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Synthesis and evaluation of organic pigments. 2. Studies of bisazomethine pigments based on planar nonmutagenic benzidine analogs

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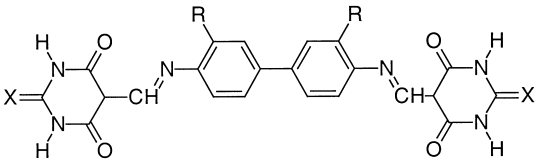
Abstract

The synthesis, characterization, genotoxicity and light fastness of thio- and oxopyrimidine based bisazomethine pigments prepared from a series of nonmutagenic benzidine congeners is reported. Whereas bisazomethine pigments based on benzidine and 3,3′-dimethylbenzidine were mutagenic in both the Ames assay and the Prival modification of the Ames assay, incorporation of bulky ($>C_2$) alkyl and alkoxy groups in the 3,3′- positions of the benzidine moiety gave nonmutagenic bisazomethine pigments.

Keywords: Bisazomethine pigment; Benzidine; Nonmutagenic

1. Introduction

Bisazomethine pigments based on oxo- and thiopyrimidines of type **1** were first introduced in 1976 by Ando et al. [1]. These pigments typically possess high thermal, chemical and photostability, and are resistant to solvent bleeding. Commonly, this pigment class is used for the mass coloration of plastics, via melt extrusion, when greenish-yellow to golden yellow hues are required. Like many bis-chromophoric pigments, this class of compounds is prepared using genotoxic benzidine-type intermediates such as 3,3′-dimethoxybenzidine, 3,3′-dichlorobenzidine and 3,3′-dimethylbenzidine [1]. Hereafter, these compounds are referred to as common benzidines.



	R	X
1	Cl, CH_3, OCH_3	O, S
2	$>C_2$ alkyl/alkoxy	O, S

Most pigments are considered environmentally benign owing to their high stability and insolubility in the media in which they are applied. In fact, evaluation of pigments in various biological protocols has, to date, provided no substantive data indicating any significant risk to human health. However, pigments prepared from genotoxic benzidines pose an occupational risk during synthesis of the pigment and may be environmentally hazardous in the event that not all the diamine is converted to pigment [2,3]. Nevertheless, pigments are still prepared on a large

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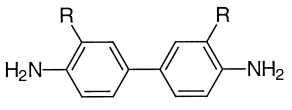
scale using genotoxic analogs of benzidine, probably because of the absence of nongenotoxic alternatives that are inexpensive and produce the same desirable technical properties as the common benzidines when converted to pigment.

In part one of this study [4] some strategies for reducing or eliminating genotoxicity of benzidine-type diamines were summarized. One approach demonstrated a reduction in mutagenic activity of aromatic amines when bulky alkyl or alkoxy substituents are incorporated in positions *ortho* to the amine [5]. More recently, Hunger et al. [6,7] reported analogs of benzidine of type 3, and demonstrated that the resultant diamines were nonmutagenic. Hence, these nongenotoxic benzidine congeners are potential intermediates for the synthesis of a variety of organic colorants.



R = > C_3 alkyl/alkoxy

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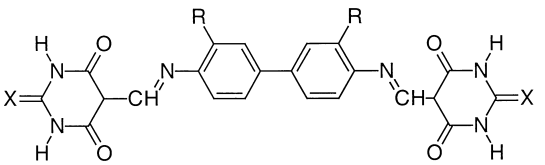
In consideration of the genotoxic and occupational risks associated with common benzidines, research in these laboratories has emphasized the development of organic pigments based on nonmutagenic analogs of benzidine. A further goal was the incorporation of nonmutagenic analogs of benzidine into pigments without compromising the desirable technical properties associated with benzidine congeners currently used. This effort led initially to the development of bisazomethine pigments of type **2**.

2. Results and discussion

2.1. Synthesis

The synthesis of type **3** was reported previously [4,6,7]. Table 1 lists the bisazomethine pigments prepared from **3** together with selected characterization data. Bisazomethine pigments were synthesized according to the route outlined in Scheme 1,

Table 1
Data pertaining to bisazomethine pigments **2a–k**

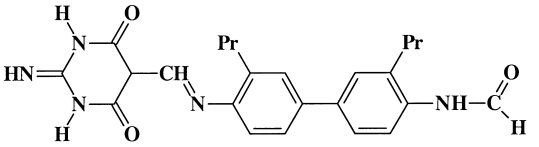


	R	X	Yield (%)	M.p. (°C)	Hue	Elemental Analysis							
						Theory				Found			
						C	H	N	S	C	H	N	S
2a	H	O	89	>400	Green-yellow	57.39	3.48	18.26		57.55	3.86	17.89	
2b	H	S	80	>400	Green-yellow	53.66	3.25	17.07	13.01	53.72	3.61	16.92	12.81
2c	CH_3	O	[a]	>400	Green-yellow								
2d	Pr	O	93	>400	Green-yellow	61.99	4.89	15.49		60.97	4.93	15.69	
2e	Pr	S	87	>400	Green-yellow	58.52	4.56	14.62	11.16	58.05	4.92	14.7	11.21
2f	OPr	O	88	>400	Yellow	58.54	4.53	14.63		58.21	5.13	14.52	
2g	OPr	S	84	385[b]	Golden yellow	55.26	4.60	13.81	10.52	55.22	4.67	13.81	10.44
2h	OBu	O	88	386[b]	Yellow	59.60	5.33	13.90		59.52	5.31	13.92	
2i	OBu	S	83	370[b]	Golden yellow	56.60	5.07	13.20	10.05	56.60	5.12	13.20	10.01
2j	OC_2H_4OMe	O	89	395[b]	Yellow	55.26	4.64	13.81		55.18	4.82	13.65	
2k	OC_2H_4OMe	S	82	360[b]	Golden yellow	52.66	4.10	13.16	10.04	52.71	4.16	13.12	9.98

[a] Supplied by Dianippon Ink and Chemicals, Inc.
[b] Decomposition temperature

and possessed good thermal stability, with decomposition temperatures exceeding 350°C.

The hues of pigments prepared from barbituric acid (BA) were hypsochromic relative to those synthesized from thiobarbituric acid (TBA). In order to obtain even greater hypsochromic shifts, the synthesis of pigments of type **2** (X = NH) based on iminobarbituric acid (IBA) was attempted. In this case, however, pigments of acceptable purity were not obtained, the product being a mixture that included a monoazomethine colorant (e.g. **4**). While a number of variables were investigated in an attempt to synthesize IBA-based bisazomethine pigments, including temperature, solvent, catalyst and reaction time, in all cases the target compound was not detected. However, analysis of the BA and TBA-based pigments by field desorption mass spectrometry (FDMS) showed the parent ion in each case [8].



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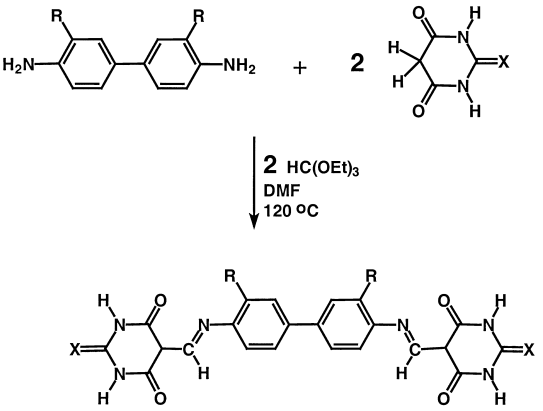
Presumably, the low reactivity of IBA was the major contributor to the failed synthesis of bisazomethines using this pyrimidine. The pK_a of each pyrimidine (TBA, BA, and IBA) can be taken as an indicator to reactivity, and, in the present synthesis, as an indicator to successful formylation and condensation. The pK_a values for the pyrimidines were measured according to a standard potentiometric titration procedure [9]. The results indicated the reactivity of the pyrimidines in this study to be in the order TBA (pK_a 3.5) > BA (pK_a 4.0) ≫ IBA (pK_a 7.2).



Scheme 1. Synthesis of type 2 pigments (R = Pr, Bu, OPr, OBu, OC_2H_4OMe; X = O, S).

2.2. Mutagenicity of bisazomethine pigments

The bisazomethine pigments were evaluated in the standard Ames Salmonella mammalian mutagenicity assay [10] and the Prival modification [11] of that method. The latter assay was developed specifically for assessing azo dyes by providing enhanced reductive cleavage of azo linkages. Since the azomethine linkage in the present pigments can be reduced, the Prival modification was employed for these compounds to further substantiate mutagenicity results from the standard protocol.

Table 2 provides a summary of results from both mutagenicity assays. Pigments prepared using benzidine itself (e.g. **2a** and **2b**) and 3,3′-dimethoxybenzidine (e.g. **2c**) were evaluated and were mutagenic in both assays in strain TA98 in the presence of rat or hamster liver enzyme, S9, indicating that the pigments are promutagens rather than direct acting mutagens. That is, the pigments were converted to mutagenic substances following treatment S9 enzyme. No pigment was

Table 2
Data from the mutagenicity testing of pigments **2a–k**

	Ames			Prival		
Pigment	TA98	TA98 (+S9)	TA100	TA100 (+S9)	TA98	TA100
2a	–	+	–	–	+	+
2b	–	+	–	–	+	+
2c	–	+	–	–	+	+
2d	–	–	–	–	–	–
2e	–	–	–	–	–	–
2f	–	–	–	–	–	–
2g	–	–	–	–	–	–
2h	–	–	–	–	–	–
2i	–	–	–	–	–	–
2j	–	–	–	–	–	–
2k	–	–	–	–	–	–

Ames positive in TA100, though **2a–c** were mutagenic in TA100 in the Prival assay. All pigments prepared from nonmutagenic benzidine analogs (**2d–k**) were nonmutagenic in all tests. Fig. 1 shows the dose response curves for the benzidine-based pigments **2a** and **2b**. In general, if the number of revertant colonies counted is more than twice that of the base count (using DMSO, and in the absence of pigment), then a mutagenic response was recorded. Fig. 2 shows the number of revertant colonies for pigment **2c** and clearly shows a positive mutagenic response.

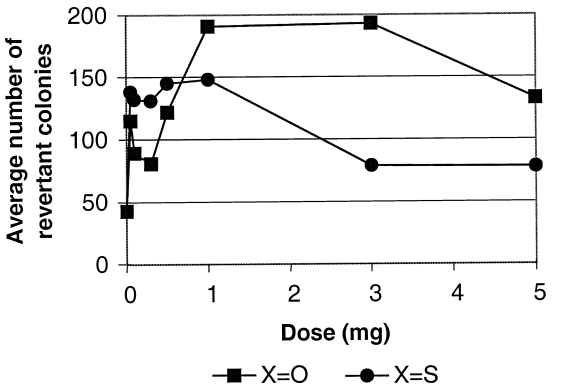


Fig. 1. Effect of dose level on the number of revertant colonies using the standard Ames assay with TA98 and S9 rat liver metabolic activation for pigments **2a** and **2b** (DMSO = base count).

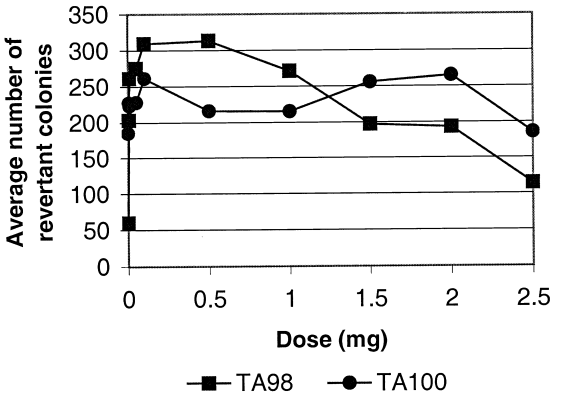


Fig. 2. Effect of dose level on the number of revertant colonies in the standard Ames assay with TA98 and TA100 with S9 rat liver metabolic activation for pigment **2c** (DMSO = base count).

While it is generally considered that pigments are chemically inert and are not a serious risk to human health or the environment, these initial in vitro test results indicate that insoluble azomethine pigments can be converted to mutagenic metabolites by reductase enzymes. Hence, an in-depth investigation into the genotoxicity of commercial pigments containing benzidine congeners may be prudent.

2.3. Photostability of bisazomethine pigments

To assess photostability, each pigment was milled in alkyd resin and draw-downs were prepared using a standardized method. After exposing the printed ink to carbon arc light for 20, 40, 60, 80 and 100 h under standardized conditions, the photostability of each pigment was determined via colorimetric assessment of the exposed area. The overall color difference after exposure was calculated using the CIE color difference (DE) formula [12]. In each case, color difference between an exposed and unexposed areas of the ink draw-down was calculated. For comparative purposes, a pigment based on 3,3′-dimethylbenzidine (**2c**) was employed, since this pigment is known to possess high photostability. In general, the target was to attain a photostability corresponding to DE value less than 10 after 100 h.

Fig. 3 shows the change in color difference upon light exposure. Pigments **2j–k**, in which β-methoxyethoxy substituents were incorporated into the biphenyl link, were relatively unstable, irrespective of whether BA or TBA was employed in the

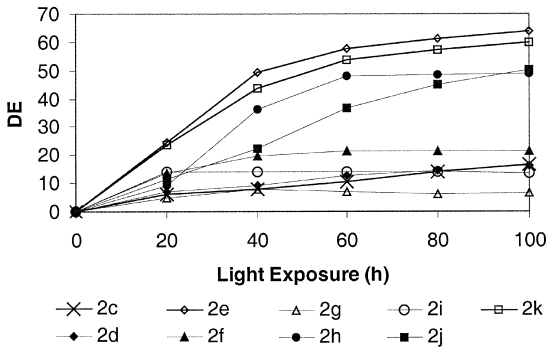


Fig. 3. Graph of CIELAB DE versus light exposure.

synthesis. Interestingly, in some cases bisazomethine pigments based on TBA possessed higher photostability (lower DE value following light exposure) than the corresponding pigments prepared using BA, whilst in other cases the reverse was evident. For example, **2e** (TBA-based) possessed the least photostability of the pigments prepared, but **2d** (BA-based) exhibited high stability, similar to the reference pigment **2c**. However, the BA-based pigment **2h** was far less stable than the corresponding TBA-based pigment **2i**. The reasons for such differences are unclear.

The most interesting of the new pigments in terms of photostability were those prepared using 3,3′-dipropoxybenzidine (**2f** and **2g**) and 3,3′-dibutoxybenzidine (**2i**).

2.4. Colorimetric assessment

Three important attributes of color that must be considered in the development of new colorants are lightness, color strength and hue. These attributes can be determined by colorimetric assessment of ink draw-downs prepared using the prototype pigment. The CIE attributes of lightness (L^*), chroma (C^*), and hue (a^* = redness/ greenness; b^* = yellowness/blueness) were calculated in the present work.

As expected, incorporation of alkoxy groups in place of alkyl produced a bathochromic shift. Fig. 4 shows a graph of CIE coordinates a^* versus b^* for selected pigments. (An increasing a^* value represents an increase in redness, while a decrease in a^* represents a green hue shift). In almost all cases, the prototype pigments displayed a bathochromic shift relative to the reference pigment **2c**. As the b^* scale represents yellowness (increasing b^* value) versus blueness (decreasing b^* value), it is evident that most of the new pigments were significantly yellower than **2c**. Fig. 5 shows a graph of CIE lightness versus chroma for selected pigments. Of the pigments studied, those prepared from intermediates 3,3′-dipropoxybenzidine and 3,3′-dibutoxybenzidine, displayed similar chroma to the reference pigment **2c**. Hence, incorporation of bulky alkyl or alkoxy groups in the positions *ortho* to the amines of the benzidine intermediate did not adversely affect the color attributes of the resultant pigment relative to bisazomethine pigments prepared from common benzidines. However, a hypsochromic shift toward bright greenish-yellow hues with high chroma represents an area of color space that is not commonly obtained with many high performance bis-chromophoric pigments. Hence, the focus of the research in these laboratories turned toward strategies for the development of new bis-chromophoric colorants prepared from nonmutagenic benzidine-type intermediates with hypsochromic shifts relative to corresponding pigments synthesized from common benzidines. Data from this work will be reported in a forthcoming paper.

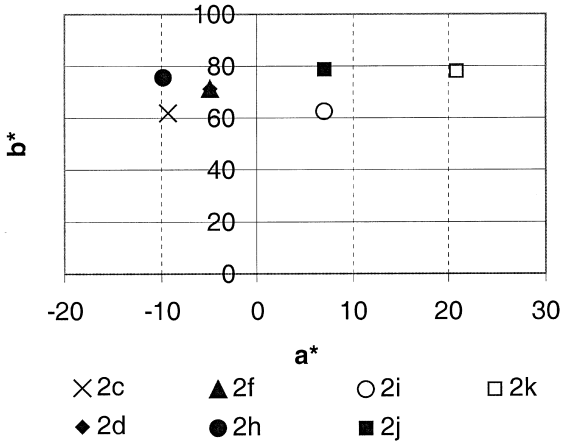


Fig. 4. Graph of CIE a^* versus b^* for selected pigments.

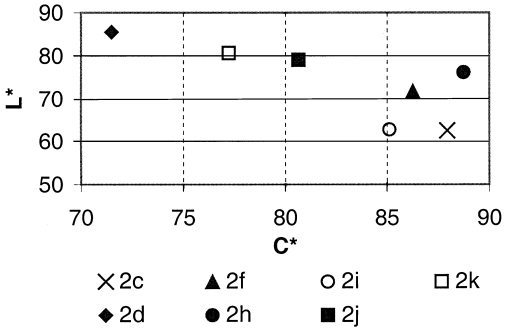


Fig. 5. Graph of CIE lightness (L^*) versus chroma (C^*) for selected pigments.

3. Experimental

3.1. General

Unless otherwise stated, all chemicals were purchased from Aldrich Chemicals (Milwaukee, WI) and used without further purification. Field desorption mass spectra (FD MS) were recorded on a Hewlett Packard 5985B GC mass spectrometer. The procedure used for FD MS was reported previously [8]. Atlantic Microlabs (Norcross, GA) performed elemental analysis. Melting points were recorded on a Mel-Temp apparatus and are uncorrected.

3.2. Preparation of draw-downs

Pigment (1 part) was milled with alkyd resin (3 parts) in a Hoover Muller mixer. The mixture was milled to 100 revolutions under 150 lbs. The milling step was repeated three times prior to preparing the draw down. The ink (12.2±0.2 mg) prepared was printed onto coated paper (20×4 cm) using a Praufbau printability tester.

3.3. Photostability testing

Photostability of pigments dispersed in standard draw-downs was assessed using a standard Fade Meter (SUGA Test Instruments, Inc.) equipped with a carbon arc UV lamp. Areas of the draw-downs were exposed separately for 10, 20, 30 and 40 h with the lamp adjusted so that the black panel temperature of the draw down was maintained at 63±3°C. Relative humidity was maintained at 35±5%.

3.4. Colorimetric analysis

Reflectance spectra of draw-downs were recorded on a Datacolor International Spectraflash 600 plus instrument using the following instrument settings: specular included, small area view, and UV included. Colorimetric data [12] were calculated using SheLyn, Inc., SLIFORM N/G software.

3.5. Mutagenicity testing

Mutagenicity testing was performed using the standard Ames *Salmonella* mammalian assay [10] and Prival modification [11]. *Salmonella typhymurium* strains TA98 and TA100 were employed in the Ames assay, with (+S9) and without (−S9) mammalian microsomal activation system.

3.6. Synthesis

The general procedure employed for the preparation of bisazomethine pigments was as follows: To a stirred solution of the appropriate alkyl/alkoxy-benzidine (0.016 mol) in 40 ml DMF at 25°C was added either barbituric acid or 2-mercapto-4,6-dihydroxypyrimidine (0.035 mol). After 30 min triethyl orthoformate (0.036 mol) was added, and the mixture was stirred for 3 h at 25°C, then heated to 80°C over 30 min. After 1 h the temperature was increased to 120°C over 30 min, and after 2 h, allowed to cool to 80°C. The product was filtered and washed with hot MeOH, then with hot H_2O, and dried at 40°C.

4. Conclusions

It has been shown that bisazomethine pigments based on 3,3′-dimethylbenzidine and benzidine are mutagenic, and that incorporation of bulky alkyl or alkoxy substituents in the 3,3′-positions of benzidine removes mutagenic activity. Several of the nonmutagenic pigments synthesized in this study possessed satisfactory thermal stability, lightfastness and hue, making them promising candidates as high performance, nongenotoxic yellow to golden yellow pigments.

Acknowledgements

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