

# FLUORESCENCE REACTIONS WITH BORIC ACID AND *o*-HYDROXY-CARBONYL COMPOUNDS, AND THEIR APPLICATION IN ANALYTICAL CHEMISTRY

## Part IV. Azo-dyes from *o*-Hydroxy-carbonyl Compounds

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IN the previous parts Neelakantam, *et al.*<sup>1</sup> showed that solutions of many compounds containing the *o*-hydroxy-carbonyl group in concentrated sulphuric acid, when treated with boric acid, developed a marked fluorescence if originally non-fluorescent, or exhibited a marked intensification of the fluorescence already present. This 'Fluorescence Effect' was noticeable in some cases in daylight but generally only under filtered U.V. light. This remarkable behaviour was found to be quite characteristic of such molecules, and among the large number and wide variety of compounds already examined, there were only two exceptions which gave a positive reaction in the absence of this group. It was also found that the introduction of the bathofloric bromine into the molecule of the compound did not lead to negative results in all cases. It was, however, noted that while a positive reaction indicated the presence of the ortho-combination of the groups, the converse was not true. With the introduction of the bathofloric nitro-group, the 'Fluorescence Effect' with boric acid also disappeared, while sulphonic group enhanced the effect.

In the light of the previous work it is of considerable interest to investigate the effect of introducing the bathofloric azo-group, by coupling the *o*-hydroxy-carbonyl compounds with diazonium salts, on the Fluorescence Effect with boric acid. Such an investigation was regarded of value for two reasons, *viz.*:—(a) it would throw light on the scope of the test for detecting the group in molecules of such great tinctorial powers as the azo-dyes are; and (b) as a necessary extension of these investigations whose primary object was the development of suitable reagents for the detection and determination of boric acid. In the course of investigations already reported, resacetophenone was found to give the best results in the test and was therefore suggested for the detection of micro-quantities of boric acid by a fluorescence reaction under filtered U.V. light.<sup>2</sup> The utility of this reagent

is limited by the fact that the fluorescence in presence of boric acid is excited only under U.V. light. A reagent which yields similar results in daylight would indeed be very useful for the detection and should also prove of value for the determination of boric acid.

A large number and variety of azo-dyes can be prepared from aniline itself by diazotising and coupling with different *o*-hydroxy-carbonyl compounds, and by using other amines, an enormous number of compounds can be produced. The present investigation, however, has been limited to seven of the commercial azo-dyes derived from salicylic acid, samples of which were available, and twenty azo-dyes which were synthesised for the purpose. Azo-dyes derived from salicylic acid have received special attention in this investigation for two reasons, *viz.*:—(a) it is a typical *o*-hydroxy-carbonyl compound which is commonly available; and (b) it is specially characterised by a marked ability to fluoresce when compared with other compounds of the same group. In strongly alkaline and concentrated solutions salicylic acid exhibits a deep violet fluorescence visible in daylight itself and very prominent under filtered U.V. light. In concentrated sulphuric acid solutions, the acid is non-fluorescent in daylight but strongly fluoresces in filtered U.V. For purposes of comparison eight azo-dyes which contained one or more phenolic hydroxyls, but the *o*-hydroxy-carbonyl group was absent, were synthesised.

TABLE I

No.	Azo-dye	Fluorescence (U.V.)		Remarks
		Blank	With boric acid	
1	Chrysamine G. ..	Pale rose	Deeper	Pink solution; deeper with boric acid; fluorescence difference quite prominent.
2	Cotton yeollw, GI.	Pale pink	„	Pale brownish yellow solution; pale orange with boric acid; fluorescence difference quite prominent
3	„ R.	„	„	Pale reddish brown solution; yellowish brown with boric acid; fluorescence difference quite prominent
4	Diamine brown M.	Nil	Nil	Pale blue solution; with boric acid pale violet
5	„ green B.	„	„	Pale bluish violet solution; deeper with boric acid
6	Diamond black F.	„	Very pale green	Pale bluish green solution; with boric acid lighter in shade; fluorescence not marked
7	„ green B.	Pale yellow	Deeper	Pale yellow solution; with boric acid no change; fluorescence difference not marked

TABLE II

No.	Azo-dye		Colour	M. P. °C.	Fluorescence (U.V.)		Remarks
	Amine (diazotised)	Coupled with			Blank	With Boric acid	
1	Aniline	Resacetophenone	Reddish brown	198	Nil	Nil	Brownish yellow solution
2	"	1-OH-2-Naphthoic acid	Chocolate	142	Pale skyblue	Pale skyblue	Bluish pink solution; with boric acid red tint increases
3	"	2-OH-3-Naphthoic acid	Deep red	205	Nil	Nil	Pink solution; with boric acid deeper
4	"	Salicylic acid	Brown	215	Pale yellow	Deeper	Yellow solution; no difference with boric acid
5	<i>p</i> -Nitraniline	"	Brownish red	254	Nil	Nil	Pale orange solution; pure yellow with boric acid
6	<i>m</i> -	"	Brownish yellow	249	Very pale yellow	Deeper	Do. fluorescence difference not prominent
7	<i>α</i> -Naphthylamine	"	Yellowish brown	212	Nil	Nil	Purple solution; with boric acid blue
8	<i>β</i> -	"	Pale brown	236	"	"	Wine red solution; with boric acid slightly deeper
9	<i>o</i> -Aminophenol	"	Brownish yellow	152	Pale violet	Deeper	Solution colourless with and without boric acid; fluorescence difference very prominent
10	<i>p</i> -	"	Light orange	159	"	"	"
11	Sulphanilic acid	"	Brownish yellow	..	Very pale yellow	"	Yellow solution; with boric acid no change; fluorescence difference not prominent
12	<i>p</i> -Chloraniline	"	Pale brown	248	Pale orange yellow	Pale orange yellow	No visible difference

13	<i>m</i> -Toluidine	..	"	Deep chocolate	210	Pale yellow	Deeper	Orange yellow solution; fluorescence difference not prominent
14	Anthranilic acid	..	"	Yellow	223	Very pale yellow	"	Pale orange solution; yellow with boric acid; fluorescence difference not prominent
15	Magenta	..	"	Chocolate	..	Pale yellow	Pale yellow	Blue solution (coupling with one mol salicylic acid); purplish with boric acid
16	"	..	"	Dark brown	..	Nil	Nil	Blue solution; purpled with boric acid (coupled with two molecules)
17	Chrysoidin	..	"	Chocolate brown	..	Very weak	Very weak	Brownish red solution; more red with boric acid
18	<i>p</i> -Amino-azobenzene	..	"	Dirty yellow	251	Flak	Deeper	Red solution; lighter with boric acid
19	Dinitro-diamino diphenylmethane	..	"	Reddish brown	..	Pale orange	Pale orange	Diazotised and coupled with one molecule of salicylic acid; brownish red solution; no colour change with boric acid; no difference in fluorescence
20	"	..	"	Do.	..	"	"	Tetrazotised and coupled with two molecules of salicylic acid; brownish red solution; no colour change with boric acid

TABLE III

No.	Azo-dye		Colour	M. P. °C.	Fluorescence (U.V.)		Remarks
	Amine (diazotised)	Coupled with			Blank	With Boric acid	
1	Aniline ..	Phenol	Orange yellow	152	Nil	Nil	Brownish yellow ; no change with boric acid
2	" ..	<i>p</i> -Hydroxybenzoic acid	Brownish yellow	205	Nil	Nil	Brownish red solution ; deeper with boric acid
3	" ..	" "	Khaki	210	Nil	Nil	Brownish yellow solution ; no change with boric acid
4	" ..	Resorcinol (Cerotine yellow R.)	Orange red	155	Pale orange yellow	Pale orange yellow	No difference
5	" ..	$\alpha$ -Naphthol	Deep chocolate	185	Nil	Nil	" "
6	" ..	$\beta$ -	Orange red	131	Pale pink	Pale pink	" "
7	Sulphanilic acid ..	" (orange II)	Orange	..	Orange	Orange	Sodium salt ; deep pink solution. No change in colour or fluorescence with boric acid
8	<i>p</i> -Nitraniline ..	Resorcinol (Magneson)	Chocolate	..	Orange yellow	Orange yellow	Orange red solution ; no change in colour or fluorescence with boric acid
9	" ..	$\beta$ -Naphthol	Red	250	Nil	Nil	Deep pink solution ; no change in colour or fluorescence with boric acid

## EXPERIMENTAL

*Azo-dyes.*—The azo-dyes were prepared in the usual manner by diazotising the amine and adding the solution gradually to an alkaline solution of the coupling component. The reaction mixture was kept distinctly alkaline and left over-night. The solution was then acidified and heated to boiling. The precipitated dyes were filtered and crystallised generally from aqueous alcohol and in a few cases from water. The dyes were characterised by their colour and melting points. They generally sintered several degrees below the temperature at which they finally melted and it is the latter temperatures that are recorded in the table.

*Fluorescence Test.*—The solutions were prepared and the tests carried out exactly as described in the previous communications. When the solutions in sulphuric acid were strongly coloured, they were diluted until pale in colour.

As the source of U.V. a mercury arc is enclosed in a cabinet provided with an U.V.-filter which transmitted mostly in the U.V. and a little in the violet-blue region was employed.

## RESULTS

The results are recorded in three tables given above, commercial samples in Table I and the prepared dyes in Table II. Compounds included in both tables contain the *o*-hydroxy-carbonyl group. For comparison, the results obtained with dyes in which this group is absent are given in Table III. With the exception of 'Magneson' (*p*-nitrobenzene-azo-resorcinol, B.D.H. sample), the dyes included in the table were also prepared for this investigation.

## DISCUSSION

It is well known that while the azo-group is bathofloric, it does not destroy the ability to fluoresce completely, as does the nitro-group, but weakens. It can be seen from the data presented above, that several dyes of the azo-group fluoresce, though weakly, in concentrated sulphuric acid solution under ultra-violet light. On the addition of boric acid, however, the intensification of fluorescence does not occur in all cases, but there are several examples in which it does (*cf.* Tables I and II). A positive result in the fluorescence test appears to be independent of the ability of the parent molecule to fluoresce in solution and no generalisation with regard to the influence of substituents is at present possible. It is, however, remarkable that though negative results have been recorded in the test with boric acid with several molecules which contained the *o*-hydroxy-carbonyl group, no positive result was obtained in the absence of this group as can be seen from Table III.

It is evident that the boric acid test for the *o*-hydroxy-carbonyl group is also applicable to the azo-compounds. However, negative results are obtained more often with the azo-dyes than with the *o*-hydroxy-carbonyl compounds from which they are derived. It may also be pointed out that the results are similar to those obtained with the brominated compounds already reported. Thus it is clear that while a positive result confirms the *ortho*-orientation of the hydroxy and carbonyl groups, a negative result does not prove the contrary.

With regard to the second object of this investigation, it may be stated that only the *o*- and *p*-hydroxy-benzene-azo-salicylic acids gave fluorescence effects with boric acid approaching those with resacetophenone. These compounds do not possess any special advantages over resacetophenone as reagents for the detection of boric acid.

#### CONCLUSION

The introduction of the azo-group into the molecule of an *o*-hydroxy-carbonyl compound does not invariably produce a negative result in the fluorescence test with boric acid carried out under filtered U.V. light. Fluorescence intensification on the addition of boric acid is observed in several cases, though there is a marked diminution in the intensity of the effect when compared with the parent *o*-hydroxy-carbonyl molecule.

The fluorescence test for the *ortho*-orientation of the hydroxyl and carbonyl groups is also applicable to the azo-dyes but a negative result does not prove the contrary.

#### REFERENCES

1. Neelakantam, *et al.* .. *Proc. Ind. Acad. Sci.*, 1942, 15A, 81 ; 1943, 18A, 364 ; 1944, 19A, 401.
2. ————— .. *Ibid.*, 1942, 16A, 349,