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Some Commercial Azo Dyes as Inhibitors of Mushroom Tyrosinase DOPA Oxidase Activity

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Abstract: The colour of mammalian skin is determined by many factors and mainly by the degree and distribution of melanin pigmentation. Tyrosinase is the key enzyme for melanin biosynthesis. Contact with different azo dyes is well known to produce contact dermatitis and some times depigmentation of the skin. In the present study we have reported the effect of known depigmenting dyes as well as some food, cosmetic and drug dyes on the activity of tyrosinase enzyme. The activity of the enzyme has been assessed in terms of oxidation of DOPA. The inhibitory effect of PPD was found to be maximum i.e., 85% while solvent yellow 3 and Brilliant crocin showed inhibition of 70 and 60%, respectively. The PPD and solvent yellow 3 were identified as noncompetitive inhibitors of tyrosinase by Linweaver Burk plot.

Key words: Azo dyes, tyrosinase, depigmentation, inhibitory activity

INTRODUCTION

The color of human skin depends on a number of factors which include the thickness of epidermis, the amount of blood supply, the degree of vasodilation and the amount of pigment present. Different endogenous pigments, such as melanin, oxyhaemoglobin, reduced haemoglobin and carotene present in the dermis, epidermis and subcutaneous tissue may contribute to the color of the normal human skin (Riley, 2003; Schroder, 1995). However, the major factor responsible for the degree of coloration of skin is the melanin content of melanocytes, the specific epidermal cells containing the organelle, the melanosome where melanin is synthesized. Melanin is secreted by melanocyte, which are distributed in basal layers of the dermis (Bolognia and Orlow, 2003; Sulaimon and Kitchell, 2003).

Tyrosinase (EC 1.14.18.1) is a melanogenic copper containing enzyme that catalyzes the transformation of tyrosine to DOPAquinone (Solano *et al*., 2006; Xie *et al*., 2003), thus plays important role in the formation of melanin pigment during melanogenesis (Ando *et al*., 2007; Marmol and Beermen, 1996; Jimbow *et al*., 2000; Riley, 1993). Melanogenesis is mainly regulated by tyrosinase and dopachrome tautomerase. Tyrosinase is a key enzyme for melanin biosynthesis in plants and animals. Tyrosinase inhibitors can therefore be clinically useful for the treatment of some dermatological disorders associated with melanin hyperpigmentation. They have become increasingly important as cosmetic (Schurink *et al*., 2007) and medicinal products (Hwang and Lee, 2007; Miao *et al*., 1997) primarily to control melanin pigmentation.

Significant information is available from literature on the modification of skin pigmentation due to contact with variety of physical and chemical agents. Paraphenylendiamine (PPD) (Fig. 1) a

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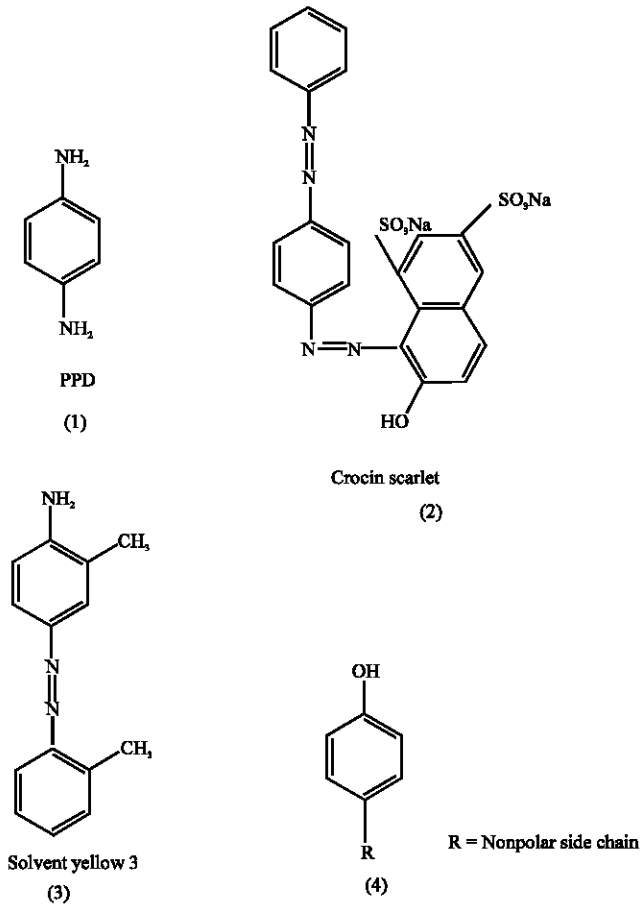

Fig. 1: p,p'-benzene substituted azo dyes interacting with CT-DNA and having potent tyrosinase inhibitory effect causing depigmentation

building block for azo dyes and major component of hair dyes has recently been implicated in producing depigmentation (Taylor *et al*., 1993; Bajaj *et al*., 1996). Black Henna touted to be a herbal product also contains approximately 16% PPD leading to depigmentation at the site of patch test after 1-3 months. Alta, a scarlet red solution applied by a certain sect of Indian woman on their feet during religious and social functions does produce the depigmentation at the site of constant application as was reported by our research group (Bajaj *et al*., 1998). We reported that an alta component viz., Crocin scarlet MOO, an azo dye (Fig. 1 and 2) to produce depigmentation at the application site. Later it was found that Solvent yellow 3 (Fig. 1and 3), another azo dye (4'-amino-2', 3'-dimethyl azo benzene or o-amino azo toluene), known as Fast Garnet GBC and used for textile dying also produces depigmentation at patch test site (Bajaj *et al*., 2000). The p-substituted phenols e.g., p-tertiary butyl phenol (PTBP) also produces depigmentation (Angeline *et al*., 1983; Bajaj *et al*., 1990).

Regarding mechanism of change in pigmentation of skin by contact with chemicals nothing significant has been reported in literature. In order to study the effect of these dyes on human skin we have conducted experiments. Earlier we have studied (Bajaj *et al*., 2004) the interaction of these dyes with Calf Thymus DNA *in vitro* in order to find out whether these pigments interact with DNA at molecular level and proposed a simple spectroscopic technique for scanning the depigmenting potential

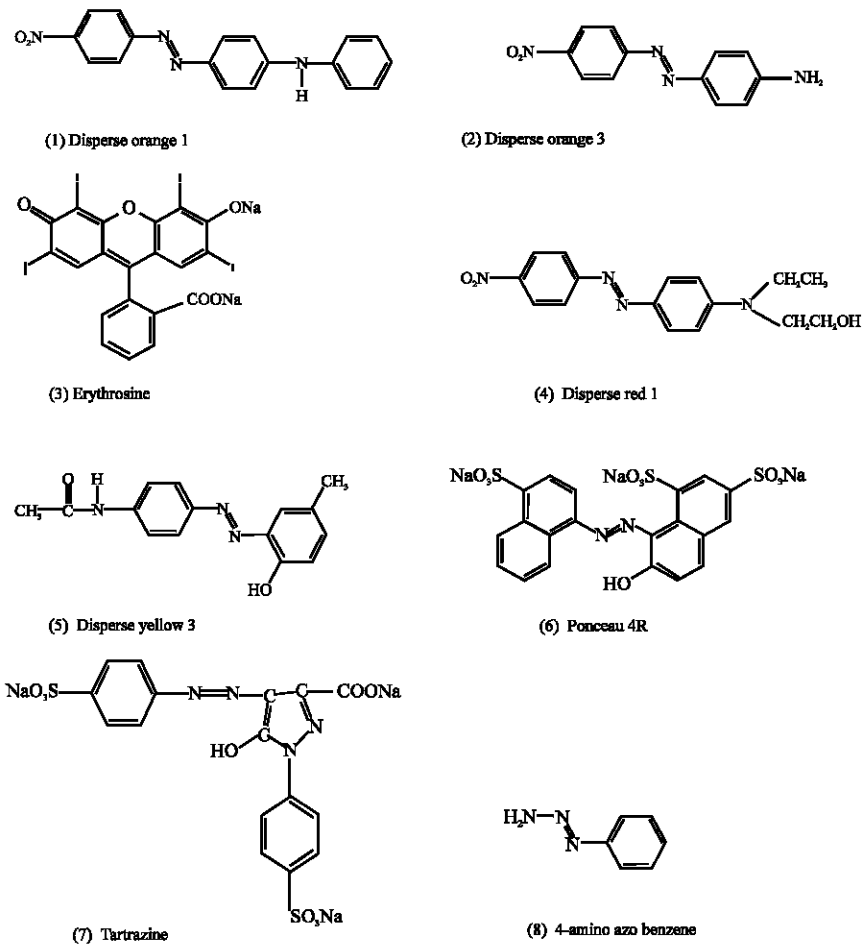

Fig. 2: Chemical structure of azo dyes screened for tyrosinase inhibitory studies

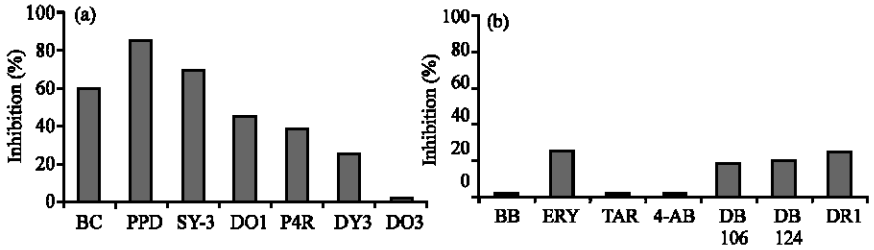

Fig. 3a and b: Comparative inhibitory effect of azo dyes on tyrosinase enzyme

of azo dyes. In this communication we have studied the inhibitory effect of these dyes on tyrosinase enzyme responsible for melanogenesis by assessing Dopa formation i.e., evaluation of tyrosinase activity by assessing Dopa quantitatively

MATERIALS AND METHODS

Materials

Mushroom tyrosinase and L-dopa (3, 4-dihydroxy phenyl alanine) were purchased from Aldrich Chemicals (USA). Azo dyes and PPD were purchased from Aldrich and Fluka. All chemicals used were of analytical grade.

Assay for Tyrosinase Activity

Inhibition of tyrosinase activity was measured by determining change in its DOPA oxidase activity using a modification of the method reported by Shono and Toda (1981). Test substances (dyes) were dissolved in methanol to a concentration of 1 mM. L-DOPA, 600 μL (8 mM, dissolved in 67 mM phosphate buffer, pH 6.8) and 200 μL of each dye solution, 100 μL of buffer, 100 μL of mushroom tyrosinase was added to make the volume to 1 mL.

The reaction mixture was incubated at 37°C for 30 min and the amount of dopachrome formed was determined spectrophotometricaly at 475 nm and inhibitory activity was calculated according to following formula:

$$\text{Inhibitory activity (\%)} = (C-S)/C\times 100$$

Where, C: The absorbance of the control at 475 nm and S is the absorbance of the sample at the same wavelength.

Inhibition Pattern of Mushroom Tyrosinase by PPD and Solvent Yellow 3

The kinetic behaviour of dyes causing inhibition of the enzyme i.e PPD (85%) and Solvent yellow-3 (70%) was studied by assaying the oxidation of L-DOPA. When different concentratios of L-DOPA were used as substrate, PPD and SY-3 decreased V_{max} value of tyrosinase in a dose dependent manner but did not change the K_m value. Therefore, PPD and Solvent Yellow-3 were identified as non competitive inhibitors by Linweaver-Burk plot analysis (Fig. 4 and 5)

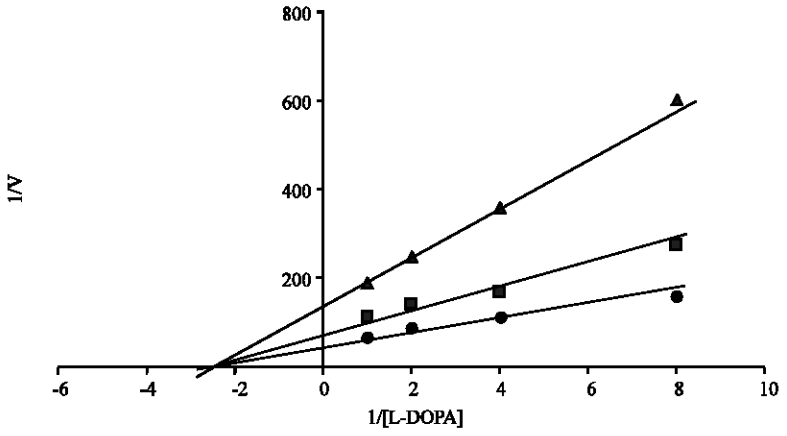

Fig. 4: Linweaver-Burk plots of mushroom tyrosinase activity changes caused by PPD 200 μM (▲), 100 μM (■) and blank (●)

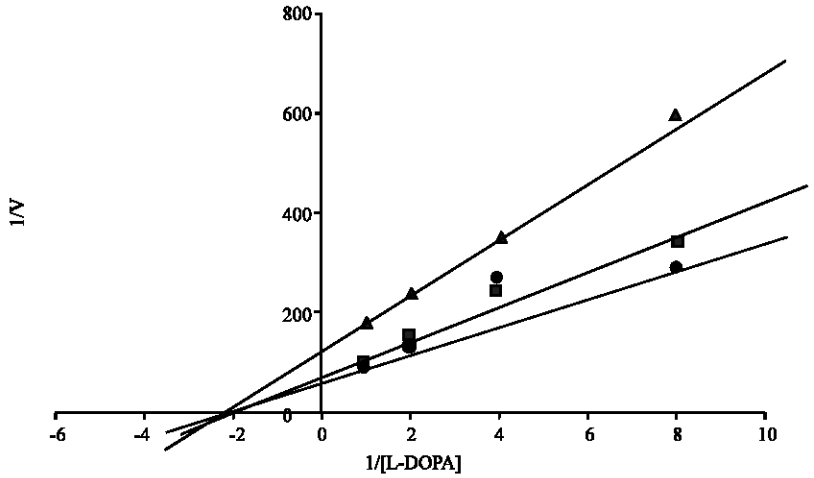

Fig. 5: Linweaver-Burk plots of mushroom tyrosinase activity changes caused by Solvent Yellow 3 200 μM (▲), 100 μM (■) and blank (●)

Table 1: Inhibitory effect of azo dyes on mushroom tyrosinase

Name of dye	% Inhibition of tyrosinase at 200 μM
Brilliant Crocin (BC)	60
Paraphenylene diamine (PPD)	85
Solvent Yellow-3 (SY3)	70
Disperse orange 1 (DO1)	45
Ponceau 4R (P4R)	38
Disperse yellow-3 (DY3)	25
Disperse orange 3 (DO3)	-
Brilliant blue (BB)	-
Erythrosine (ERY)	25
Tartrazine (TAR)	-
4-Amino azobenzene (4-AB)	-
Disperse Blue 106 (DB106)	18
Disperse Blue 124 (DB124)	19
Disperse Red 1 (DR1)	25

RESULTS

A number of dyes were screened for their inhibitory potential which is presented in Table 1. The majority of azo dyes inhibited tyrosinase activity. PPD has highest inhibitory effect, while Solvent Yellow-3 and Brilliant Crocin shows, respectively 70 and 60% inhibition of the enzyme. Some other azo dyes also showed inhibitory activities (Table 1) while some had no change at conc. of 200 μM (Bar diagrams are shown in Fig. 3a and b).

DISCUSSION

The primary material of melanin biosynthesis is tyrosine which is subsequently oxidized to dopaquinone, catalysed by tyrosinase enzyme. Thus inhibition of tyrosinase can regulate melanin synthesis. The non-production of melanin may be due to mimicking (Isosterism) of tyrosine by the p-substituted phenols or amines (PPD) or the PPD unit of azo dyes Although p-substituted benzene nucleus is an essential structural component of all azo dyes as evident from Fig. 1 and 2 the structural

similarity between all the azo dyes used and PPD may explain the cross sensitization of some dyes of azo group which are reported to cross react with para amino benzene compound and cause group sensitization. The p-substitution of benzene ring may be the minimum requirement for enzyme binding resulting in decoloration of skin. It appears that optimal depigmentation from substituted phenols occur when one position of an aromatic ring is hydroxylated and position-4 has a nonpolar side chain (Mcguvie and Hendee, 1971) (Fig. 1 and 4). Their structural similarity (Fig. 1, (4) and Fig. 2, (1-8)) to tyrosine, the building block of melanin is important. This can further be evaluated by seeing the effect of dyes on cultured melanocytes from human skin.

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