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Synthesis and Evaluation of Non-genotoxic Direct Dyes

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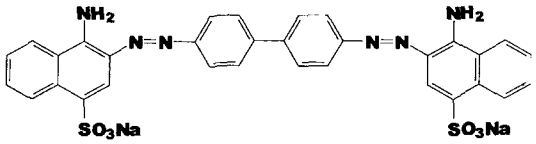
Abstract: Non-genotoxic diamines 2,2'-dimethyl-5,5'-dipropoxybenzidine and 5,5'-dipropoxy-benzidine were employed as potential alternatives to benzidine in the synthesis and evaluation of new direct dyes for cotton. Assessment of the resultant dyes indicated that both diamines can be used to prepare new direct dyes having colors and fastness properties that make them comparable to commercial direct dyes, and that the structures of the new direct dyes can be confirmed by negative ion electrospray mass spectrometry (ESMS). The mutagenic properties of new direct dyes were established using the standard Ames Salmonella mammalian mutagenicity assay.

Keywords: Direct dyes, Benzidine replacement, Electrospray ionization mass spectrometry, Mutagenicity, Wash- and light-fastness

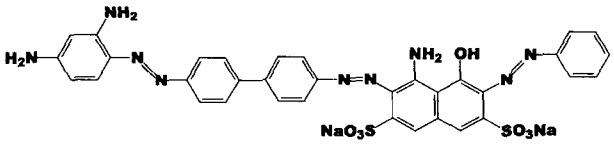
Introduction

Direct dyes have been used to dye cellulose for over 100 years. Because of the ease of their application and the wide gamut of products available at a modest cost, direct dyes are still a popular dye class[1]. Most direct dyes have disazo and trisazo structures, with each hue dominated by unmetallized structures[2]. For many years, direct dyes included those made from benzidine and its analogs. Nowadays, it is well known that benzidine is both a mutagenic amine and a human carcinogen[3,4]. Prior to the realization of these facts, many dyestuffs were produced that employed benzidine or a benzidine congener such as *ortho*-tolidine or *ortho*-dianisidine as a precursor[5-12]. By the 1980s, however, dyes derived from benzidine and certain of its derivatives were designated as genotoxic diamines. Colorants prepared from genotoxic intermediates can be either direct acting mutagens or promutagens. By genotoxic is meant interaction between DNA and substances that produce heritable changes in a cell or organism. A promutagen is a compound exhibiting mutagenic activity following metabolic activation[13]. Hence, the manufacture and use of genotoxic intermediates and colorants presents a potential occupational and environmental risk[14,15]. Dyes based on benzidine (**1-3**) and its congeners (**4-5**) can no longer be manufactured for textiles in the United States because of their carcinogenic potential[10,11, 16,17]. However, concern over exposure to this carcinogen and its congeners led to the search for non-genotoxic alternatives[18].

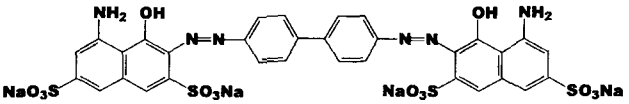
The commercial significance of benzidine-based colorants has caused the search for viable nonmutagenic analogs of benzidines to continue to be an important research problem. Significant progress in this area was made by Shahin *et al.*, who found that the mutagenicity of aromatic meta-phenylenediamine (**6**) could be lowered or removed by placing bulky alkyl or alkoxy substituents *ortho* to an amino



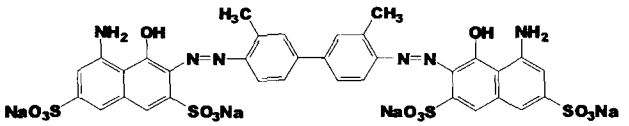
1. Congo Red



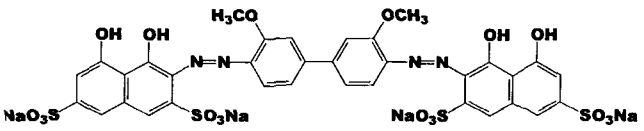
2. C.I. Direct Black 38



3. C.I. Direct Blue 6



4. C.I. Direct Blue 14



5. C.I. Direct Blue 10

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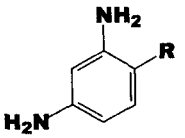
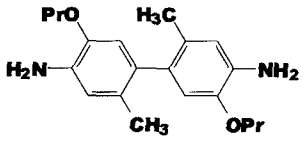


6 R = $CH(CH_3)_2$, $CH_2CH_2CH_3$, $OCH_2CH_2CH_2CH_3$, OCH_2CH_2OH



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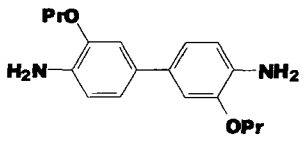

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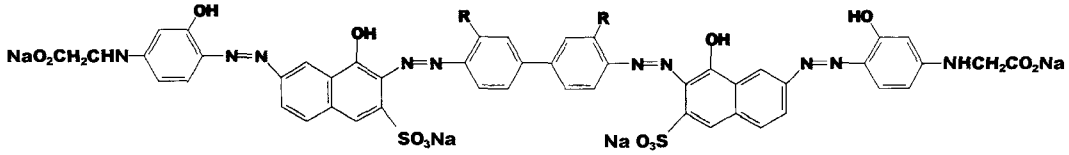


7. R = $OCH_2CH_2CH_2CH_3$, $CH_2CH_2CH_3$

group in the molecule[19-21]. The results of that study showed that mutagenicity was reduced when the *ortho*-substituent became larger. This discovery was followed by patents describing the synthesis of nonmutagenic benzidines[22,23].

Two groups, working independently, found that benzidine derivatives containing C-3 to C-4 alkyl or alkoxy groups *ortho* to the amino groups were nonmutagenic, and also reported that azo dyes based on the propyl and butoxy benzidine derivatives (**7**) were nonmutagenic in the standard Ames test[11,13]. One application example is the nonmutagenic black ink jet dye[24].

In related studies it has been shown that 2,2'-dimethyl-5,5'-dipropoxybenzidine (**8**) is non-mutagenic[25] and gives non-mutagenic azo and azomethine pigments[26].

The present paper pertains to the synthesis and evaluation of direct dyes in which 2,2'-dimethyl-5,5'-dipropoxybenzidine (**8**) and 5,5'-dipropoxybenzidine (**9**) are employed as potential alternatives to benzidine. The two benzidine analogs were diazotized and coupled with widely used dye intermediates to generate a group of direct dyes giving red to blue shades on cotton. Dye structures were confirmed using negative ion electrospray mass spectrometry (ESMS). A summary of the ESMS data and fastness properties of the new direct dyes are reported. Mutagenicity data is also provided for the new direct dyes synthesized from 2,2'-dimethyl-5,5'-dipropoxybenzidine and 5,5'-dipropoxybenzidine.

Experimental

All of the chemicals used in this work were obtained from Aldrich Chemical Co., Milwaukee, WI. The mercerized cotton fabric was obtained from Test Fabrics, Inc. and the style number was 400 M. The apparatus used to dye the cotton fabric was an Ahiba Texomat dyeing machine. Washfastness and lightfastness were measured by using an Atlas Launder-ometer and Atlas 3 SUN Hi 35 high irradiance Xenon Weather-ometer, respectively. Absorption spectra were recorded on a Varian Cary 3 UV-Visible spectrophotometer and thin layer chromatography (TLC) was conducted using Whatman 250 μm silica gel 60 A plates.

Dye Intermediates 8 and 9

Both compounds were synthesized in three steps from 4-methyl-2-nitrophenol (**10**) and 2-nitrophenol (**11**), via alkylation, alkaline-reduction and acid-mediated benzidine rearrangement[25].

Dye Synthesis

Compounds **8** and **9** were dissolved in a mixture of water and conc. HCl and the temperature was lowered to 0-5 °C. Sodium nitrite solution (1.7 M) was added dropwise with stirring for 30 minutes, and the resultant solution was added to an alkaline (pH 8-9) solution of the appropriate coupler. After 4 hours, the dye was precipitated by using sodium chloride and collected by vacuum filtration.

Dye Application

A 1 % dyeing (owf) was carried out at pH 7 and a 60:1 liquor ratio. The cotton fabric was wet out with hot water and added to the dyebath at 60 °C. The temperature was raised to 95 °C and held for 30 minutes. Na_2SO_4 solution (10 %) was added to the dyebath and dyeing was continued for an additional 30 minutes. The fabric was removed, rinsed in cold water and air-dried.

Washfastness Determination

The washfastness of dyed fabric was evaluated using AATCC test method 61-1996 No. 2 A[27]. The dried fabric was evaluated for color change and staining of adjacent undyed multifiber fabric. The rating scale was 1 (poor) to 5 (excellent).

Lightfastness Determination

The lightfastness of dyed fabric was evaluated using AATCC test method 16-1998 option E[27]. The fabric was

evaluated for color change using a scale of 1 (poor) to 5 (excellent).

Mutagenicity Test

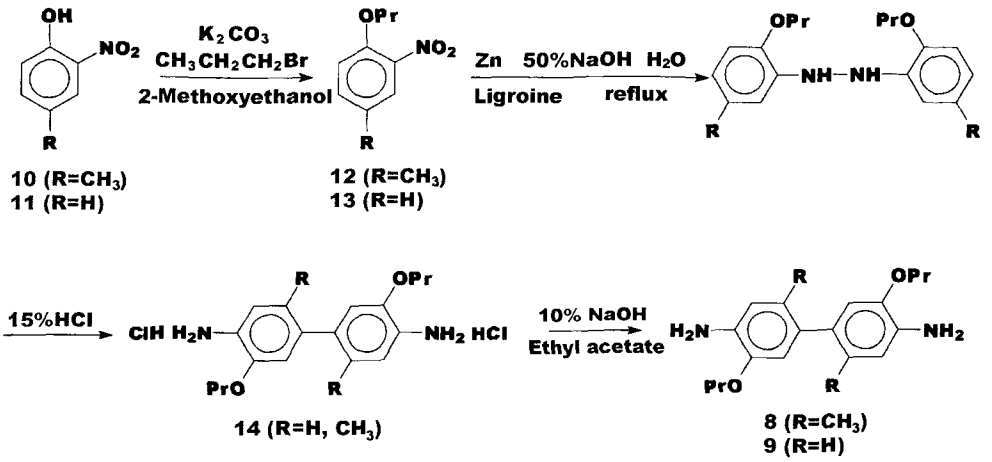
The assay employed was based upon those developed by Ames and co-workers[28]. For this assay, the introduced rat liver used in the S9 mix was prepared using male Sprague-Dawley rats. Two bacterial strains of Salmonella typhimurium were used (TA 98 and TA 100) in the presence and absence of metabolic activation (S 9 mix). Strain TA 98 was used to detect frameshift mutations, while strain TA 100 was used to detect base-pair substitutions. A plus or minus sign after the strain type represents the presence or absence of metabolic activation. To be designated as mutagenic, a dye must have produced an average revertant count that was more than two times the background average (i.e., number of colonies at the 0-μg dose), which have shown a dose-response effect, and the test result must have been reproducible. The initial dose range for the standard mutagenicity assay was 0.1-5 mg.

Results and Discussion

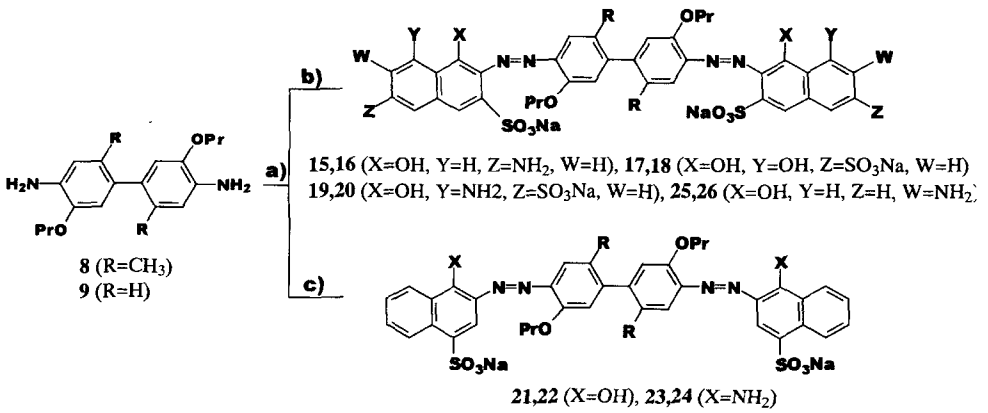
Synthesis

Benzidine derivatives **8** and **9** and direct dyes **15-26** were synthesized according to the route shown in Schemes 1 and 2. 4-Methyl-2-nitrophenol (**10**) and 2-nitrophenol (**11**) were alkylated to give compounds **12** and **13** in > 95 % yield. Alkaline reduction and benzidine rearrangement gave the dihydrochlorides of **8** and **9**. The free amines were produced by treatment of the dihydrochlorides (**14**) with aq. NaOH.

Tetrazotization of compounds **8** and **9** was carried out using $NaNO_2$/HCl at 5 °C. The tetrazotized solutions were added into and alkaline solution of couplers (such as J-acid, Chromotropic acid, H-acid, Neville-Winther acid, Naphthionic acid, and Gamma acid) to give the desired direct dyes. Dye purity was confirmed by TLC using BuOH:EtOH:NH_4OH:



Scheme 1. Synthesis of benzidine derivatives.



Scheme 2. Synthesis of direct dyes.

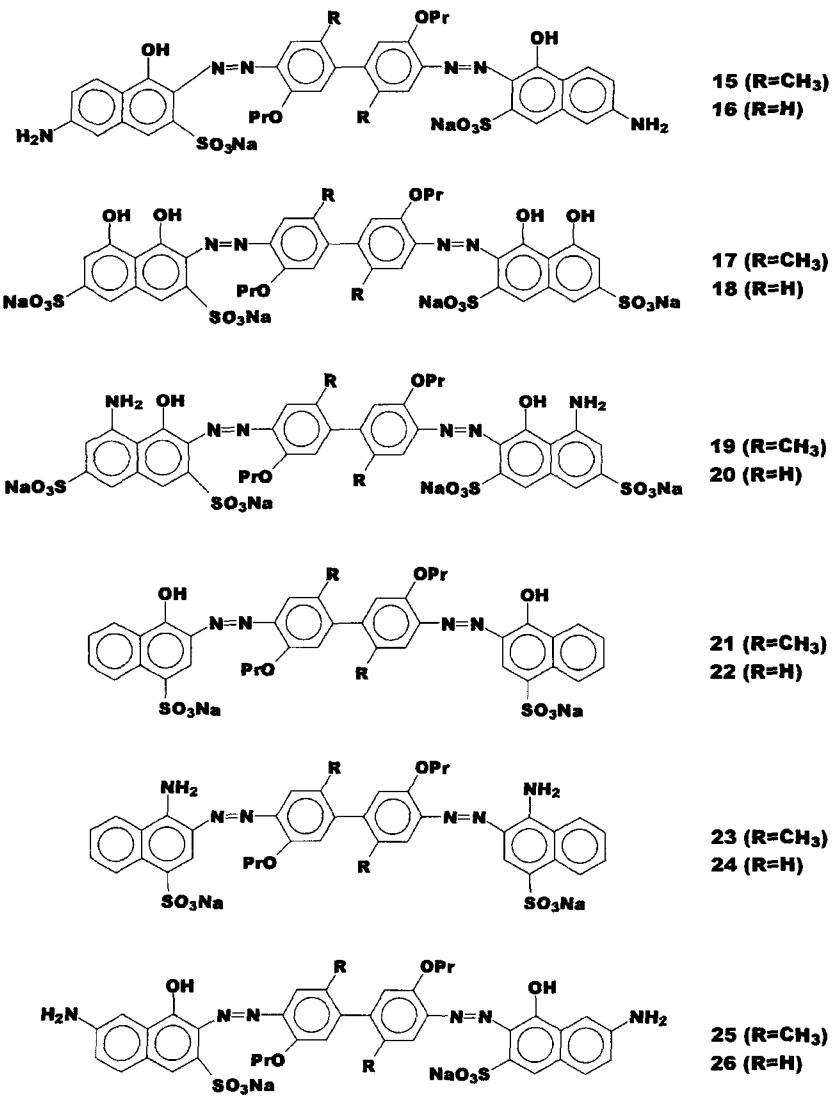


Figure 1. Structures of direct dyes made in this study.

H_2O (3:1:0.5:1.5) or (3:1:0.5:2.2) as the eluent. The structures of dyes synthesized are shown in Figure 1.

Absorption Spectra

The visible absorption spectra of all dyes were recorded in distilled water, the results of which are summarized in Table 1. When 5,5'-dipropoxybenzidine (**9**) was used, instead of 2,2'-dimethyl-5,5'-dipropoxybenzidine (**8**), a 10-50 nm higher λ_{max} was generally observed. Exceptions involved dyes **17** and **18**, where Chromotropic acid was used. All of the dyes, except **23** and **24** gave purple to blue shades (500-600 nm) on cotton, like the benzidine-based disazo dyes. Dyes **23** and **24**, in which the benzidine analogs were coupled with naphthionic acid, gave orange to red shades on cotton. The H-acid based dyes (**19** and **20**) gave comparable colors to commercial dyes **3** and **5**.

Table 1. Spectral data for dyes prepared in this study

Dye	Color	λ_{max} (nm)	E_{max} (*l*/mole.cm)
15	Purple	509	6.4×10^4
16	Violet	547	4.3×10^4
17	Blue	584	5.1×10^4
18	Blue	578	4.6×10^4
19	Blue	586	5.0×10^4
20	Blue	592	3.2×10^4
21	Purple	530	4.1×10^4
22	Blue	565	4.3×10^4
23	Orange	485	2.9×10^4
24	Red	505	2.2×10^4
25	Violet	537	2.6×10^4
26	Blue	585	3.3×10^4
3	Blue	583	3.6×10^4
5	Blue	601	3.0×10^4

Table 2. ESMS data for dyes prepared in this study

Dye	M/2 ions	M/3 ions	M/4 ions
15	413.3 (2Na)		
16	399.1 (2Na)		
17		336.5 (4Na)	246.6 (4Na)
18	479.0 (4H)	319.0 (4H)	239.4 (4H)
19		335.9 (4Na)	246.3 (4Na)
20	491.0 (4Na)	327.2 (4Na)	239.6 (4Na)
		319.6 (3Na + H)	
21	398.0 (2Na)		
22	384.0 (2Na)		
23	397.0 (2Na)		
24	383.0 (2Na)		
25	413.3 (2Na)		
26	399.0 (2Na)		

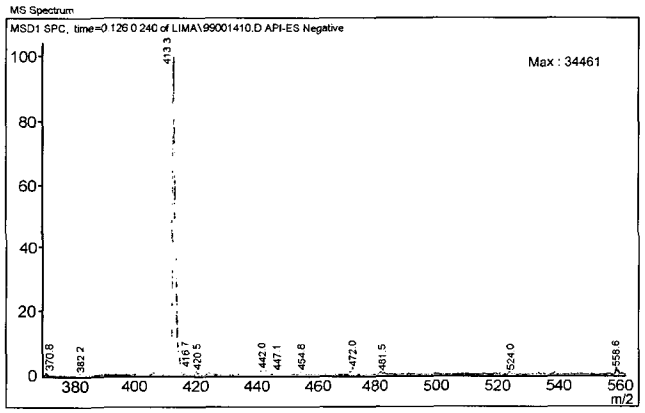


Figure 2. ESMS Mass spectrum of dye 15.

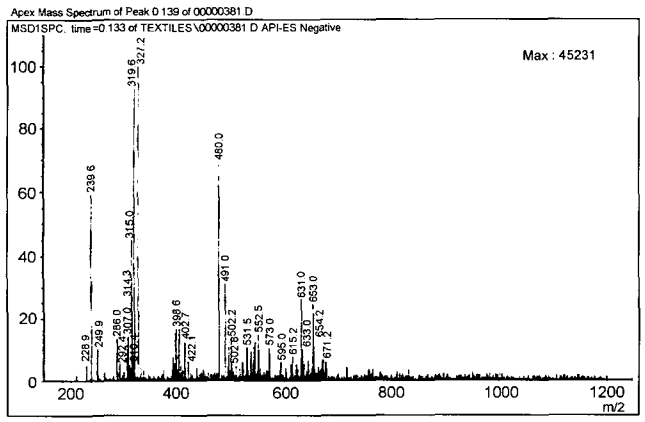
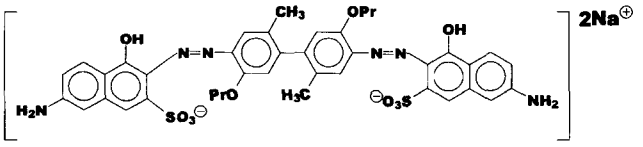


Figure 3. ESMS Mass spectrum of dye 20.

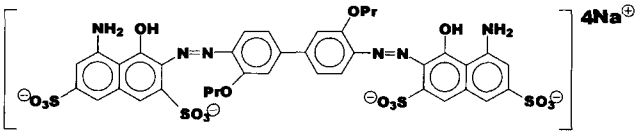
Mass Spectrometry

Table 2 contains a summary of the electrospray ionization mass spectrometry (ESMS) data produced on dyes **15-26**, and Figures 2 and 3 show representative spectra. Data presented correspond to the major analyte signals observed for m/2, m/3, and m/4 species. As expected, dyes **15, 16** and **21-26** gave only on m/2 signal as shown in Figure 4, while dyes **17-20** gave signals arising from 2^-, 3^-, and 4^- charges. In some cases, multiple signals having the same charge were observed. For instance, dye **20** gave two m/3 signals, which correspond to the structures shown in Figure 5. Clearly, ESMS is an excellent method for confirming direct dye structures.

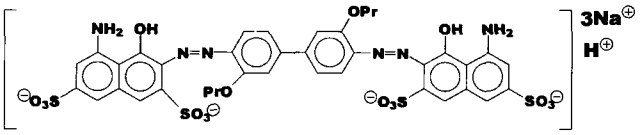


m/2 = 413.3

Figure 4. m/2 Species observed in the ESMS mass spectrum of dye 15.



m/2 = 491.0 m/3 = 327.2 m/4 = 239.6



m/3 = 319.6

Figure 5. m/z Species observed in the ESMS mass spectrum of dye 20.

Table 3. Fastness data for the dyes prepared in this study

Dye	Light fastness	Wash fastness		
		Change in shade	Staining on cotton	Staining on wool
15	2	1-2	2	3
16	3	3-4	2	5
17	2	1	5	5
18	2	2	3	5
19	1-2	1	4	5
20	2-3	1-2	2-3	5
21	1	1	3	3
22	2-3	2-3	2	5
23	1	1	3-4	4
24	2	3	2	4
25	2	1	3	5
26	3-4	3-4	2	5
3	3	3-4	2-3	5
5	3	3	3	5

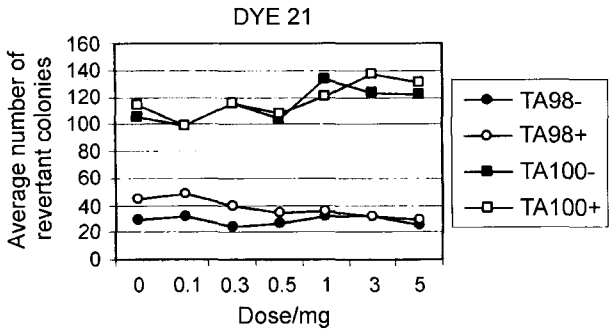


Figure 6. Dose response of Dye 21 using standard mutagenicity assay with bacteria strains TA98 and TA100 with (+) and without (−) S9 rat liver enzyme metabolic activation. DMSO = base count.

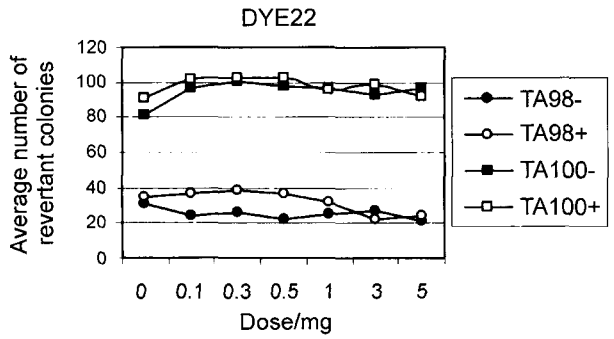


Figure 7. Dose response of Dye 22 using standard mutagenicity assay with bacteria strains TA98 and TA100 with (+) and without (−) S9 rat liver enzyme metabolic activation. DMSO = base count.

Fastness Properties

The results of fastness studies for the new dyes and related commercial dyes are summarized in Table 3. It can be seen from the data that the washfastness and lightfastness properties of the dyes are better when R=H. As would be anticipated, staining of cotton was better when the less linear dyes ($R=CH_3$) were used. The washfastness and lightfastness were acceptable when R=H, with a rating of 2-4 for color change. The best dyes, overall, were dyes **16** and **26**, which gave good lightfastness as well as washfastness. In this case, fastness was comparable to the commercial dyes that were employed in this study.

Mutagenicity Test

The dyes were evaluated in the standard salmonella mammalian mutagenicity assay. Figures 6 and 7 show the representative (**21**, **22**) dose response curve for each of the dyes tested. The background count is established for a control test in which no dye is present. A mutagenic response is recorded if the number of revertant colonies counted is at least twice the background count. As can be seen from Figures 6 and 7, all clearly established the dyes as nonmutagenic with and without S 9 activation for TA 98 and TA 100. This data provides evidence that bulky substitutents are required to reduce genotoxicity of benzidine-type compounds.

Conclusions

Nonmutagenic diamines such as 2,2'-dimethyl-5,5'-dipropoxybenzidine and 5,5'-dipropoxybenzidine were synthesized and evaluated as potential replacements for benzidine in direct dye synthesis. It was found that both diamines couple with naphthalene-based compounds that are frequently used to prepare direct dyes and the resultant dyes have colors and fastness properties that are comparable to certain commercial direct dyes. The introduction of a methyl group in the 2,2'-positions gives the expected hypsochromic shifts in disazo direct dyes, and gives favorable results with regard to staining of adjacent fabrics. The structures of the new direct dyes can be confirmed by negative ion electrospray mass spectrometry (ESMS). In this regard, the data obtained correspond to the formation of m/2, m/3 and m/4 species. It is also clear that the resultant direct dyes are generally nonmutagenic in the standard Ames Salmonella mammalian mutagenicity assay, and that several of them are potential replacements for commercial direct dyes prepared from mutagenic benzidine congeners.

Acknowledgements

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