

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

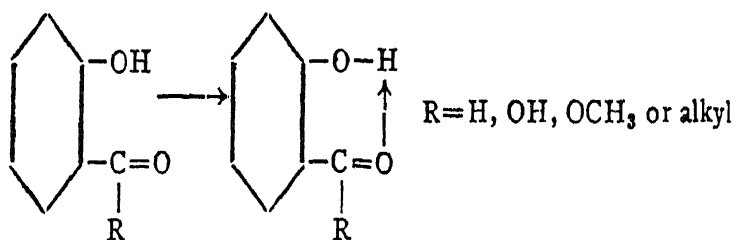
## Part II. Detection of $\begin{array}{c} \text{OH} \quad \text{O} \\ | \quad | \\ -\text{C}-\text{C}-\text{C}- \\ | \quad | \end{array}$ in Aromatic Compounds containing C, H and O only

BY K. NEELAKANTAM, L. RAMACHANDRA ROW AND V. VENKATESWARLU  
(From the Department of Chemistry, Andhra University, now at Madras)

Received October 14, 1943

It is well known that a hydroxyl ortho to the carbonyl group in aromatic

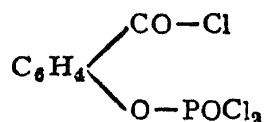
compounds,  $\begin{array}{c} \text{OH} \quad \text{O} \\ | \quad | \\ -\text{C}-\text{C}-\text{C}- \\ | \quad | \end{array}$ , exhibits certain peculiarities, viz., that it is not readily methylated and that it is less phenolic than when it is present in other positions. This behaviour has been observed not only with the simpler compounds such as resacetophenone, salicylic acid, etc., but also with the hydroxy-flavones, -flavonols, -flavanones, -chromones, -chalcones, -xanthenes, and -anthraquinones containing this group. These peculiarities have been ascribed to the existence of chelation between the hydroxyl hydrogen and the carbonyl oxygen thus:



leading to a diminution of ionisability of the phenolic hydrogen atom and also causing fixation of the double bonds. When the hydroxyl is in the meta or the para position to the carbonyl it behaves in the normal manner. Consequently the qualitative detection of the presence of a hydroxyl ortho to the carbonyl group is of considerable interest.

No attempt seems to have been made in the past to develop a general reaction for the detection of this group in various types of compounds. However, a few reactions which distinguish ortho-hydroxy carbonyl compounds from the meta- and para-compounds have been recorded and these could be utilised, though to a limited extent for this purpose.

Anschtütz<sup>1</sup> found that while the, *o*-, *m*-, and *p*-hydroxy benzoic acids themselves reacted with phosphorous pentachloride yielding the phosphorous oxychloride derivative of the phenolic carboxylic acid chloride of the following type:



ortho-hydroxybenzoic acids in which the second ortho position to the hydroxyl is already occupied give the free acid chlorides. Obviously, the reaction is of no value for detecting the presence of the ortho-hydroxy-carbonyl group in the simple unsubstituted hydroxy-benzoic acids. However, it could be employed, for example, with *o*-cresol-carboxylic acids.

Jean-Renaud<sup>2</sup> found that only esters of *o*-hydroxybenzoic acids yield with hydroxylamine and alkali in aqueous solution the corresponding hydroxamic acids, R-CO-NHOH, while the *m*- and *p*-compounds do not. Since the hydroxamic acids can be readily detected by means of the characteristic property which they possess of yielding a red or violet colour with ferric chloride, this difference could be utilised to distinguish the three types of hydroxy acids. However, it fails with the ester of  $\beta$ -naphthol- $\beta$ -carboxylic acid, and Angelli and Castellana<sup>3</sup> showed that if an alcoholic solution is employed, the ester of *m*-hydroxybenzoic acid also gives the hydroxamic acid. In this connection it is of interest to note that among the unsubstituted *o*-, *m*-, and *p*-hydroxybenzoic acids the ortho acid can be easily and directly distinguished by means of its colour reaction with ferric chloride. While the ortho compound yields a violet colour, the *m*-, and *p*-compounds give none. It may, however, be pointed out that substitution brings about a marked change in the colour obtained with the ferric chloride as, for example, in the case of 5-nitro-salicylic acid which yields a red colour and hence the ferric chloride colour reaction is not a sure test for the *o*-hydroxybenzoic acids.

A third reaction distinguishing the *o*-hydroxy-carboxylic acids from the *m*-, and *p*-acids has been reported by Nölting.<sup>4</sup> Anilides of the ortho acids on treatment with dimethylaniline and phosphorous oxychloride yield compounds of the malachite green series whereas those of the *m*- and *p*-compounds yield derivatives of dialkyl-amido-benzophenones.

With regard to the hydroxy-ketones Pfeiffer *et. al.*<sup>5</sup> found that while carbonyl compounds and *m*- and *p*-hydroxy-carbonyl compounds yielded with stannic chloride additive compounds, in the presence of an ortho-hydroxyl internally complex (chelate) compounds were obtained. The latter compounds are closely related to the tin lakes and are coloured unlike the additive compounds.

In the group of the hydroxy-anthraquinones, Dimroth *et al.*<sup>6</sup> found that

the  $\alpha$ -hydroxy compounds containing the  $\begin{array}{c} \text{OH} \quad \text{O} \\ | \quad | \quad || \\ -\text{C}-\text{C}-\text{C}- \\ | \quad | \end{array}$  group gave on treatment with boracetic acid in acetic anhydride solution characteristic colour changes, in many cases with fluorescence, due to the formation of boracetate complexes (chelation) involving the hydroxyl and carbonyl and that when the hydroxyl was not in the ortho position to the carbonyl only acetylation of the former occurred. Brass *et al.*<sup>7</sup> utilised this reaction for proving the absence of *l*-hydroxyl in the dihydroxyphenanthraquinone (2 : 4) they had prepared.

Feigl and Krumholz<sup>8</sup> observed that boric acid itself reacts with  $\alpha$ -hydroxy-anthraquinones dissolved in concentrated sulphuric acid yielding characteristic colour changes which can be used either for the detection of boric acid or the  $\alpha$ -hydroxyanthraquinones. These authors explained these colour changes as due to the formation of inner complex (chelate) boric esters or of similar boric-sulphuric acid esters involving the ortho hydroxyl and the carbonyl group. From the data given by Meyer<sup>9</sup> it is clear that generally in the absence of a hydroxyl ortho to the carbonyl group, no prominent colour changes occur on the addition of boric acid. However, a few exceptions are known. 2 : 3-dihydroxy-anthraquinone gives a colour change, though not a prominent one (red-brown to brown) on adding boric acid, but it does not contain a hydroxyl ortho ( $\alpha$ ) to the carbonyl group, while 1 : 3-dihydroxy- and 1 : 4 : 5 : 8-tetrahydroxy-anthraquinones fail to give a colour change on adding boric acid though they contain the  $\alpha$ -hydroxyl.

Rangaswami and Seshadri<sup>10</sup> showed that Wilson's boric-citric acid colour test is quite useful for the detection of the presence of a 5-hydroxyl in hydroxy-flavones and -flavonols and that a positive reaction is given by the methyl ethers also. Similar results were obtained with the *o*-hydroxy-chalkones and their methyl ethers. However, the 5-hydroxy-flavanones, naringenin and hesperetin, and the simpler *o*-hydroxy compounds, salicylic acid and resacetophenone fail to give the test. These authors pointed out that for a compound to yield a positive reaction the molecule should contain: (1) a keto-group, (2) an ortho hydroxyl or methoxyl and (3) an external double bond in conjugation with the C=O. This reaction is obviously

limited to those compounds containing the  $\begin{array}{c} \text{OH} \quad \text{O} \\ | \quad | \quad || \\ -\text{C}-\text{C}-\text{C}- \\ | \quad | \end{array}$  group in which there is present in addition an external double bond in conjugation with the

C=O as in the case of 5-hydroxy-flavones, -flavonols and the 2-hydroxy-chalkones.

To the above may be added the ferric chloride colour reactions for the *o*-hydroxy-carbonyl compounds, acids, aldehydes and ketones which serve to a limited extent to distinguish the *o*- from the *m*- and *p*-compounds.

It is clear from the above that the available reactions for the detection of the  $\begin{array}{c} \text{OH} \quad \text{O} \\ | \quad | \quad || \\ -\text{C}-\text{C}-\text{C}- \\ | \quad | \end{array}$  group have been developed with reference to particular types of compounds and have consequently a limited range of applicability.

In a previous communication<sup>11</sup> which may be regarded as Part I of this series, Neelakantam and Row reported that addition of boric acid to solutions of the *O*-hydroxy-carbonyl compounds in concentrated sulphuric acid, except in a few cases, brought about either a marked intensification or a change in colour of the fluorescence exhibited by them in daylight or under the ultra-violet lamp. In some cases fluorescence appeared only on the addition of boric acid, the solutions (H<sub>2</sub>SO<sub>4</sub>) of the compounds themselves being non-fluorescent. These results were given not only by the simpler compounds such as salicylic acid, resacetophenone, etc., but also by most of the 5-hydroxy-flavones, -flavonols and -flavanones examined. In the

absence of the  $\begin{array}{c} \text{OH} \quad \text{O} \\ | \quad | \quad || \\ -\text{C}-\text{C}-\text{C}- \\ | \quad | \end{array}$  group no fluorescence effects (*loc. cit.*) were observed except in one case (3:7-dihydroxy-flavone). These authors suggested that the reaction might serve for the detection of this group. It was, however, pointed out by them that a negative result does not necessarily indicates the absence of this group.

Since the previous work was published several other *o*-hydroxy-carbonyl compounds became available as a result of synthetic work done in our laboratories and it was felt desirable to examine the scope of this fluorescence

reaction as a general reaction for the detection of the  $\begin{array}{c} \text{OH} \quad \text{O} \\ | \quad | \quad || \\ -\text{C}-\text{C}-\text{C}- \\ | \quad | \end{array}$  group in aromatic compounds. The present investigation is confined to compounds containing carbon, hydrogen and oxygen only. Feigl (*loc. cit.*) examined the colour reactions of the  $\alpha$ -hydroxyanthraquinones with boric acid in daylight only and hence the few available compounds of this series were included in the present investigation. In all twenty-six compounds including some

*o*-hydroxychalkones recently synthesised in our laboratory have now been examined. Further, a variety of aromatic compounds containing the hydroxyl and carbonyl groups in other than the ortho position to each other and also those in which the hydroxyl or the carbonyl is absent have been examined to elucidate the limitations of the reaction.

#### *Experimental*

*Solutions.*—The boric acid solution and the solutions of the substances under investigation were prepared as described already.<sup>11</sup>

*Procedure.*—The procedure adopted was also identical with the one previously described.

*Results.*—The colour of the fluorescence obtained with the *o*-hydroxy-carbonyl compounds is recorded below. The results obtained with compounds in which the two groups are not ortho to each other and also those in which one of them is absent are also recorded in order to test the specific nature of the reactions.

*o*-Hydroxy-ketones.—2-Hydroxy-6-methoxy-acetophenone gave a bright yellow and 2-hydroxy-4:6-dimethoxy-acetophenone a greenish yellow fluorescence with boric acid under the lamp. 2-Hydroxy-3:6-dimethoxy-acetophenone and  $\omega$ -methoxy-phloracetophenone gave no fluorescence with boric acid even under the lamp. With all these compounds the blanks exhibited no fluorescence either in daylight or under the lamp.

*o*-Hydroxy-aldehydes.—2:4-Dihydroxy-6-methyl-benzaldehyde gave a bright greenish yellow and 2-hydroxy-4-methoxy-6-methyl-benzaldehyde a greenish yellow fluorescence with boric acid under the lamp. With the two compounds no fluorescence was obtained either in daylight or under the lamp in the blank test. 2-Hydroxy-4:6-dimethoxy-benzaldehyde, however, gave a pale yellow fluorescence in the blank test and a deeper yellow fluorescence with boric acid under the lamp.

*o*-Hydroxy-carboxylic acids and esters.—Atranorin and ethyl rhizonate gave no fluorescence in the blank test but with boric acid they gave a pale blue fluorescence under the lamp. Montagnetol and lecanoric acid gave a pale violet fluorescence under the lamp even without boric acid and on its addition this became deeper in both cases. The methyl ester of karanjic acid gave a pale blue fluorescence in the blank and a deeper blue with boric acid under the lamp.

*o*-Hydroxy-chalkones.—2:4:3':4'—Tetrahydroxy-chalkone (butein) dissolved in sulphuric acid gave a green fluorescence visible in daylight itself and on treatment with boric acid, there was appreciable increase in its intensity; the blank appeared greenish yellow under the lamp and with boric

acid a deeper greenish yellow. 2:4-Dihydroxy-chalkone gave a pale greenish yellow fluorescence in the blank and a deeper greenish yellow with boric acid under the lamp. 2:4:4'-Trihydroxy-3'-methoxy-chalkone gave an yellow fluorescence with boric acid under the lamp and none without it; in daylight no fluorescence was obtained with or without boric acid.

The following chalkones recently synthesised by Seshadri *et al.* (unpublished work) gave no fluorescence with or without boric acid in daylight or under the lamp :—

2:6-Dihydroxy-, 2:4'-dihydroxy-6:3'-dimethoxy-, 2:5:4'-trihydroxy-6:3'-dimethoxy-, 2-hydroxy-3:6-dimethoxy-, 2-hydroxy-3:6:4'-trimethoxy-, 2:4'-dihydroxy-3:6:3'-trimethoxy-, and 2-hydroxy-5:6:3':4'-tetramethoxy-chalkones.

*α-Hydroxy-anthraquinones.*—Quinizarin gave a pale yellow fluorescence in daylight itself without any boric acid and on its addition it was deepened; there was no appreciable colour change with the boric acid. The blank gave an orange-yellow fluorescence under the lamp and with boric acid this became deeper yellow. Alizarin, quinalizarin and *rheum*-emodin (2-methyl-4:5:7-trihydroxy-anthraquinone) gave no fluorescence with or without boric acid in daylight or under the lamp. In these cases prominent colour changes occurred on the addition of boric acid. Alizarin gave a pale pink solution which became violet on adding boric acid, with quinalizarin the change was from violet to blue and in the case of *rheum*-emodin from orange to violet-red.

$$\begin{array}{c} OH \quad . \quad O \\ | \quad | \quad || \\ -C-C-C- \\ | \quad | \end{array}$$

Compounds in which the —C—C—C—group is absent.—The following compounds gave no fluorescence with or without boric acid in daylight or under the lamp :—

*p*-Hydroxy-benzaldehyde, vanillin, isovanillin, protocatechuic aldehyde, benzoin, benzil, *α*-naphthol, 5:6:3':4'-tetramethoxy-flavanone, anisic acid and gallic acid.

In the following cases the solutions of the substances in concentrated sulphuric acid gave fluorescence under the lamp but on the addition of boric acid there was no change in intensity or in colour:—

*m*-, and *p*-Hydroxy-benzoic acids, mandelic acid, benzophenone, phthalic acid, *β*-naphthol and 3-methoxy-7-hydroxy-8-methyl-flavone.

Other compounds examined for negative evidence were the following:—

Benzilic acid, umbelliferone, 4-methyl-, 5-methyl-, 4:8-dimethyl-, and 8-allyl-umbelliferone; 2-methyl-3-methoxy-7-hydroxy-, and 2-methyl-3-

methoxy-7-allyloxy-chromone, and 6:8-diallyl-7-hydroxy-flavone. All these gave fluorescence when dissolved in sulphuric acid even in daylight but addition of boric acid produced no change.

7-Methoxy-3-hydroxy-flavone, however, behaved in an exceptional manner. Its solution in concentrated sulphuric acid exhibited a light greenish blue fluorescence in daylight which on the addition of boric acid became a bright blue; under the lamp it was bright green in the blank and deep blue with boric acid.

### Discussion

In the case of the *o*-hydroxy-ketones, -aldehydes, and -acids, the results now obtained generally support the conclusions reported in the previous communication (*loc. cit.*). Among the compounds now exam-

ined also there are a few cases in which though the  $\begin{array}{c} \text{OH} \quad \text{O} \\ | \quad | \quad || \\ -\text{C}-\text{C}-\text{C}- \\ | \quad | \end{array}$  group is present negative results are obtained. Among the simpler *o*-hydroxy-carbonyl compounds it appears that when the 3-position is occupied either by a methyl or a methoxy group as in 3-methylresacetophenone, 2-hydroxy-3:6-dimethoxy acetophenone and 3-methyl-4-methoxy-2-hydroxybenzoic acid, no fluorescence is observed with boric acid either in daylight or under the lamp.  $\omega$ -Methoxy-phloracetophenone also gives a negative result with boric acid.

In the group of the *o*-hydroxy-chalkones, out of the ten cases examined positive fluorescence effects were obtained only with three and it is interesting to note that all these are derived from  $\beta$ -resacetophenone. A comparison of the results obtained with the chalkones and the corresponding ketones shows that the behaviour of the two classes is not always parallel and while the ketones may exhibit the fluorescence effects the corresponding chalkones may not. Thus  $\gamma$ -resacetophenone itself yields positive results while the chalkones derived from it do not but 2-hydroxy-3:6-dimethoxy-acetophenone itself gives negative results and so also the chalkones derived from it. It may be stated in general that conversion of a ketone to a chalkone reduces the capacity to exhibit fluorescence.

The  $\alpha$ -hydroxy-anthraquinones yield strongly coloured solutions when dissolved in concentrated sulphuric acid and even when diluted largely with the acid they do not give any fluorescence effects with boric acid except in the case of quinizarin. It appears that when addition of boric acid produces colour changes, no fluorescence effects are obtained.

Compounds which do not contain the  $\begin{array}{c} \text{OH} \quad \text{O} \\ | \quad | \quad || \\ -\text{C}-\text{C}-\text{C}- \\ | \quad | \end{array}$  group gave negative results with boric acid, with one exception, in all the cases examined.

The results presented in this paper in conjunction with those already reported in Part I (*loc. cit.*) show clearly that a large number of compounds containing

the  $\begin{array}{c} \text{OH} \quad \text{O} \\ | \quad | \quad || \\ -\text{C}-\text{C}-\text{C}- \\ | \quad | \end{array}$  group such as the *o*-hydroxy-acids, -ketones and -aldehydes; the 5-hydroxy-flavones, -flavonols and -flavanones; *o*-hydroxy-chalkones and  $\alpha$ -hydroxy-anthraquinones give positive fluorescence effects with boric acid. However, there are cases in which though this group is present negative results are obtained. With the data available at present no general explanation could be given for these exceptions.

Among the compounds examined so far only in the cases of 3:7-dihydroxy-flavone reported in Part I and 7-methoxy-3-hydroxy-flavone in the present paper were fluorescence effects obtained with boric acid in the

absence of the  $\begin{array}{c} \text{OH} \quad \text{O} \\ | \quad | \quad || \\ -\text{C}-\text{C}-\text{C}- \\ | \quad | \end{array}$  group. It is of interest to note in this connection that in the boric-citric acid test also both compounds gave a deep blue fluorescence but no colour.<sup>10</sup> 7-Hydroxy-flavone itself gives a negative result in the fluorescence as well as in the boric-citric acid test. Obviously, therefore, the 3-hydroxyl group in 3:7-dihydroxy-flavone and in its 7-methyl ether is responsible for their exceptional behaviour in the fluorescence test. From the data available, therefore, it could be deduced that, with these two exceptions, a positive result with boric acid indicates the presence of the

$\begin{array}{c} \text{OH} \quad \text{O} \\ | \quad | \quad || \\ -\text{C}-\text{C}-\text{C}- \\ | \quad | \end{array}$  group in aromatic compounds containing carbon, hydrogen and oxygen only but the converse is not true.

It is evident that this reaction is of greater applicability than any so far reported in the literature. It is much more general than the others and is not confined to any one type of compounds. Further the test is very easily carried out and it does not involve further substitution as in Anschütz's method or preparation of derivatives as in Jean-Renaud's and Nölting's methods.



### Conclusion

1. Addition of boric acid to aromatic compounds (C, H, and O only)

of various types containing the group  $\begin{array}{c} \text{OH} \quad \text{O} \\ | \quad | \quad || \\ -\text{C}-\text{C}-\text{C}- \\ | \quad | \end{array}$ , dissolved in concentrated sulphuric acid generally produces a marked intensification or a change in colour of the fluorescence exhibited by them in daylight or under the lamp. In several cases the solution of the compound in sulphuric acid is itself non-fluorescent and fluorescence appears on adding boric acid.

2. In a few cases even when this group is present no fluorescence effects are obtained with boric acid.

3. 3:7-Dihydroxy-flavone and its 7-methyl ether are exceptions in that they give positive reactions with boric acid even though the above group is absent.

4. The fluorescence effects obtained with boric acid could be utilised

for the detection of the  $\begin{array}{c} \text{OH} \quad \text{O} \\ | \quad | \quad || \\ -\text{C}-\text{C}-\text{C}- \\ | \quad | \end{array}$  group in various types of aromatic compounds containing carbon, hydrogen and oxygen only. A positive reaction indicates the presence of this group but the converse is not true.

5. This reaction is more general than all the others described in literature and is also easily carried out.

Further work is in progress.

The authors wish to express their thanks to Prof. T. R. Seshadri for valuable help rendered in the course of this investigation.

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