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

Part III. Effect of Bromination of the *O*-Hydroxy-Carbonyl Molecule on the Appearance of Fluorescence Effects with Boric Acid

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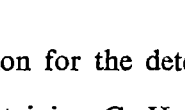
IN a previous communication[1] Neelakantam, Row and Venkateswarlu suggested a new fluorescence reaction for the detection of the  group in aromatic compounds containing C, H and O only. This test is carried out by dissolving the substance in concentrated sulphuric acid, diluting with more acid if the solution is coloured and then adding boric acid to one half of the solution, leaving the other half for blank test. The solutions placed in quartz test-tubes are examined for fluorescence effects produced on adding boric acid, *viz.*, (*a*) appearance of fluorescence if the blank is non-fluorescent, and (*b*) an increase in intensity or (*c*) a change in colour of the fluorescence when the blank is itself fluorescent. Observations are made both in daylight and under ultra-violet light using the Cenco Black Light Source described as argon-filled gas glow lamp fitted with U.V. Filter.[2] If the results are positive, except in the case of the simple 3 hydroxy-flavones (*loc. cit.*), it could be deduced that the molecule contained the *O*-hydroxy-carbonyl group while negative results using this lamp, which is a very poor source of ultra-violet light, though cheap and readily handled, did not necessarily indicate the absence of this group. This reaction has been shown to be more general and of greater applicability, not being confined to any one type of compounds, than any other reaction previously reported in the literature. It is, therefore, of considerable interest to investigate the effect of introducing groups and atoms such as the sulphonic, nitro, amino and halogens into the molecule of the *O*-hydroxy-carbonyl compound on the applicability of this reaction.

Neelakantam and Row[3] pointed out that sulphonation of the *O*-hydroxy-carbonyl compound generally produced a marked increase in the intensity

of fluorescence while nitration and bromination damped the fluorescence considerably and in the cases examined the latter two caused the disappearance of fluorescence. Thus 5-nitrosalicylic acid and nitro-sulphosalicylic acid did not exhibit any fluorescence effects with or without boric acid in daylight or under the lamp while salicylic acid gave prominent effects with boric acid under the lamp, and with sulphosalicylic acid the effects were relatively more prominent. 5-Bromosalicylaldehyde, the only bromo com-

Compound	Under U.-V. Lamp		Remarks
	Without boric acid	With boric acid	
1. 3 : 5-Dibromo-resaceto-phenone	Nil	Greenish yellow	Resacetophenone—bright blue with boric acid under lamp ; blank nil
2. 2-Hydroxy-4-methoxy-5-bromo-benzaldehyde	Nil	do.	2-Hydroxy-4-methoxy-benzal-dehyde—greenish yellow with boric acid under lamp ; blank nil
3. 2-Hydroxy-4-methoxy-3 : 5-dibromo-benzaldehyde	Nil	Nil	
4. 2 : 4-Dihydroxy-3 : 5-di-bromobenzaldehyde	Nil	Nil	β-Resorcylic aldehyde—green with boric acid under lamp ; blank nil
5. 3 : 5-Dibromo-resorcylic acid	Nil	Pale blue	β-Resorcylic acid—bright violet with boric acid under lamp ; blank nil
6. Do. Methyl ester ..	Nil	do.	
7. 3 : 5-Dibromo-orsellinic acid-ethyl ester	Nil	do.	Orsellinic acid and ethyl or-sellinate—deep violet with
*8. Do. Methyl ester ..	Nil	do.	boric acid under lamp ; blank nil
9. 3 :5-Dibromo-orsellinic acid	Nil	do.	
10. 3 : 5-Dibromo-orc-aceto phenone	Nil	Pale yellow	
11. 3 : 5-Dibromo-orcylic aldehyde	Nil	Nil	Orcylic aldehyde—bright greenish yellow with boric acid under the lamp ; blank nil
12. 3 : 5-Dibromo-2 : 4 : 4'-tri-hydroxy-3'-methoxy-chalkone	Nil	Pale yellow	2 : 4 : 4'-trihydroxy-3'-methoxy-chalkone—yellow with boric acid under lamp ; blank nil
13. 3 : 5-Dibromo-6-methyl-2 : 4 : 4'-trihydroxy-3'-methoxy-chalkone	Nil	Nil	
14. 3 : 5-Dibromo-2 : 4-dihydroxy-chalkone	Pale yellow	Deeper yellow	2 : 4-Dihydroxy-chalkone—pale greenish yellow in blank and deeper greenish yellow under the lamp
15. 3 : 5-Dibromo-2 : 4-di-hydroxy-4'-methoxy-chalkone	do.	do.	

*Compounds 8 to 15 were recently synthesised by Seshadri and Venkateswarlu—unpublished work.

pound then examined, showed that introduction of bromine into salicylaldehyde led to complete disappearance of fluorescence effects with boric acid while salicylaldehyde itself gave a positive reaction. Further it was found that bromide interfered with the resacetophenone—boric acid reaction using concentrated sulphuric acid and the explanation was offered that this was due to the production of bromine and bromination of resacetophenone.[4]

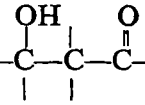
The bathofloric effect of halogens on fluorescence of organic compounds is well known.[5] Thus fluorescein is much more fluorescent than eosin, erythrosin and Rose-Bengal which are its brominated, iodinated and chlorinated derivatives. The ability of free halogens to inhibit (or quench) the fluorescence of fluorescein is a well-known reaction for the detection of the halogen, especially bromide in presence of large amounts of chloride[6] and also iodide. The object of the present investigation, however, is to find out the effect of introducing one or more halogen atoms, *e.g.*, bromine, into the molecule of an *O*-hydroxy-carbonyl compound on the " boric acid reaction ". It may be pointed out that this investigation is not concerned with the differences in fluorescence of the parent compound and its halogen derivative but with the effect of adding boric acid to the latter.

Experimental

The solutions were prepared and the tests carried out exactly as described in the previous communications. With none of the compounds examined was any fluorescence observed with or without boric acid in daylight. In the following table, therefore, the results obtained under the lamp and the colour of the fluorescence are reported. For comparison the results previously reported with the bromine-free compounds are given under remarks.

Discussion

It may be stated in general that the fluorescence effects obtained were much less marked in the case of the bromo-compounds than with the corresponding halogen-free compounds. Consequently care must be exercised in the interpretation of the results obtained. It must be emphasised in this connection that the lamp used is a very weak source of U.-V. light and the filter provided with the lamp transmits a little in the violet-blue region. Further it was observed that some samples of boric acid dissolved in concentrated sulphuric acid showed a very faint blue fluorescence. These, however, were of little significance when the bromine-free compounds were examined as the effects were prominent but could not be disregarded in the present investigation. Careful testing with the aid of blanks showed that there was an increase in intensity of fluorescence on the addition of boric acid

in several cases given in the table. From the results now reported taken in conjunction with those previously published it could be deduced that the introduction of bromine atoms into the molecule of an *O*-hydroxy-carbonyl compound does not invariably lead to complete disappearance of fluorescence effects with boric acid and though there is a marked diminution of intensity. The results depend on the nature of the compound and the number of bromine atoms introduced. From this it is clear that the boric acid reaction serves for the detection of the  group in compounds containing bromine in addition to C, H and O. However it must be pointed out that negative results with boric acid are obtained more often when bomine is present than in its absence. Attention may be drawn in this connection to the fact recorded in the previous paper that a negative fluorescence reaction with boric acid using the Cenco lamp as the source of U.-V. light does not necessarily indicate the absence of the *O*-hydroxy-carbonyl group.

Conclusion

The introduction of bromine atoms into the molecule of an *O*-hydroxy-carbonyl compound does not invariably result in absence of fluorescence effects on the addition of boric acid.

The fluorescence test for the *O*-hydroxy-carbonyl group is also applicable in the case of brominated compounds but as with the bromine-free compounds a negative result does not necessarily mean absence of *O*-hydroxy-carbonyl group. Negative results are obtained more often when bromine is present than in its absence.

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