# Short Communication

## Chromogenic activity of carbonyl-substituted resorcinols with Ehrlich reagent

In our previous communication[1] we reported color reactions of 4-alkylresorcinols and some naturally occurring phenols with modified Ehrlich reagent. Study of the former compounds indicated that generally with an increase in alkyl chain a decrease in color reaction was observed. Testing of the latter revealed the usefulness of the reagent for the identification of micro amounts of some natural phenols.

To study possible relationship of color and structure, we have tested* over one hundred phenolic compounds with modified Ehrlich reagent[2]. Herein are reported our results of color reactions of carbonyl-substituted resorcinols.

Among the carbonyl group containing compounds ACHESON *et al.*[3] have examined the three resorcinol carboxylic acids. $\gamma$-Resorcylic acid was observed to give pale blue color in an unspecified amount. However, STEELINK[4], MCGEER *et al.*[5] and later ACHESON AND TURNER[6] reported that all of the three carboxylic acids yielded no color reaction. Although no other carbonyl substituted resorcinols were examined, ACHESON AND TURNER[6] concluded that this substituent in general deactivated the benzene ring and thus inhibited the color tests. Phloroglucinolcarboxylic acid and 2,4,6-trihydroxyacetophenone, however, gave a pink color, indicating that additional activation by a phenolic group could mitigate the adverse effect of the carboxyl or other carbonyl group.

Preliminary work performed by us[1] on some carbonyl substituted resorcinols appeared to confirm the finding of the previous workers[4–6] that the presence of a carbonyl group in resorcinol inhibited the color reaction. However, only some 3,5-dihydroxy- and 2,4-dihydroxy-carbonyl group containing compounds were then tested. Subsequent study of 2,6-dihydroxy-carbonyl phenols and comparative tests on all the three types of compounds showed varying and noteworthy results.

*Testing technique*

For studying color reactions we preferred to use spot tests on paper rather than the paper chromatographic technique employed by the previous workers[3–5]. Generally six different amounts, *viz.* 1,2,5,10, 50 and 100 $\mu$g, of each compound were spotted on a Whatman No. 1 filter paper strip. Two $\lambda$ of the modified Ehrlich reagent (0.5 g *p*-dimethylaminobenzaldehyde in 90 ml absolute ethyl alcohol, 2.8 ml concentrated hydrochloric acid added and volume made to 100 ml with absolute alcohol) was applied on a 3–4 mm diameter spot of each compound. The paper was placed in an oven for one minute at 100°C.

* These results were recently presented to the Annual Meeting of the American Pharmacognosy Society, held at Chapel Hill, North Carolina (U.S.A.), in July 1963. Abstract of the paper is published[2].

In case of some compounds that were colored or found to yield light coloration on heating, a blank spot of each concentration of the test substance was made. Reagent was not applied on this spot. Two $\lambda$ of the reagent was also separately spotted. After comparing the colors of the separate test compound and reagent controls with the color of these combined, differences were recorded. The color recorded was of the lowest amount of the test compound yielding a noticeable coloration.

TABLE I

COLOR REACTIONS OF CARBONYL SUBSTITUTED RESORCINOLS WITH EHRLICH REAGENT

| *No.* | *Compound* | *Color* | *Amount of test compound* ($\mu$g)* |
|---|---|---|---|
| 1 | 2,4-Dihydroxybenzaldehyde | Light pink | 50 |
| 2 | 2,4-Dihydroxybenzamide | Light yellow | 5 |
| 3 | 2,4-Dihydroxybenzoic acid | Light pink | 50 |
| 4 | 2,4-Dihydroxyacetophenone | Light violet | 50 |
| 5 | 2,4-Dihydroxypropiophenone | Light pink | 100 |
| 6 | 2,4-Dihydroxybenzophenone | Orange-brown | 100 |
| 7 | 2,4-Dihydroxybenzaldoxime | Yellow-brown | 50 |
| 8 | 2,6-Dihydroxyacetophenone | Pink | 1 |
| 9 | 2,6-Dihydroxybenzamide | Pink | 1 |
| 10 | 2,6-Dihydroxybenzoic acid | Light violet | 1 |
| 11 | Methyl 2,6-dihydroxybenzoate | Light pink | 1 |
| 12 | 3,5-Dihydroxybenzamide | Light yellow | 50 |
| 13 | 3,5-Dihydroxybenzoic acid | Light yellow | 100 |
| 14 | Methyl 3,5-dihydroxybenzoate | Light yellow-gray | 100 |
| 15 | 3,5-Dihydroxy-4-methylbenzoic acid | Pale yellow** | 100 |

* Spot tests on paper on six different quantities, 1, 2, 5, 10, 50 and 100 $\mu$g, of each compound were performed and the lowest amount of the compound that gave noticeable color reaction is recorded.

** This color could not be distinctly differentiated from the reagent blank and might be considered as doubtful.

*Color reactions of carbonyl substituted resorcinols*

Resorcinol derivatives with electron withdrawing carbonyl substituents such as aldehyde, amide, carbomethoxyl, carboxyl, and ketone functions were examined. The carbonyl substituted phenols with hydroxyl groups in 2,4-, 2,6-, and 3,5- were tested. Color reactions of the spot test are listed in Table I.

The results (Table I) reveal the varying chromogenic activity of the three types of carbonyl substituted resorcinols. 2,6-Dihydroxy-carbonyl containing phenols show the most sensitive color reaction and pink to violet coloration is noticeable even in an amount of 1 $\mu$g. The compounds in which hydroxyl groups are in 3 and 5 positions show the least sensitive test and the colored products are yellowish. The variation of chromogenic activity of the three types of carbonyl substituted phenols to yield coloration with modified Ehrlich reagent can be summarized as follows:

$$\text{2,6-dihydroxy-} > \text{2,4-dihydroxy-} > \text{3,5-dihydroxy-}$$

The usefulness of the reagent in the identification of 2,6-dihydroxy-carbonyl substituted phenols is also indicated from the results (Table I).

*Color reactions of carbonyl substituted trihydroxyphenols*

To study the compensating effect of an additional hydroxyl group comparative examination of trihydroxy-carbonyl substituted compounds has also been made. Several carbonyl substituted trihydroxyphenols with hydroxyl groups in 2,3,4-, 2,4,5-, 2,4,6-, and 3,4,5- have been tested. It was found that sensitive pink-violet color reactions were observed only in the case of carbonyl containing phloroglucinols. This indicated that the location of the phenolic hydroxyl group was important in mitigating the adverse effect of the carbonyl group. More details on trihydroxy carbonyl substituted compounds and results on many other phenols tested will be published.

*Color reactions of 5,7-dihydroxyflavans and related compounds*

The difference in chromogenic activity of some carbonyl group containing resorcinol derivatives as against those that do not contain such deactivating function can be used to distinguish natural phenols such as 5,7-dihydroxyflavans and related compounds that have resorcinol moiety in their structure. To determine this we have tested some more such compounds than previously reported[1]. For comparison results of color tests on all these are recorded in Table II.

TABLE II

COLOR REACTIONS OF 5,7-DIHYDROXYFLAVANS AND RELATED COMPOUNDS WITH EHRLICH REAGENT

| *No.* | *Compound* | *Colour* | *Amount of test compound* (μg)* |
|---|---|---|---|
| 1 | D-Catechin** | Violet | 1 |
| 2 | Chrysin | Light yellow | 10 |
| 3 | Cyanidin chloride** | Pink | 1 |
| 4 | Epicatechin** | Violet | 1 |
| 5 | Fisetin | Yellow-orange | 2 |
| 6 | Genistein | Orange | 50 |
| 7 | Hesperetin | Orange-yellow | 5 |
| 8 | Hesperidin methyl chalcone | Light yellow | 10 |
| 9 | Kaempferol | Light yellow | 10 |
| 10 | Malvidin chloride** | Pink | 1 |
| 11 | Morin | Yellow-green | 5 |
| 12 | Naringenin | Orange-yellow | 5 |
| 13 | Quercetin | Yellow | 5 |
| 14 | Quercitrin | Yellow | 5 |
| 15 | Rhamnetin | Yellow | 10 |
| 16 | Rutin | Yellow | 10 |

* Amount spotted and color observations were made similarly as given in footnote* under Table I.

** The lowest amount that could be detected was 0.1–0.2 μg.

It is indicated from the results (Table II) that the modified Ehrlich reagent is valuable in identifying 5,7-dihydroxyflavans and related compounds which do not have a carbonyl group in 4 position. This reagent gives pink to violet color with such compounds (*e.g.* catechin, epicatechin, cyanidin chloride, and malvidin chloride). Anthocyanidins (such as cyanidin and malvidin) are colored flavan derivatives as compared to catechins which are colorless. The two classes of compounds after detection with our reagent can be additionally distinguished from each other.

In connection with tests on catechin and epicatechin it might be indicated that this type of compounds can also be detected in crude extract of such plants as tea. A spot test on an infusion of tea, which is known to contain several catechins, shows a distinct pink coloration suggesting their presence.

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## Separation of saturated and unsaturated fatty acid esters of cholesterol by gas–liquid chromatography

While several procedures for the separation of sterols and their derivatives by gas–liquid chromatography (GLC) have been published[1], the separation of long-chain fatty acid esters of cholesterol has not been specifically reported. This communication deals with the separation of cholesteryl palmitate, stearate and oleate, prepared by trans-esterification of cholesteryl acetate and the appropriate fatty acid ester using sodium methoxide[2].

GLC analysis was carried out on an F & M model 500 temperature-programmed gas chromatographic unit with flame ionization attachment. Of several supports and stationary phases examined under a variety of conditions, the system here described gave the best separation. A 4-ft. spiral stainless steel column (0.3 in. diam.) was packed with 60/70 mesh Anakrome ABS (an acid-washed, base-washed and silicone-treated flux-calcined diatomaceous earth) coated with 2 % SE 30 (silicone rubber gum). The temperatures at the injection port and detector block were 320° and 350° respectively. Flow rates for air, helium and hydrogen were 400, 100 and 30 ml/min respectively. A mixture of cholesteryl palmitate, stearate and oleate (5 mg each) was made up to 0.3 ml with chloroform and a sample of 1–3 $\mu$l was injected. Attenuation was kept at 800. The column was programmed from 200–340° at 3°/min.

Cholesteryl palmitate was eluted at 270° and cholesteryl stearate and oleate in a single peak at 290°. The mixture of these three esters (*ca.* 20 mg) was oxidized with