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Direct Dyes Derived from 4,4'-Diaminobenzanilide Synthesis, Characterization and Toxicity Evaluation of a Disazo Symmetric Direct Dye

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The synthesis of a new symmetric disazo direct dye containing the non-carcinogenic 4,4'-diaminobenzanilide as the middle component and salicylic acid as the coupling component is presented. The synthesized dye is an analog of the benzidine based dye C. I. Direct Yellow 1 (C. I. 22,250) and was analyzed by thin layer chromatography (TLC), electronic spectra (VIS) and mass spectroscopy (FAB-MS). The coloristic and fastness properties of the synthesized dye were determined and compared with those of C. I. Direct Yellow 1. The toxicities of the dye and of its precursors were evaluated by biological tests, using the process of metamorphosis in the marine hydrozoon *Hydractinia echinata*. The concentrations (termed MRC₅₀) at which the synthesized dye and its precursors antagonize metamorphosis induction were determined.

Key Words: Direct dyes, 4,4'-diaminobenzanilide, fastness properties, *Hydractinia echinata*, toxicity, MRC₅₀.

Introduction

It is well known that benzidine-based dyes' manufacture is prohibited due to the carcinogenicity of the precursor. Thus, extensive research has been conducted to find alternative dyes whose production and use would meet high environmental and safety requirements¹⁻¹².

Our studies are focused on the possibilities of using aromatic diamines, which are not benzidine congeners, for the synthesis of non-toxic direct dyes having similar dyeing and fastness properties to their benzidinic homologs.

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One such diamine is 4,4'-diaminobenzanilide, which has already been used as a benzidine substitute in the synthesis of some direct dyes^{4,6,13}.

The aim of the present study was to establish the best reaction conditions for the synthesis of an analog dye 'C. I. Direct Yellow 1 (C. I. 22,250), using 4,4'-diaminobenzanilide as a substitute for benzidine. Secondly, we determined the physical-chemical and fastness properties of this dye in order to compare them with those of C. I. Direct Yellow 1. The toxicity of the synthesized dye was evaluated by biological tests, using the process of metamorphosis in the marine hydrozoon *Hydractinia echinata*, and the concentration at which the synthesized dye (and its precursors) antagonize metamorphosis induction was determined.

Experimental

General

The chemicals employed in this study were obtained from Merck Co, Fluka Co, Aldrich Chemical Co and Reactivul Bucuresti. The thin layer chromatography (TLC) data were taken from silica gel plates (Merck 60F-25). Electronic spectra were recorded on a Specord M-40 Karl Zeiss Jena spectrophotometer. Melting points were recorded on a Boetius PHMK 05 melting point apparatus and are uncorrected. IR spectra were recorded on a Specord M-80 Karl Zeiss Jena spectrophotometer. Mass spectra were recorded on a Nermag R 10-10 spectrometer.

Procedures

Synthesis of 4,4'-diaminobenzanilide

A mixture of 34 g (0.2 mol) of 4-nitrobenzoic acid was heated with 35.7 g (0.3 mol) thionyl chloride in the presence of 1.6 mL (0.02 mol) dimethylformamide as catalyst. The process was carried out at a temperature of 65–70 °C for 4–5 h. Once the reaction was terminated, 30 mL of CCl₄ was added to the mixture and excess thionyl chloride was distilled off. The 4-nitrobenzoic acid chloride was obtained with 97% yield. Recrystallization from ligroin gave m. p. 72–73 °C (lit. 73–74 °C)¹⁴.

A mixture of 210 mL of anhydrous chlorobenzene, 30 g (0.162 mol) of 4-nitrobenzoic acid chloride and 16.4 g (0.165 mol) of triethylamine was stirred and gently heated to 60 °C. At this temperature, 22.8 g (0.165 mol) of p-nitroaniline was then added to the mixture over 1 h. The mixture was heated to 90–95 °C and maintained at this temperature for 4–4.5 h. The pH of the mixture was brought to 7–7.5 by the addition of a 10% NaOH solution. Excess chlorobenzene and triethylamine were removed by steam distillation. The product was collected by filtration and washed with water to give 40 g of 4,4'-dinitrobenzanilide (89% yield). Recrystallization from acetic acid gave m.p. 272–273 °C (lit. 271–273 °C)¹⁵.

The resulting dinitrocompound was subjected to hydrogenation, by stirring under reflux for three h 20 g (0.12 mol) of 4,4'-dinitrobenzanilide in an aqueous FeCl₂ solution containing 100 mL water, 35 g of Fe and 3.5 mL of 32% HCl. The reaction mixture was maintained for 2 h at 95–98 °C and the solution was made alkaline using 20% Na₂CO₃ until pH 7.5–8 and was filtered hot. The filtrate was treated with a 30% sulphuric acid solution until pH 1–1.5, when solid 4,4'-diaminobenzanilide sulfate precipitated. 4,4'-diaminobenzanilide was obtained by neutralization, cooling and filtration. Some 11 g of 4,4'-diaminobenzanilide was obtained, m.p. = 203–205 °C (lit. 204–205 °C (DMF:water = 5:1)¹⁵. The IR spectrum showed carbonyl absorption

($\nu_{C=O}$) at 1690 cm^{-1} and an amide stretch (ν_{NH}) at 2980 cm^{-1} (asym); 3320 cm^{-1} (sym). The positive ion EI-MS spectra in ammonia gave the molecular peak $[M+H^+] = 228\text{ m/z}$.

Synthesis of salicylic acid, 4,4'-diaminobenzanilide salicylic acid

A suspension of 11.5 g of 4,4'-diaminobenzanilide (0.05 mol), 70 mL of water, 26 mL (0.27 mol) of 35% HCl and 50 g of ice was cooled to $0\text{ }^\circ\text{C}$ and stirred. Then, 7.2 g (0.102 mol) of 98% NaNO_2 in 30 mL of water was added to the above suspension over 30 min. The resulting solution was stirred at $0\text{--}2\text{ }^\circ\text{C}$ for 1 h. A clear acid yellow-brown solution was obtained. Thereafter, 15 g (0.108 mol) of salicylic acid was dissolved in a hot solution ($40\text{ }^\circ\text{C}$) containing 6 g (0.0566 mol) of Na_2CO_3 in 90 mL water. To the obtained solution, 39 g (0.30 mol) of 30% NaOH solution was then added and the mixture was cooled with ice to $5\text{ }^\circ\text{C}$. Half of the resulting salicylic acid solution was added to the bis-diazonium salt at such a rate that the reaction temperature did not exceed $5\text{ }^\circ\text{C}$ in a 45 min period. The pH of the reaction was maintained at 7.5–8 by periodic addition of 0.5 M Na_2CO_3 aqueous solution. The mixture was kept cold for 2 h. The coupling reaction was controlled in the usual way (drop reaction with an alkaline solution of 1-amino-8-naphthol-3,5-disulphonic acid (H acid) respectively with the diazonium salt of 4-nitroaniline) and by TLC. Thereafter, the pH of the reaction was brought to 8.5–9 by the addition of 0.5 M Na_2CO_3 solution and the other half of the salicylic acid was added dropwise to it over 45 min. The temperature was maintained in the range of $8\text{--}12\text{ }^\circ\text{C}$ for 3 h. The dye was precipitated without salting, by adjusting the mixture's pH to 6–7 with the aid of 10% HCl. The crude dye was collected by vacuum filtration, and purified by several recrystallizations from water. The pure yellow dye was obtained in 82% yield. It was analyzed by means of TLC (silica gel and *i*-propanol/methylethylketone/ NH_4OH 25% = 4:3:3), electronic spectra and mass spectroscopy. It had $R_f = 0.88$. The VIS spectra were examined in the region of 300–800 nm. The spectra contain a maximum at about $\lambda_{\max} = 441\text{ nm}$ ($E_{1\text{cm}}^{1\text{g/L}} = 62.8$) in aqueous 0.1 M NaOH solution and respectively 1 maximum at $\lambda_{\max} = 440\text{ nm}$, ($E_{1\text{cm}}^{1\text{g/L}} = 62.4$) in 1% $\text{CH}_3\text{COONH}_4$ aqueous solution. The mass spectrum of the dye was recorded by FAB-MS in negative mode (-) using nitrobenzylalcohol and glycerol as the matrix. The molecular peak of the dye was $m/z = 524.38$ [M-1].

The coloristic and application properties, e.g., dyebath uptake, exhaustion degree, migration, levelling and fastness properties of the synthesized dye' were determined according to Romanian standards¹⁶.

Biological tests

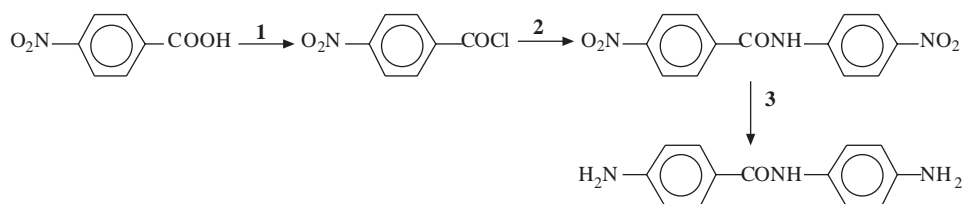
Colonies of *H. echinata* were used to obtain eggs and larvae. The culture medium was artificial seawater¹⁷. For 3 h the larvae were exposed to seawater containing 20 mM CsCl and simultaneously one of the test substances (the disazo dye, and its precursors, e.g., salicylic acid, 4,4'-diaminobenzanilide, 4-nitrobenzoic acid and 4-nitroaniline). A dish contained 30 larvae in 3 mL of seawater. The percentage of animals that underwent metamorphosis (development into polyps) was determined the next day. For each substance concentration experiments were performed in triplicate. The experiment was repeated at least twice. Based on these values, the concentration of the test substance (in mol per liter) at which the frequency of induction was reduced by 50% was determined.

Results and Discussion

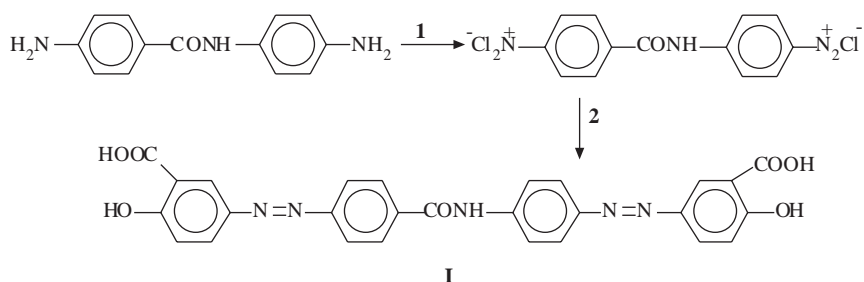
In the present study, the optimal reaction conditions for the synthesis of 4,4'-diaminobenzanilide and of a symmetrical disazo dye based on this intermediate were established.

The usual method for the preparation of 4,4'-diaminobenzanilide involves the condensation of 4-nitrobenzoyl chloride with 4-nitroaniline followed by the reduction of the bis-nitro compound^{15,18}. It can be also prepared by the use of 1,4-phenylenediamine instead of 4-nitroaniline.

In the present work 4,4'-diaminobenzanilide was prepared as shown in Scheme 1, with an overall high yield, namely 79%, using the first mentioned process, with some improvements in order to increase the yield of the reaction.



Scheme 1. 1. SOCl_2 , DMF, 65–70 °C, 4–5 h, 2. p-nitroaniline, $\text{C}_6\text{H}_5\text{Cl}$, triethylamine, 90–95 °C, 4.5 h, 3. Fe, H_2O , HCl, 95–98 °C, 2 h.



Scheme 2. 1. NaNO_2 , HCl, 0 °C, 1 h, 2. Salicylic acid, final pH = 9, 5–12 °C, 5 h.

From a series of preliminary studies, it was found that bis-diazotization of 4,4'-diaminobenzanilide by the direct method, in an aqueous HCl solution, was the best method for this compound.

The amount of acid used was more than 4.0 moles per mole of 4,4'-diaminobenzanilide. A slight excess of sodium nitrite was used for a complete bis-diazotization. It was found that the bis-diazotization occurred with the best yield when temperature was maintained in the range 0–3 °C and the pH of the mixture was around 1. The amount of the bis-diazonium salt of 4,4'-diaminobenzanilide was calculated from the amount of sodium nitrite consumed, and was used to determine the quantity of couplers employed in subsequent steps.

In the coupling step with salicylic acid, a 3% excess of the coupling component was used. During the syntheses, it was found that if the pH value was too high, undesirable by-products may be formed. To decrease impurities, an alkaline solution of salicylic acid was added to the bis-diazonium salt solution fast initially, and then slowly. The first coupling reaction required a temperature below 5 °C, 2 h and a final pH ranging from 7.5 to 8.

For the next coupling step, the pH of the mixture was brought to 8.5. The best reaction conditions were 8–12 °C and pH 8.5–9. The reaction time was about 3 h. The progress of the coupling reactions was

monitored in the usual way and by TLC, when the formation of the coupled compounds was examined. The final product was separated, without salting out, by filtration.

The dye was characterized by means of TLC and VIS spectra and its structure was confirmed by negative (-) FAB – MS.

The coloristic and application properties of the disazo direct dye were determined in order to compare them to those of a similar benzidinic dye, e. g. Direct Yellow 1. It was noted that the untreated dyeing on cotton was reddish yellow and the dye has fairly good migration and good levelling properties. The percentage uptake on cotton was found to be 50 %. The results of the fastness studies for the synthesized dye and for C. I. Direct Yellow 1 are summarized in Table 1.

Table 1. Fastness data for the 4,4'-diaminobenzanilide based disazo dye and for its benzidinic homolog C. I. Direct Yellow 1 (C. I. 22,250).

| Fastness: | Disazo dye I New compound | | C. I Direct Yellow 1 (C. I. 22,250) Reference compound | |
|-------------------------|------------------------------|---|--|---|
| | Direct dyeing | After treatment with CuSO ₄ | Direct dyeing | After treatment with CuSO ₄ |
| Light | 4 | 4-5 | 4 | 4-5 |
| Water | 3-4 | 4-5 | 2-3 | 4 |
| Wash 40 °C | 3-4 | 4 | 1-2 | 2-3 |
| Perspiration (alkaline) | 3 | 3 | 2 | 1-2 |
| Perspiration (acid) | 3 | 3 | 2-3 | 3 |
| Hot pressing | 4-5 | 4-5 | 4 | 3-4 |

These results show that the application and coloristic properties of the synthesized disazo dye are typical for the direct class of dyes. The after-treatment of the dyed samples with CuSO₄ shows some improvements, especially in the case of wet fastness. Light fastness values are the same as those of Direct Yellow 1, but the wet fastness as well as the fastness to acid perspiration values are even better. According to these results, we can conclude that disazo direct dye containing 4,4'-diaminobenzanilide as the diazo component is an excellent substitute for its benzidinic homolog Direct Yellow 1.

As described in the Experimental, the toxicity of the disazo direct dye and of its precursors was evaluated by biological tests. The larvae of *H. echinata* have an elongated spindle-shaped body of about 1 mm in length and a diameter of 100 μ m and are easy to obtain. They consist of about 10, 000 cells. They have no mouth, no gut, no extremities, no sense organs but have nerve cells that may serve to sense environmental signals. In the laboratory the adults produce several thousand eggs per week all year long. Metamorphosis into polyps can be triggered artificially by a 3- h treatment with seawater enriched with Cs⁺ ions or depleted by Mg²⁺ ions¹⁹. Within 1 day a larva transforms into a polyp that looks similar to the well known freshwater polyp Hydra. When certain substances (our test substances for instance) are applied during the triggering treatment, induction is antagonized. In this case the larvae remain at the larval stage: they are prevented from completing their life cycle. Thus, the chemicals antagonize the species from surviving and this is one of the reasons justifying the choice of our testing system. On the other hand, substances that display a strong toxicity in well-known test systems also display a strong antagonizing influence on metamorphosis induction and vice versa¹⁶. The results of the biological tests expressed by the logarithm of the reciprocal value of the metamorphosis reducing concentration, log 1/MRC₅₀, are shown in Table 2.

Table 2. Results of the toxicity tests for the 4,4'-diaminobenzanilide direct dye and for its precursors.

| Compound | log 1/MRC ₅₀ |
|-------------------------|-------------------------|
| 4-nitroaniline | 2.85 |
| 4- nitrobenzoic acid | 3.11 |
| 4,4'-diaminobenzanilide | 3.39 |
| Salicylic acid | 2.73 |
| Disazo dye | 3.44 |

The values of log 1/MRC₅₀ are quite close for all the test substances and no major difference was observed when comparing them.

Conclusions

A yellow disazo direct dye containing 4,4'-diaminobenzanilide as the middle component was synthesized in an attempt to complete the range of colors of the direct dyes free of benzidine.

The application, coloristic and fastness properties on cotton of the synthesized dye were similar or even better in comparison to those of Direct Yellow 1, and indicated that the synthesized disazo direct dye is an excellent substitute for its benzidinic homolog.

Metamorphosis reducing concentrations, MRC₅₀, of the synthesized dye and of its precursors were calculated by means of biological tests using *H. echinata* larvae. The values of the logarithm of the reciprocal value of the metamorphosis reducing concentration, log 1/MRC₅₀, show that the synthesized dye may be an ecological product and this is in agreement with the major concerns of Green Chemistry.

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