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Thin-layer chromatography of fluorinated 4-aminoazobenzene and its N-methylated and 4'-ethyl derivatives

In support of a program[1] which is studying the metabolism and carcinogenic mechanism of certain azo dyes related to 4-dimethylaminoazobenzene (DAB), we have synthesized a number of fluorinated derivatives of 4-aminoazobenzene (AB)[2,3], with two fluorine atoms (2,6 or 3,5 positions) and four fluorine atoms (2,3,5,6 positions). We have also made the corresponding dyes with a 4'-ethyl substituent, which enhances the carcinogenic potential of DAB[4]. In each group we have made the primary amine, the N-methyl derivative (MAB) and the N,N-dimethyl dye. Ultraviolet and infrared spectral data have been[2] or are being[3] presented and we felt that a survey of TLC data should be recorded for these compounds. This is a field where much is already known about the relationship of structure to biological activity, but where there are few final answers.

A TLC study of DAB itself and some of its metabolites has already appeared[5]; we have included DAB and AB from this study as reference point, although ours is a different solvent and adsorbent system. The same authors[4] also have reported the R_F(azo dye)/(R_F(DAB) ratio for five 4'-substituted dyes including 4'-ethyl-DAB. This ratio, for the latter dye, in their study is 1.07; in our system it is 0.98 (*trans* form) and 0.90 (*cis* form).

To obtain reproducible activity[6], silica gel sheets (100 μ, Eastman Chromagram No. 6061) were equilibrated in a closed vessel with the vapors of a saturated aqueous solution of sodium bromide at 24° (58% relative humidity) for at least 24 h.

The dyes were spotted (1 μg in absolute ethanol) 2 cm from the lower edge of the 15 cm sheets. The chromatograms were developed in an S-chamber at 26–28° with a solvent mixture of benzene–heptane (2:1). The sheets were left in the solvent for 15 min after the solvent had reached the top of the sheet, then were dried in air and exposed to HCl fumes for a few minutes for visualization.

We are including values for the illuminated (*cis*) forms as well as the relaxed (*trans*) forms (Table I). Values for the *trans* forms of the dyes were obtained from solutions which had been in the dark for at least two days. The plates were spotted in subdued light and developed in the dark. Values for the *cis* forms were obtained from solutions which had been illuminated for at least 3 h by UV light from a GE Purple X bulb 5 in. from the glass vials. The solutions were kept relatively cool by a stream of air from a portable air-conditioner. The plates were spotted under illumination from a 100 W incandescent bulb, 15 in. away, and were developed under the same light. While it is improbable that the R_F values obtained are for pure *cis* forms, the results do show that the *cis–trans* isomers are distinguishable under these conditions.

As shown in Table I, there is decreasing adsorption in the series with substituents as follows:

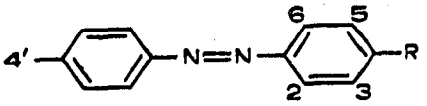
$R = NH_2 > R = NHCH_3 > R = N(CH_3)_2$ for a given fluorine substitution pattern;

2,6-Difluoro > no fluoro > 2,3,5,6-tetrafluoro > 3,5-difluoro for a given R (except 3,5-difluoro DAB's > 2,3,5,6-tetrafluoro DAB's);

cis and *trans* forms have the same relative order throughout;

TABLE I

R_F VALUES FOR AZO DYES ON SILICA GEL DEVELOPED WITH BENZENE–HEPTANE (2:1)



Ring substitution		*Trans*			*Cis*		
		$R = NH_2$	$R = NHCH_3$	$R = N(CH_3)_2$	$R = NH_2$	$R = NHCH_3$	$R = N(CH_3)$
	2,6-Difluoro-	0.12	0.24	0.48	0.12	0.24	0.44
4'-Ethyl-	2,6-Difluoro-	0.11	0.24	0.49	0.12	0.24	0.45
		0.19		0.54	0.19		0.52
4'-Ethyl-				0.53			0.47
	2,3,5,6-Tetrafluoro-	0.46	0.64	0.81	0.44	0.64	0.74
4'-Ethyl-	2,3,5,6-Tetrafluoro-	0.47		0.81	0.44		0.75
	3,5-Difluoro-	0.63	0.74	0.76	0.58	0.69	0.73
4'-Ethyl-	3,5-Difluoro-	0.62	0.75	0.77	0.57	0.71	0.69

The 4'-ethyl substituent has no consistent effect on migration, only a slight variable effect in some cases.

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