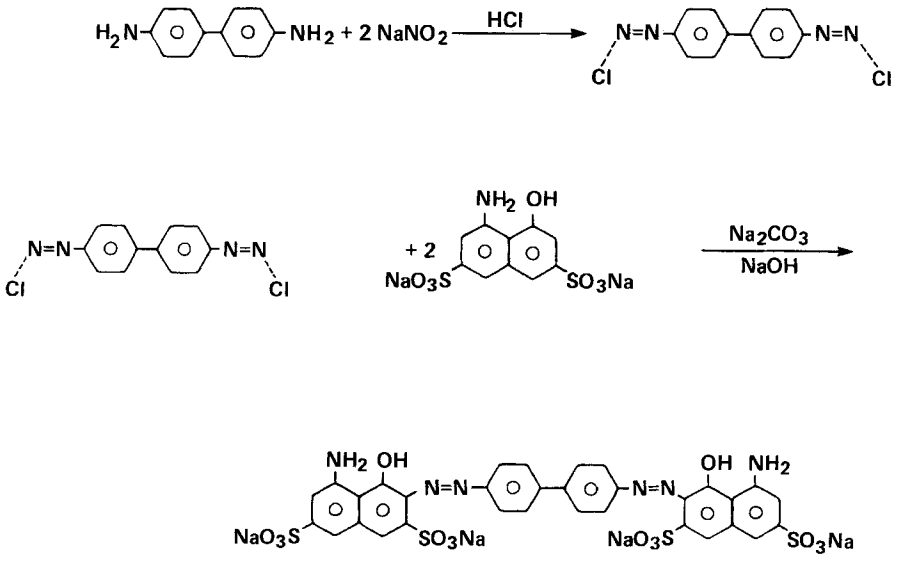
principles. Since ultra-pure compounds were required for our biological studies and since azo dye synthesis often leads to a wide variety of products with similar physical characteristics, special problems in purification were encountered.

The focus of our studies has been the biological fate of benzidine or substituted benzidine produced by metabolic azo reduction of bis-azo biphenyl dyes (6). Hence, these dyes were prepared from uniformly ring-labelled ^{14}C-benzidine or ^{14}C-3,3'-dimethylbenzidine to allow detection of the benzidine moiety by liquid scintillation counting. The chromophores used were unlabelled so that their eventual distribution, after azo reduction, would not interfere with the detection of the benzidine base.

The starting materials, benzidine and 3,3'-dimethylbenzidine, were tetra azotized by the usual procedure using sodium nitrite and HCl (7). The resulting electrophilic species was then allowed to react with two equivalents of H acid in the case of Direct Blue 6 (Figure 1), or sequentially with one equivalent of

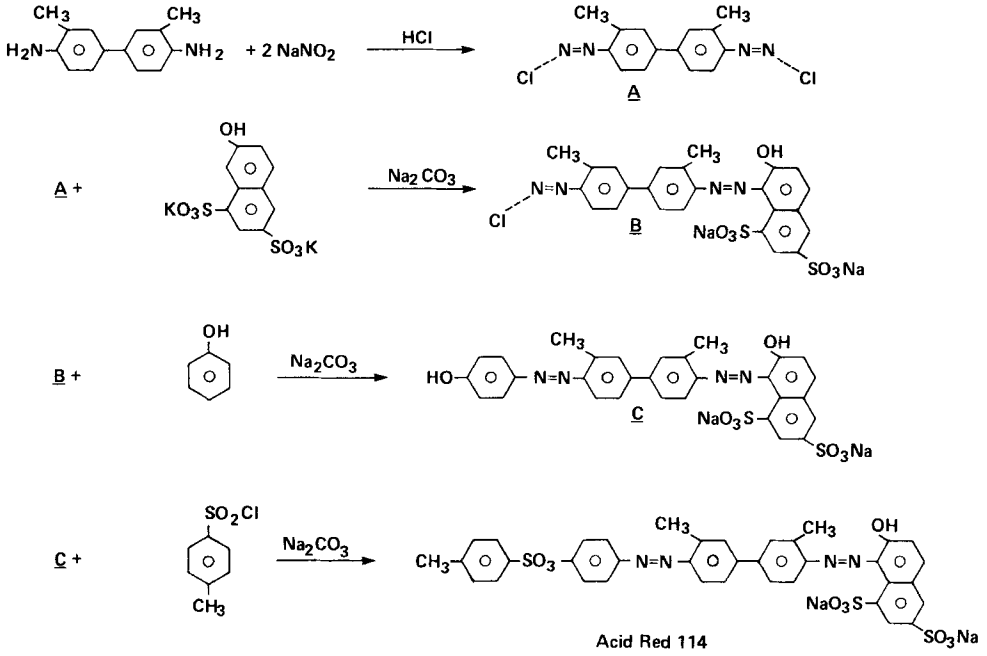
Figure 1



Direct Blue 6

G acid, followed by one equivalent of phenol in the case of Acid Red 114 (Figure 2). The product of the phenol coupling was then converted to the p-toluene sulfonate using p-toluenesulfonyl chloride.

Figure 2



The coupling of the tetra-azo compounds with the chromophores was accomplished under basic conditions to insure that the attack occurred in a position ortho to the naphthol and para to the phenol. However, in spite of this effort, several products were formed from these syntheses. These apparently resulted from the variety of available sites for attack on the chromophore. As dye stuffs are often difficult to crystallize, it was found that these compounds were best purified by chromatographic techniques.

EXPERIMENTAL

High pressure liquid chromatography was performed on a Hewlett Packard 1084B liquid chromatograph using 0.01 M phosphate buffer, pH 6, and acetonitrile as solvents. Preparative scale TLC was performed on Analtech Inc. 2000 μ silica gel G plates. Uniformly ring-labelled ^{14}C-benzidine and ^{14}C-3,3'-dimethylbenzidine were provided by Pathfinder Laboratories, Inc., St. Louis, MO. Unlabelled 3,3'-dimethylbenzidine was obtained from Fluka Chemical Corp., Hauppauge, NY, and was greater than 98% pure. G-acid (2-naphthol-6,8-disulfonic acid dipotassium salt) was obtained from Pfaltz and Bauer, Inc., Stamford, CT, as were p-toluenesulfonyl chloride and H-acid (8-amino-1-naphthol-3,6-disulfonic acid monosodium salt). Phenol was greater than 99% pure and was obtained from Aldrich Chemical Co., Milwaukee, WI.

Direct Blue 6. Benzidine, uniformly ^{14}C-labelled, 2024 μCi, 28 mg (0.15 mmol), was dissolved in 600 μl of 1.5 N HCl at 80°C. The solution was then cooled to 10°C and 150 μl of 2 M $NaNO_2$ was added slowly with stirring. This solution of tetra-azotized benzidine was added slowly with stirring to an ice-cold solution of 120 mg (0.35 mmol) H-acid and 120 mg Na_2CO_3 in 600 μl of 0.5 N NaOH. This solution was stirred for 20 min at 0°C and was then allowed to warm to room temperature with stirring for 1 hr. Finally, the solution was heated to 80°C and stirred for 5 hr. It was then allowed to cool to room temperature and was stirred overnight.

The following day, the reaction mixture was diluted to 50 ml with water and was neutralized with 1 N HCl. Pure ^{14}C-labelled Direct Blue 6 was then obtained by preparative scale HPLC on a Waters C18 Bondapak column. The percent acetonitrile in phosphate buffer was increased from 5% at the rate of 2/3% per min at a flow rate of 2 ml/min. The product was eluted in the 16% acetonitrile fraction. Yield 66% by u.v. detection at 295 nm.

Acid Red 114. ^{14}C-3,3'-dimethylbenzidine, uniformly ring-labelled, 1230 μCi, 19.7 mg (0.095 mmol) was combined with unlabelled 3,3'-dimethylbenzidine, 86.4 mg

(0.415 mmol) and dissolved in 2 ml of 1.5 N HCl at 30°C. The solution was then cooled in ice and 0.5 ml of 2 M $NaNO_2$ was added slowly with stirring. The mixture was allowed to continue stirring for 20 min after the addition was complete and was then added dropwise to a stirred, ice-cold solution of 190 mg (0.500 mmol) of G acid dipotassium salt and 220 mg (2.07 mmol) of sodium carbonate in 2.5 ml of water. After this mixture had stirred for 30 min, phenol, 60 mg (0.63 mmol) was added and the resulting solution was stirred for 3 hrs with ice cooling, followed by 15 hrs of stirring at room temperature.

The following day, p-toluenesulfonyl chloride, 250 mg (1.31 mmol) and sodium carbonate, 145 mg (1.36 mmol) was added to the reaction mixture with stirring. The reaction was monitored by TLC and additional aliquots of p-toluenesulfonyl chloride and sodium carbonate were added hourly until the reaction was complete. This required a total of 500 mg p-toluenesulfonyl chloride and 290 mg of sodium carbonate.

The pH of the solution was then adjusted to 8.0 with 0.5 M H_2SO_4 and the product was salted out at 60°C with 360 mg NaCl per ml of solution. The mixture was allowed to stir at this temperature for 10 min after the salt was added and was then refrigerated overnight.

The precipitated dye was recovered by suction filtration and dried over P_2O_5 at 0.025 torr for 24 hr. This dry product was then washed with chloroform to remove excess p-toluenesulfonyl chloride and purified by preparative TLC on silica gel using 16:4:3:2 butanol:methanol:ammonium hydroxide:pyridine. Yield: 278 mg (67%).

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REFERENCES

1) Wynder E.L., Onderdonk J., and Mantel N. - Cancer 16: 1388 (1963).

2) Cole P., Hoover R., and Friedell G.H. - Cancer 29: 1250 (1972).

3) Viadana, E., Bross, I., and Houten, L. - J. Occupat. Med. 18: 787 (1976).

4) Robens J.F., Dill G.S., Ward J.M., Joiner J.R., Griesemer R.A., and Douglas J.F. - Toxicol. Appl. Pharmacol. 54: 431 (1980).

5) Lynn R.K., Milam D.F., Eastman C.L., Rodgers R.M., and Garvie-Gould C. - Pharmacologist (1982), in press.

6) Lynn R.K., Donielson D.W., Ilias A.M., Kennish J.M., Wong K., and Matthews H.B. - Toxicol. Appl. Pharmacol. 56: 248 (1980).

7) Fierz-David H.E. and Blangey L. - Fundamental Processes of Dye Chemistry, Transl. by Vittum P.W., Interscience, New York, 1949.