13rd International Electronic Conference on Synthetic Organic Chemistry (ECSOC-13), 1-30 November 2009.
http://www.mdpi.org/ecsoc-13/ & http://www.usc.es/congresos/ecsoc/13/

[c009]

SYNTHESIS AND *IN VIVO* IMAGISTIC SKIN EVALUATION OF A NEW DISAZO DYE DERIVED FROM 4,4'-DIAMINOSTILBENE-2,2'-DISULFONIC ACID

SIMU Georgeta Maria[1], DRAGOMIRESCU Anca[1], GRAD Maria Elena[2] and ANDONI Mihaiela[1]

[1] University of Medicine and Pharmacy Victor Babeş Timişoara, Faculty of Pharmacy, Piata Eftimie Murgu 2-4, RO-300034 Timişoara, Romania
[2] Institute of Chemistry Timisoara of the Romanian Academy, B-dul Mihai Viteazul 24, 300223 Timişoara, Romania

The synthesis of a new disazo dyes containing 4,4'-diaminostilbene-2,2'-disulfonic acid as middle component is presented. The synthesized dye was analyzed by thin layer chromatography (TLC), electronic spectra (VIS) and HPLC technique. The structure was elucidated by FT/IR and ^{13}C-NMR spectroscopy. The colouristic evaluation of the synthesized dye indicates that this dye could be ranged as a direct dye, suitable for application on cellulosic substrates. Further, an in vivo imagistic skin study was performed in order to evaluate the individual human skin tolerance for this new disazo dye.

Key words: Azo direct dyes, 4, 4'-diaminostilbene-2,2'-disulfonic acid, Colouristic properties, Imagistic skin evaluation

Introduction

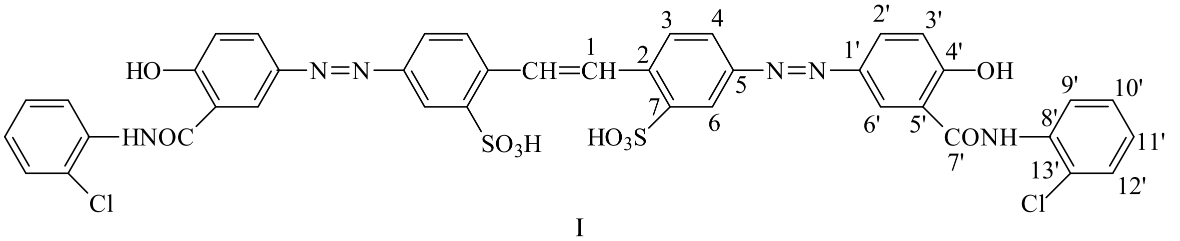
Azo dyes are considered as one of the most important class of synthetic dyes due to their availability, ease of synthesis, low costs and their wide field of application. According to literature, these dyes are used as colorants for a great a variety of products such as food, paper, leather, textiles, paints, printing inks, plastics, medicines, cosmetics, etc [1-17].

It was proved that the main hazard of the azo dyes arises from the reduction of the azo group, by the action of intestinal anaerobic bacteria or the hepatic azo reductases with the release of some aromatic amines which can react in cells, leading to the initiation of the carcinogenic process [2,3,11,13,16]. So, the design and the synthesis of new compounds which do not exhibit occupational and environmental risk present a great interest [17-28].

Our studies are focused on the possibility of developing new azo dyes with good colouristic and application properties, as alternative compounds to those which have proved their adversity against human health (the benzidinic dyes by instance). Moreover, an *in vivo* imagistic skin evaluation study was performed, in order to evaluate the individual human skin tolerance for this new disazo dye.

Results and discussion

Optimum reaction conditions for the synthesis of a new disazo dye (with structure **I**), derived from 4,4’-diaminostilbene- 2,2’-disulfonic acid were established.



I

For the synthesis of dye I, a two step procedure was developed. The preparation procedure involved the bis-diazotization of 4,4’-diaminostilbene-2,2’-disulfonic acid by the direct method of diazotisation, and thereafter the coupling reaction of the resulting bis-diazonium salt with 2-chlorosalicylanilide (as coupling component) in a 1:2 molar ratio.

The bis-diazotization of 4,4'-diaminostilbene-2,2'disulphonic acid was carried out by the direct method, in a HCl aqueous solution and the resulting bis-diazonium salt was separated by filtration for the complete removal of the salts, unlike the classical method.

In the azo coupling reaction, the diazonium ions formed during diazotization give rise to azo compounds in an electrophilic aromatic substitution. As diazonium ions are relatively weak electrophilic reagents, the phenolate ion (Ar-O$^-$) and the free amine (Ar-NH$_2$) react more easy as coupling components than free phenol (Ar-OH) and the ammonium ion (Ar-NH$_3$ $^+$). In this regard the coupling reactions were performed in an alkaline aqueous medium (pH around 8), in presence of Na_2CO_3 at a temperature around 10ºC. It was noticed that the direct adding of the alkaline solution of 2-chlorosalicylanilide to the acid suspension of the bis-diazonium salt of 4,4'-diaminostilbene-2,2'-disulphonic acid was optimum for the disazo dye's I synthesis.

In the coupling step, a 3% excess of 2-chlorosalicylanilide was used. The reaction time was about 2.5 h. The progress of the coupling reaction was monitored in the usual way and by TLC, when the formation of the coupled compound was examined. The obtained dye was purified by recrystallization from dioxane:pyridine 90:10 (v:v). This mixture was selected according to the lower solubility of the basic dominant product in dioxane as compared to the higher one in pyridine, at high temperatures.

The synthesized disazo dye was obtained in 80 % yield and was characterized by means of TLC, VIS, FT/IR , ^{13}C–NMR, spectroscopy and HPLC technique.

As shown in the experimental part, the FT/IR and ^{13}C–NMR spectroscopy data agree well with the dye's chemical structure.

The chromatographic study was carried out in different elution conditions: absolute methanol, methanol–water 90%; 85% (v/v) and flow rate ranging from 0.75-1 of mL/min. As presented in the experimental part, the chromatographic separation reveals the presence of a single dominant product. The obtained results by means of two distinct analysis methods (spectroscopic and chromatographic), showed unsignificant differences.

The colouristic and application properties of the disazo (I) were determined and compared to those exhibited by some azo dyes with close chemical structures and which are known as classical direct dyes (by instance C. I. Direct Orange 1).

The untreated dyeing on cotton was reddish-orange, the dye had good migration and good levelling properties. The percentage uptake on cotton was found to be 58 %. The obtained results are presented in Table 1.

Table 1

Application properties on cotton of dye (I) and of C. I. Direct Orange 1 (C. I. 22.250).

	Disazo dye **I**		C. I Direct Orange 1* (C. I. 22.430)
Fastness:	Direct dyeing	After treatment with $CuSO_4$	Direct dyeing
Light	2-3	3	2
Water	3-4	4	3-4
Wash 40 °C	2-3	3	1-2
Perspiration (alkaline)	2-3	3	2
Perspiration (acid)	2-3	3	4
Hot pressing	3	3	3

* Literature data [20]

As it can be seen in Table 1, the application properties of the disazo dye (I) are quite similar to the reference dye Direct Orange 1. Moreover, the after-treatment of the dyed samples with $CuSO_4$ shows improvements in the case of the wet fastness of dye (I). The obtained results indicate that the synthesized dye can be ranged as a direct dye.

Further, dye (I) was used in some *in vivo* imagistic skin evaluation tests, as described in the Experimental part. The possibilities of apparition of adverse effect were monitorized with an imagistic skin evaluation, *in vivo,* daily after each application [29-31]. The image of the external forearm skin in an elected region of photoinduced aging skin of an admitted volunteer, before the beginning of the tests is shown in Figure 1, where the lines signify the wrinkles graph, realized by ProDerm II Skin Analyzer.

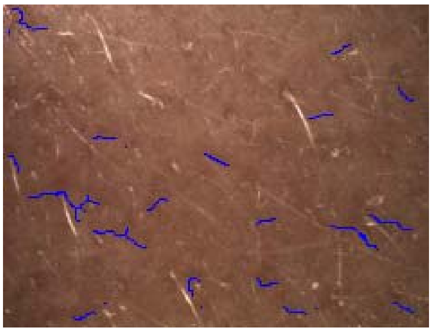


Figure 1. The 1 cm^2 skin forearm area of an admitted volunteer of 42 old years.

For all the volunteers involved in this study, and for all concentrations applied, any pathological sign were registered. Concerning to skin coloration, no modification was noticed, excepting the transitory color of the dye (I). Because of the rapid regeneration of the epidermal layer (the daily desquamation of the corneocyte layer), dye I did not colored the skin, even in the greatest concentrations.

Concerning the skin texture, no peeling effect was registered, and the epidermal desquamative registrations were no more differenced than the blank skin areas (see Figure 2).

Figure 2. Normal texture of skin after all test application. The normal quadriladge of the skin can be observed.

Moreover, during all the period of skin determination, no case of contact dermatitis was registered.

Materials and Methods

Most of the chemicals used in this study were of analytical grade and were obtained from Merck Co, Fluka Co, AcrŌs Organic Co, Lachema Co, and Aldrich Chemical Co respectively.

The thin layer chromatography (TLC) was performed on silica gel plates (Merck 60F-254) and the eluting system isopropanol: methyl-ethyl-ketone: NH_3 25%.

Electronic absorption spectra (VIS) of the synthesized dye in 50% aqueous MeOH solution ($c=1.0\text{x}10^{-5}$ mol/dm^3) were performed on a CECIL CE 7200 spectrophotometer in the range 300−800nm.

The HPLC study has been performed on a JASCO ensemble, using a diode–array UV–VIS detector. The dye purity was investigated using a C18 (25x0.46mm), 5μm column and an 83% aqueous MeOH solution as mobile phase. The HPLC method consists of an isocratic elution and a flow rate of 0.9-1mL/min.

The FT/IR spectra were recorded on a JASCO FT/IR−4200 spectrometer in the range 400-4000cm^{-1}.

The ^{13}C-NMR spectra were recorded as solution in DMSO−d6 using a BRUKER AVANCE DRX-400 spectrometer with tetramethylsilane (TMS) as internal standard.

Synthesis of the disazo dye I

A mixture of 1.95 g of 95% 4,4'-diaminostilbene- 2,2′-disulphonic acid (0.005 mol), 10 mL distilled water and 30% NaOH aqueous solution was cooled to 5°C. Then, 3mL (0.03 mol) of 32% HCl were added to the mixture. The resulting suspension was bis-diazotised with 0.71 g of 98% solid $NaNO_2$ (0.0101 mol) at 5°C and a pH value around 1. The mixture was stirred for 1 hour for the completion of the diazotation reaction. Finally, the excess of the nitrous acid was destroyed by urea. The obtained bis-diazonium salt was filtered out and the precipitate was washed with distilled water. Thereafter, 2.86 g of 95% 2-chlorosalicylanilide (0.011 mol) were dissolved in 10% NaOH aqueous solution and the previously obtained bis-diazonium salt was suspended in 20 mL distilled water. The coupling reaction was carried out by direct treating of the bis-diazonium salt suspension with the alkaline solutions of the coupling component (2-chlorosalicylanilide). The pH of the reaction mixture was maintained around 8 by periodic addition of 10% Na_2CO_3 aqueous solution. The temperature did not exceed 10°C.

The coupling reaction was monitored by the conventional method (drop reaction with an alkaline solution of 1-amino-8-naphtol-3,5-disulphonic acid and with the diazonium salt of 4-nitroaniline). After the complete addition of the alkaline solution of 2-chlorosalicylanilide, the mixture was maintained under stirring for 2 hours for the completion of the coupling reaction. The synthesized dye was separated by precipitation from an acid solution (pH=6-6.5) by addition of 10% HCl solution. The crude dye was collected by filtration, washed with dimethyl-ketone, methanol and dried. Thereafter, the disazo dye was purified by recrystallization from dioxane:pyridine 90:10 (v:v). The homogeneity of the purified dye was confirmed by TLC, using silica gel plates and isopropanole: methyl-ethyl-ketone: ammonia 25 %= 4:2:3 as eluting system.

TLC (silicagel plates 60F−254, *iso*−propanol:methyl−ethyl ketone:ammonia 25%=4:3:5 (v:v:v)) (R_f): 0.72;

VIS (50% aqueous MeOH) (λmax/nm, (ε/L mol^{-1} cm^{-1})): 458 (23437);

IR (KBr, cm^{-1}): 3416 (stretching of –OH group), 1635(C=O stretching of –CO−NHgroup), 1543(II amide band), 1228(III amide band), 693(-NH deformating of secondary amide), 1188(SO_2 asym. stretching of $-SO_3H$ group), 1075(SO_2 sym. stretching of $-SO_3H$ group), 1319(-OH deformating of -OH group), 900,829,744 (CH−deformating of aromatic ring), 533,439 (skeletal deformating of aromatic ring);

^{13}C-NMR (100MHz, $(CD_3)_2SO$, δ/ppm): 125.16(C_1), 135.34(C_2), 127.80(C_3;$C_{10'}$), 123.51(C_4), 122.87(C_6), 146.00($C_{1'}$), 117.13($C_{3'}$), 156.78($C_{4'}$), 119.65($C_{6'}$), 164.10($C_{7'}$), 133.81($C_{8'}$), 129.34($C_{12'}$), 130.66(C_{13}');

HPLC (C18, 83% aqueous MeOH, isocratic elution, flow rate:0.9mL/min) (λ/nm, (t_R/min)): 458 (1.43)

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 standards.

The degree of dye exhaustion by cellulose fibres was determined by dyeing samples of bleached cotton fabric at 95 °C, using 1 % of dye in relation to fibres with addition of 0.5 % Na_2SO_4 at the dye liquor at a fibre ratio 30: 1. The dye concentrations in the dyebath before (c_1) and after dyeing (c_2) were determined spectrophotometrically.

In vivo skin evaluation

In order to evaluate the possible skin adverse effects of the disazo dye I, a study group of 45 healthy volunteers, all women, aged between 25 – 51 years was selected. All volunteers agreed to apply the disazo dye I as dilute aqueous solution of 5 %, 2.5 %, 1 % and 0.5 %, on 4 areas of forearms, for 6 days, daily, 1 time a day. In the volunteer selection, the subjects presenting skin pathologic signs (seborrhea dermatitis, psoriasis lesions) were eliminated. However, physiological signs, like photoaging signs, dilatation of sebaceous channel glands, or other physiological signs were admitted. No restrictions regarding to the human skin phototype were considered. For all volunteers, a blank skin area was also chosen, on which we applied distillated water. The possibilities of apparition of adverse effect were monitorized with an imagistic skin evaluation, *in vivo,* daily after the previous application. The tests were performed using a ProDerm II Skin Analyzer. The following parameters were evaluated: the skin coloration (normal or the apparition of a rush); the skin texture (preservation of the normal skin quadrilagde, and eventually irritation signs – a peeling effect), and the possible skin dryness, eventually induced by this dye (involution of density of seboceous glands channels – pointed out by the device in green points).

Conclusions

Optimum reaction conditions were established for the synthesis of a new orange disazo dye derived from 4,4’-diaminostibene- 2,2’- disulfonic acid as diazo component and 2-chlorosalycilamide as coupling component.

The colouristic and application properties of the synthesized dye on cotton fibre, indicate that this compound could be ranged in the direct dye class.

According to the *in vivo* imagistic skin evaluation of the new disazo dye, one could conclude that, from dermatologic point of view, the new dye may be used as colorant in the textile industry.

References

1. Szadowski, J., Niewiadowski, Z., *Dyes Pigments*, **50**, 2001, p.87

2. Wainwright, M., *J. Antimicrob. Chem*., **47**, 2001, p. 1

3. Klink, S.I., Alink, P.O., Grave, L., Peters, F.G.A., Hofstraat, J.W., Geurts F., Van Veggel, F.C.J.M., *J. Chem. Soc., Perkin Trans*., **2**, 2001, p.363

4. Gong, G., Gao, X., Wang, J., Zhao, D., Freeman, H. S., *Dyes Pig*ments, **53**, 2002, p.109

5. Hinks, D., Freeman, H. S., Nakpathom, M., Sokolowska, J., *Dyes Pigments*, **44**, 2000, p.199

6. Bello, K. A., Shen, K., Zhao, D., Esancy, M., Freeman, H., *Dyes Pi*gm., **46**, 2000, p. 121

7. Zhang, S., Cheng, X., Yang, J., *Dyes Pigm*., **43**, 1999, p. 167

8. Hinks, D., Freeman, H.S., Nakpathom, M., Sokolowska, J., *Dyes and Pigments*, **44**, 2000, p. 199

9. Wojciechowski, K., Wyrebak, A., Gumulak, J., *Dyes Pigments*, **56**, 2003, p. 99

10. Wojciechowski, K., Gumulak, J., *Dyes Pigments*, **56**, 2003, p. 195

11. Rudyk, H., Knaggs, M.H., Vasiljevic, S., Hope, J., Birkett, C., Gilbert, I.H., *Eur. J. of Med. Chem.,* **38**, 2003, p.567

12. Pielesz, A., Baranowska, I., Rybak, A., Wlochowicz, A., *Ecotoxicology and environmental safety*, **53**, 2002, p. 42

13. Mittal, A., Kurup, L., Mittal, J., *Journal of Hazardous Materials*, **146**, 2007, p. 243

14. Wainwright, M., *Dyes and Pigments,* **76**, 2008, p. 582

15. Ahmed, A.E.I., Hay, J.N., Bushell, M.E., Wardell, J.N., Cavalli, G., *Reactive & Functional Polymers*, **68**, 2008, p. 248

16. Stoltz, A., *Appl. Microbiol. Biotechnol*, **56**, 2001, p. 69

17. Pielesz, A., Weselucha-Birczynska, A., *J. Molec. Structure*, **555**, 2000, p. 325

18. Simu, G., Chicu, S. A., Morin, N., Schmidt, W., Sisu, E., *Turk. J. Chem.*, **28**, 2004, p. 579

19. Grad, M., Simu, G., Hora, S., Chicu, S.A., Sisu, E., *Chem. Bull. Politehnica Univ. (Timişoara)*, **63(1-2)**, 2004, p. 31

20. Color Index, 2nd Edition, Percy Lund Humphries & Co Ltd, Vol. 2, Bradford, 1956, p. 2037

21. Simu, G., Funar-Timofei, S., Hora, S., Schmidt, W., Kurunczi, L., Sisu, E., Morin, N., *Rev. Chim.-Bucharest*, **53**, 2002, p. 826

22. Simu, G. M., Hora, S. G., Grad, M. E., Sisu, E. N. V., *Rev. Roum. Chim.*, **50(2)**, 2005, p.113

23. Bae, J.-S., Freeman, H. S., *Fibres and Polymers*, **3(4)**, 2002, p. 140

24. Bae, J.-S., Freeman, H. S., El-Shafei, A., *Dyes and Pigments*, **57**, 2003, p. 121

25. Combellas, C., Kanoufi, F., Pinson, H., Podyorica, F., *Am. Chem. Soc.*, **34**, 2008, p. 429

26. Pinson, J., Podyorica, F., *Chem. Soc. Rev.*, **34**, 2005, p. 429

27. Dabbagh, H. A., Teimouri, A., Chermahini, A. N., *Dyes Pigments*, **73**, 2007, p. 239

28. Murray, P. S., Ralph, S. F., *Electrochimica Acta*, **51**, 2006, p. 2471

29. Dragomirescu, A., Dehelean, C., *RSCC Magazine*, **3(2)**, 2003, p. 12

30. Dragomirescu, A., Andoni, M, Muresan, A. R., *TMJ*, **58**, suppl 2, 2008, p. 396

31. Martini M.-C., Introduction à la dermatopharmacie et à la cosmetologie. Ed. Lavoisier, 2006, p. 107-139