

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

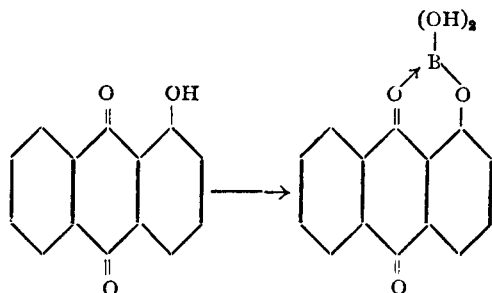
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FLUORESCENCE reactions for the detection of inorganic anions and cations are very few in number, and among these may be mentioned two examples involving boric acid. Of these two, the most important was proposed by Szebellédy and Gáál¹ for the detection of boric acid. This reaction depends on the fact that in a suitably buffered solution and in the absence of carbonates, heavy metals, etc., which interfere, boric acid yields with tincture cochineal an orange-yellow fluorescence visible under the ultra-violet light. The second example, mentioned by Meyer,² is that of the yellow fluorescence visible in daylight yielded by quinizarin (1:4-dihydroxy-anthraquinone) dissolved in concentrated sulphuric acid.

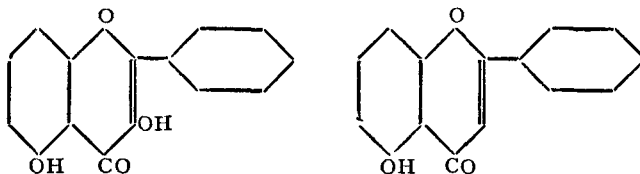
Feigl³ observed that boric acid reacts with many hydroxy-anthraquinones yielding characteristic colour changes which can be used for either the detection of boric acid or the hydroxy-anthraquinones.⁴ From a study of these reactions, he came to the conclusion that the colour changes are apparently due to the formation of inner complex (chelate) boric acid esters of the type shown below,



or similar boric-sulphuric acid esters.

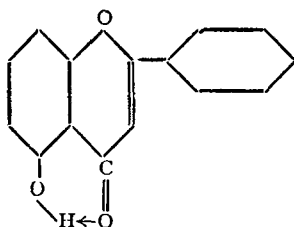
In the course of the work which is in progress on the detection of boric acid in qualitative analysis, it was considered desirable to investigate the

reaction with boric acid of the 5-hydroxy-flavonols and -flavones which also

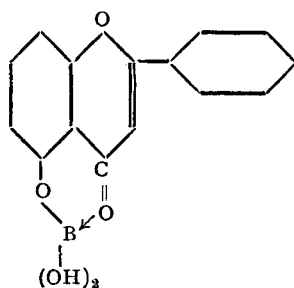


contain an identical reactive grouping $\begin{array}{c} | & | & | \\ -C & -C & -C- \\ | & | & || \\ OH & & O \end{array}$. It is well known

that in these compounds the 5-hydroxyl group exhibits a peculiarity in that it is difficult to methylate, and this is attributed to the fact that chelation exists between the hydrogen of the hydroxyl and the carbonyl oxygen as shown below. It, therefore, appeared quite probable that boric acid will



yield chelate compounds of the type shown below involving these two groups as in the case of the hydroxy-anthraquinones. Further, scattered through the literature are several examples of hydroxy-flavones and -flavonols which yield fluorescence visible in daylight when dissolved in sulphuric acid. Com-



parison of the constitutions of these compounds in relation to the fluorescence exhibited by them indicated that the 5-hydroxyl group generally exerted a batho-floric effect. From this again it appeared probable that chelation of the above kind would lead to interesting fluorescence changes which might serve for the detection of boric acid. A preliminary investigation showed that this was the case. Since the 5-hydroxyl group reacts as an ortho-group

to the carbonyl, the investigation was extended to the simple ortho-hydroxy-carbonyl compounds such as the ortho-hydroxy-acids, -aldehydes, and ketones with or without other substituents in the molecule, with a view to find out whether a satisfactory reagent for boric acid could be found among them. To throw light on the reaction, a few flavylum salts, polyhydric phenols and hydroxy-carbonyl compounds containing the hydroxyl in positions other than ortho to the carbonyl were examined. In all over forty compounds were examined.

Experimental

Solutions—

1. *Boric Acid.*—A solution of boric acid in concentrated sulphuric acid containing 20 mg. of the former acid per c.c. was used.

2. *Reagent Solution.*—About 10–15 mg. of the substance under investigation was dissolved in concentrated sulphuric acid (5 c.c.) either in the cold or by slight warming. In case the solution was strongly coloured it was diluted with more acid. The solution was divided into approximately equal parts in each case and one of them used as blank.

Procedure—

The two halves of the reagent solution were placed in quartz test-tubes of approximately the same dimensions and to one was added 1 c.c. of boric acid solution. After mixing in the cold, the two solutions were examined for any fluorescence first in daylight and then under the Cenco Black Light Source.⁵

Results—

The colour of the fluorescence obtained is recorded in Table I.

Of all the compounds examined resacetophenone yields the best results with boric acid. It gives no fluorescence even under the lamp, when dissolved in sulphuric acid, but in the presence of boric acid in the cold it yields a bright blue fluorescence visible under the lamp. A preliminary examination of the applicability of this reaction for the detection of boric acid gave the following results:—

(a) Limit of Identification = 100 γ .

(b) Limit of Sensitiveness = 1 : 10,000.

It also appears probable that with a better source of ultra-violet light the sensitiveness can be considerably increased.

TABLE I

Reagent	Without Boric Acid		With Boric Acid		Remarks
	Day-light	Under lamp	Day-light	Under lamp	
	<i>Flavones and Flavonols</i>				
1 Kaempferol ..	Blue	Greenish yellow	Blue	Bright greenish yellow	Yellow solution, deeper yellow with boric acid,
2 Quercetin ..	Green	Greenish yellow	Deep green	Intense greenish yellow	Yellow colour deepened with boric acid. The green fluorescence of quercetin in conc. H ₂ SO ₄ has not been observed before. Working with quartz test-tubes, however, this fluorescence is observed.
3 Morin ..	Bright green	Bright green	Bright yellowish green	Intense yellowish green	Addition of boric acid caused a deepening in the yellow colour of the solution.
4 Quercetagetin ..	Nil	Pale green	Nil	Pale green	Deep yellow solution. Difference is not prominent.
5 Gossypetin ..	Nil	Nil	Nil	Nil	Solution deep orange yellow.
6 3 : 7-Dihydroxyflavone ..	Light blue	Bright blue	Bright blue	Intense blue	Pale yellow solution. This result is surprising in view of the fact that the molecule does not contain a 5-hydroxyl.
7 7-Hydroxy-flavone ..	Blue	Intense blue	Blue	Intense blue	Almost colourless solution. No difference has been observed in the test from the blank.
8 Herbacetin ..	Nil	Greenish yellow	Nil	Greenish yellow	Orange solution. Difference is not marked.
9 Cannabiscetin ..	Nil	Yellow	Nil	Deep yellow	Yellow solution.
<i>Flavanones</i>					
1 Naringenin ..	Pale green	Greenish yellow	Deep green	Deep greenish yellow	Pale yellow solution; deeper yellow with boric acid.
2 Butin ..	Nil	Nil	Nil	Nil	Yellow solution; deeper yellow on adding boric acid.

<i>O-Hydroxy-ketones</i>									
1 Resacetophenone	Nil	Nil	Nil	Bright blue	This solution is colourless and the fluorescence with boric acid is very prominent.				
2 2 : 6-Dihydroxy-acetophenone	Nil	Nil	Nil	Yellowish green	Yellow solution. The fluorescence obtained is not so very prominent as with resacetophenone.				
3 <i>w</i> -Methoxy-resacetophenone	Nil	Nil	Nil	Light green	Very pale yellow solution.				
4 Phloracetophenone	Nil	Nil	Nil	Green	Yellow solution; fluorescence not so intense as with resacetophenone.				
5 2-Methoxy-3 : 6-dihydroxyacetophenone	Nil	Nil	Nil	Pale yellow	Yellow solution; deeper yellow on adding boric acid.				
6 2 : 4-Dihydroxy-3-methylacetophenone	Nil	Nil	Nil	Nil					
7 <i>w</i> : <i>p</i> -Dihydroxyacetophenone	Nil	Nil	Nil	Nil					
<i>O-Hydroxy-aldehydes</i>									
1 Salicyl aldehyde	Nil	Nil	Nil	Greenish yellow	Deep orange solution.				
2 β -Resorcylic aldehyde	Nil	Nil	Nil	Green	Brownish yellow solution.				
3 γ -Resorcylic aldehyde	Nil	Nil	Nil	Nil	Deep orange solution.				
4 4-Methoxy-2-hydroxy-benzaldehyde	Nil	Nil	Nil	Greenish yellow	Pale yellow solution.				
5 7-Hydroxy-coumarin-8-aldehyde	Nil	Nil	Nil	Nil					
6 7-Hydroxy-3-methoxy-flavone-8-aldehyde	Nil	Nil	Nil	Nil					
7 5-Bromo-salicylaldehyde	Nil	Nil	Nil	Nil					
8 2-Benzoyl-phloroglucin-aldehyde	Nil	Nil	Nil	Greenish yellow	Yellow solution.				
<i>O-Hydroxy-Carboxylic Acids</i>									
1 Salicylic acid	Nil	Violet	Nil	Intense violet	Colourless solution.				
2 Sulpho-salicylic acid	Nil	Violet	Violet	Intense violet	Colourless solution; fluorescence obtained is more intense than with salicylic acid.				

TABLE I—(Contd.)

Reagent	Without Boric Acid		With Boric Acid		Remarks	
	Day-light	Under lamp	Day-light	Under lamp		
3 Nitro-sulpho-salicylic acid	Nil	Nil	Nil	Nil	Colourless solution. Colourless solution; fluorescence not so intense as with β -resorcylic acid. Colourless solution; fluorescence deeper than with β -resorcylic acid. Pale yellow solution.	
4 Nitrosalicylic acid ..	Nil	Nil	Nil	Nil		
5 β -Resorcylic acid ..	Nil	Nil	Nil	Bright violet		
6 3-Methyl-4-methoxy-2-hydroxy-benzoic acid	Nil	Nil	Nil	Nil		
7 4-Methoxy-2-hydroxy-benzoic acid	Nil	Nil	Nil	Violet		
8 Orsellinic acid ..	Nil	Nil	Very pale violet	Deep violet		
9 Ethyl orsellinate ..	Nil	Nil	Very pale violet	Deep violet		
10 Dimethyl ether of orsellinic acid	Nil	Nil	Nil	Nil		
<i>Polyhydric Phenols</i>						
1 Catechol ..	Nil	Nil	Nil	Nil		No difference.
2 Resorcinol ..	Nil	Nil	Nil	Nil		
3 Hydroquinone ..	Pale blue	Blue	Pale blue	Blue		
4 Pyrogallol ..	Nil	Nil	Nil	Nil		
<i>Flavylium Salts</i>						
1 7-Methoxy-3:4'-di-hydroxy flavylium chloride	Bright green	Yellow	Bright green	Yellow	Yellow solution. No difference.	
2 7-Methoxy-3:3':4'-tri-hydroxy flavylium chloride	Green	Yellow	Green	Yellow	Brown solution. No difference.	
3 7-Methoxy-3:3':4':5'-tetrahydroxy flavylium chloride	Very pale green	Yellow	Very pale green	Yellow	Deep brown solution. No difference.	

Discussion

In agreement with the observations of Rangaswami and Seshadri,⁶ in the group of flavones and flavonols examined, it is found that an increase in the number of hydroxyl groups as in the case of quercetagenin and gossypetin is unfavourable for the appearance of fluorescence with or without boric acid. The existence of a group of three hydroxyls in 5:6:7 and 5:7:8 positions has a considerable damping effect on the fluorescence. These results are similar to those obtained with aluminium recorded in a previous paper.⁵ In the absence of the 5-hydroxyl, with the exception of 3:7-dihydroxy-flavone, no fluorescence change was observed either in the intensity or the colour. The flavanones also gave results consistent with the above conclusions. Besides these fluorescence effects it was observed that the addition of boric acid led to a deepening in the yellow colour of the sulphuric acid solution of these compounds.

In the case of the simple O-hydroxy-carbonyl compounds, while the results obtained were similar, it is remarkable that with the exception of salicylic and sulpho-salicylic acids, in the absence of boric acid none of them showed any fluorescence either in daylight or under the lamp; with boric acid, however, fluorescence was observed only under the lamp. In several cases it has been noticed that heating the solution of the substance in concentrated sulphuric acid with boric acid produced either an increase in intensity or a change in the colour of the fluorescence obtained in the cold. This may probably be due to the molecule undergoing sulphonation. This inference is supported by the observation that sulpho-salicylic acid in cold concentrated sulphuric acid yields under the lamp a violet fluorescence which is considerably more intense than that obtained with salicylic acid under almost identical conditions; an intensification of the fluorescence under the lamp was also observed with salicylic acid on heating with concentrated sulphuric acid. On the introduction of a nitro-group into the molecule of salicylic acid as well as sulphosalicylic acid, the fluorescence disappears completely thereby indicating that the nitro-group is considerably bathofloric in character.

In the group of O-hydroxy-carbonyl compounds examined, a few cases are exceptional in that they do not give any fluorescence effects with or without boric acid either in daylight or under the lamp. A common constitutional feature of these compounds is the existence of a substituent group between two hydroxyls one of which may be in the form of an ether or ester linkage as in the cases of γ - and α -benzopyrones. It appears, therefore, that the absence of fluorescence effects with boric acid need not necessarily indicate the absence of hydroxyl ortho to the carbonyl group.

Examination of the flavylium salts, polyhydric phenols and *p*-hydroxy-carbonyl compounds showed that in the absence of a hydroxyl ortho to the carbonyl, no fluorescence effects with boric acid are obtained.

Conclusion

(1) Addition of boric acid to O-hydroxy-carbonyl compounds dissolved in concentrated sulphuric acid, except in a few cases, brings about either a marked intensification or a change in colour of the fluorescence exhibited by them in daylight or under the lamp. In a few other cases fluorescence appears for the first time on the addition of boric acid. Since even the simpler O-hydroxy-carbonyl compounds yield these results with boric acid, it appears reasonable to conclude that this reaction will serve as a suitable method for the detection of O-hydroxy-carbonyl group. It must, however, be borne in mind that while the appearance of fluorescence, its intensification or a change in colour indicates the presence of a hydroxyl group ortho to the carbonyl, the non-appearance of the fluorescence effects described does not indicate the absence of this group.

(2) No fluorescence changes are observed with boric acid if the molecule of the compound under investigation does not contain a ketonic group ortho to the hydroxyl, so also in the absence of either of them or when they are present in positions other than ortho to each other.

(3) Sulphonation of the simpler O-hydroxy-carbonyl compounds brings about a marked change in the colour and intensity of the fluorescence; the sulphonic acid group appears to be markedly auxo-floric in character. It is remarkable that nitration or bromination, on the other hand, damps the fluorescence considerably.

(4) From the brilliant fluorescence effects given by resacetophenone, it is expected to develop into a useful reagent for the detection of boric acid on a semi-micro scale.

Further work along these lines is in progress.

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