

## Colour reactions of chalcones and their mechanism (A Review)

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(Received: December 18, 2007; Accepted: February 22, 2008)

### ABSTRACT

Chalcones are open analogues of flavonoids and give bright red to purple colours with different reagents which can be used to distinguish them from other flavonoids such as flavanones, flavones, aurones etc. Studies show that the bathochromic shift arises due to the formation of carbonium ion at the carbonyl group (>C=O) of the chromogen (ArCOCH=CHAr) as an intermediate which produces deeper colour during the reaction.

**Key words:** Chalcone, halochromy, benzylideneacetophenone.

### INTRODUCTION

Benzylideneacetophenones constitute a class of naturally occurring pigments, which are often referred to as "Chalcones" (Figure 1). The term was first coined by Kostanecki<sup>1</sup> who did pioneering work in the synthesis of natural coloring compounds.

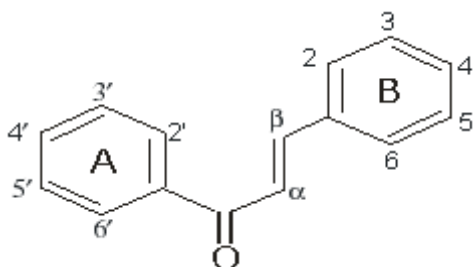
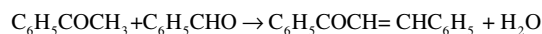


Fig. 1

The synthesis of chalcone, the parent member of the series, has been accomplished in a variety of ways, but perhaps the simplest method is the one involving the Claisen-Schmidt reaction. This is the reaction of acetophenone with benzaldehyde in the presence of aqueous alkali or Sodium ethylate, resulting in the formation of a, b - unsaturated ketone<sup>2</sup>.



Chalcones undergo a colour change to a deeper shade (by deeper shade is meant a displacement of absorption maxima towards longer wavelength), when treated with different reagents. This phenomenon is known as "halochromy"<sup>3</sup>.

The present review is intended to present some observations on the colour reactions given by chalcones with different reagents which can be used to distinguish them from other flavonoids.

### Chemistry of the colour tests for chalcones with different reagents

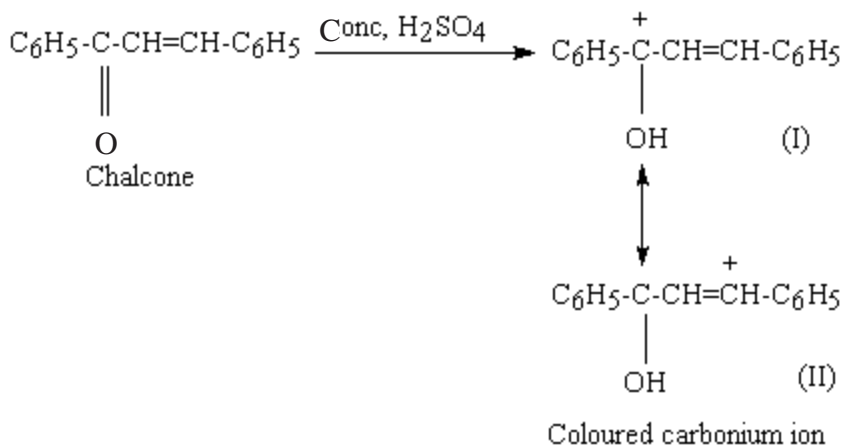
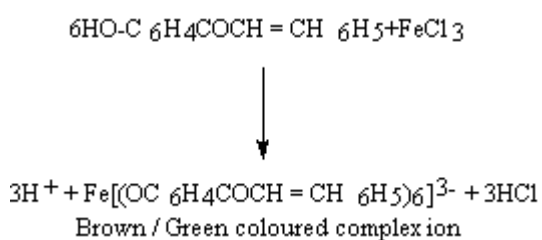
The commonly used reagents for colour tests of chalcones are (1) Alcoholic Ferric Chloride, (2) Concentrated sulfuric acid, (3) Sulfuric acid-Nitric acid, (4) Sulfuric acid-Acetic anhydride, (5) Sodium Borohydride and HCl acid, (6) Wilson's boric test and (7) Antimony pentachloride.

### Alcoholic ferric chloride test

This test is applicable to only hydroxy chalcones and is fact the characteristic of phenolic

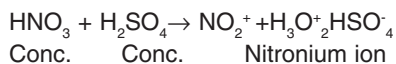
group and is due to the formation of complex iron salt<sup>4</sup>. The nature of the colour developed have however a definite relation with the orientation of the phenolic-OH in the chalcone molecule.

When a small amount (nearly 50 mg) of the chalcone was dissolved in 3ml of ethanol and then two drops of freshly prepared alcoholic FeCl<sub>3</sub> solution was added to it. Instantaneously definite shades of colours (i.e. blue, wine red, blue black, violet or green colours) were produced.



exhibits weaker halochromy, as compare to unsubstituted/substituted chalcones.

This test involves the formation of nitronium ion which attack on the 3'-position of chalcone molecule and give nitrochalcone which exhibit weaker halochromy as compare to parent chalcone.



It is worth while to mention that in certain cases the colours produced were not permanent but transient so careful observation is descramble in performing this test.

#### Concentrated Sulfuric acid<sup>5</sup>

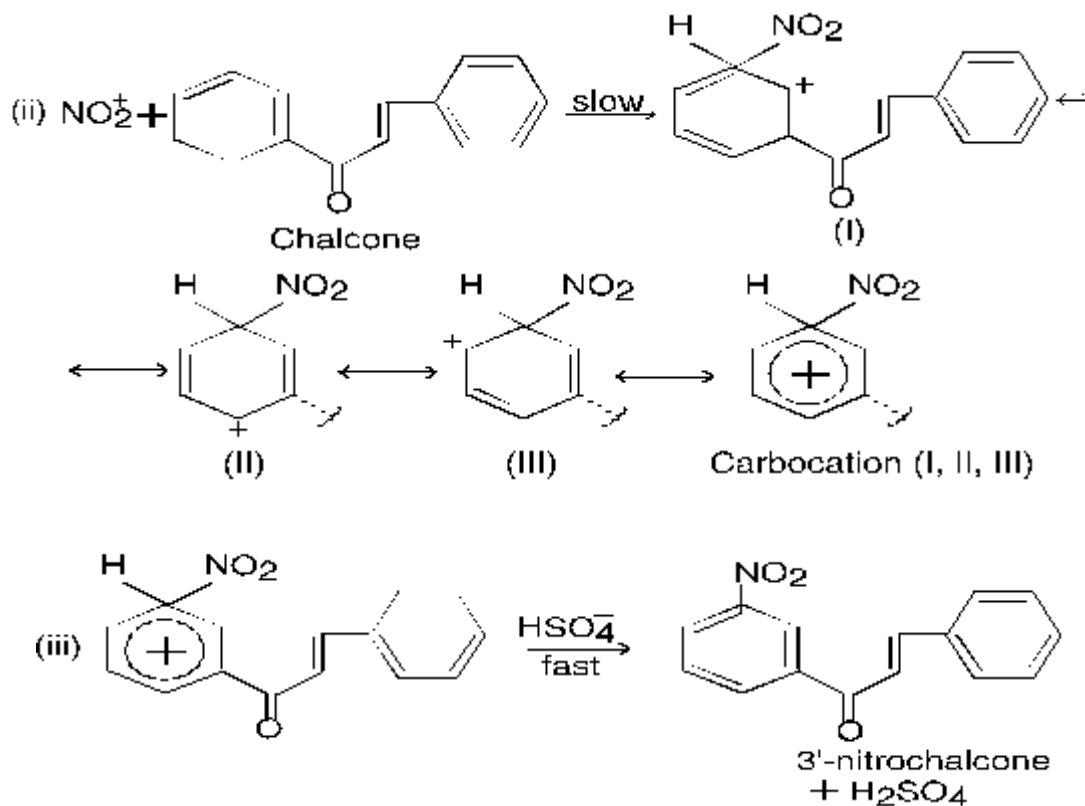
Chalcones exhibit beautiful halochromic effect when wetted with conc. H<sub>2</sub>SO<sub>4</sub>. The colours imparted are usually transient. When chalcone dissolved in conc. H<sub>2</sub>SO<sub>4</sub> the coloured carbonium ion produced. The resonance must of course be regarded as extended to the benzene ring.

#### Sulfuric acid-Nitric acid<sup>6</sup>

When the intensely coloured solution of chalcone in Conc. H<sub>2</sub>SO<sub>4</sub> acid is treated with a little Conc. HNO<sub>3</sub> acid, characteristic colour changes occur. This change involves nitration of chalcones rather than oxidation, and the resulting nitrochalcone

The nitration of chalcone takes place at 3'-position which is supported by the nitration of chalcone with small amount (1mole) of copper nitrate-acetic anhydride<sup>7</sup> as well as with ceric ammonium nitrate in chloroform<sup>8</sup>.

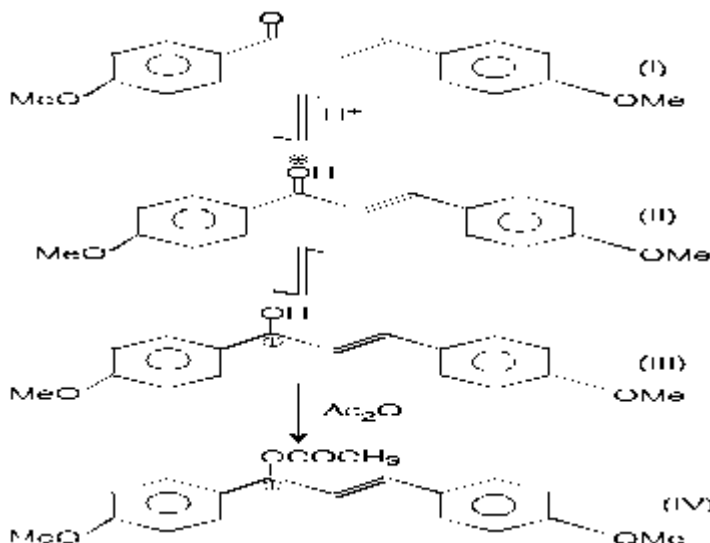
Flavanones and flavones do not under go nitration with H<sub>2</sub>SO<sub>4</sub>-HNO<sub>3</sub> acid so this can be used to distinguish chalcones from flavanones and flavones. The chalcone gives orange to yellow colour while methoxy chalcone gives reddish orange to yellow colour with this reagent.



#### Sulfuric acid–acetic anhydride

King and White<sup>9</sup> found that substituted (hydroxy, methoxy) chalcones in acetic acid solution produce deep colour (orange to purple) when treated with a drop or two of Conc.  $\text{H}_2\text{SO}_4$  acid. The test is negative with other flavonoids except

flavanones and aurones. The bathochromic shift arises due to the addition of acetic anhydride to the chalcone (in conc.  $\text{H}_2\text{SO}_4$ ) has been rationalized in terms of stability conferred on the carbonium ion (III→IV) by acetylation with acetic anhydride.



On treatment with an  $\text{Ac}_2\text{O}$  solution (0.2% W/V) containing two drops of conc.  $\text{H}_2\text{SO}_4$  at 10–15°C. Chalcones range from orange to purple colours which are listed in table 1.

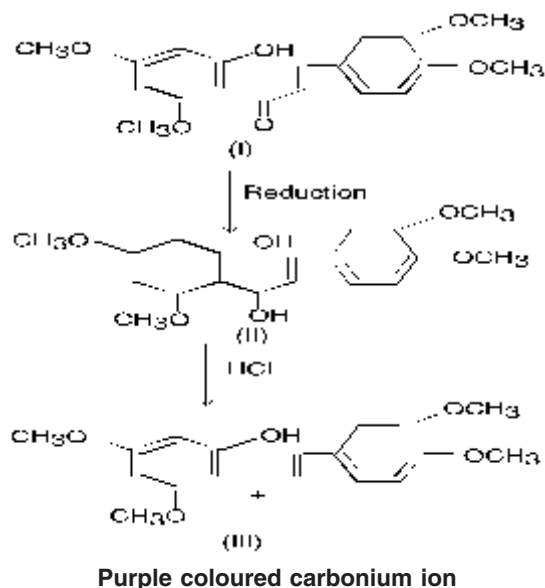
**Table 1: Colour obtained with Conc.  $\text{H}_2\text{SO}_4$ - $\text{Ac}_2\text{O}$  Solution**

Chalcone	Colour
Chalcone (unsubstituted)	Yellow
4'-Methoxy	Orange
4-Methoxy	Red
4, 4'-Dimethoxy	Purple
3, 4, 4'-Trihydroxy	Orange
3, 4'-Dihydroxy-4-methoxy	Cerise
2'-Hydroxy-3, 4, 4'-trimethoxy	Blue change into Red
2'-Hydroxy-3, 4, 4'-trimethoxy	Deep pink
4-Dimethylamino	Wine red
3, 4, 4'-Tribenzyloxy	Red

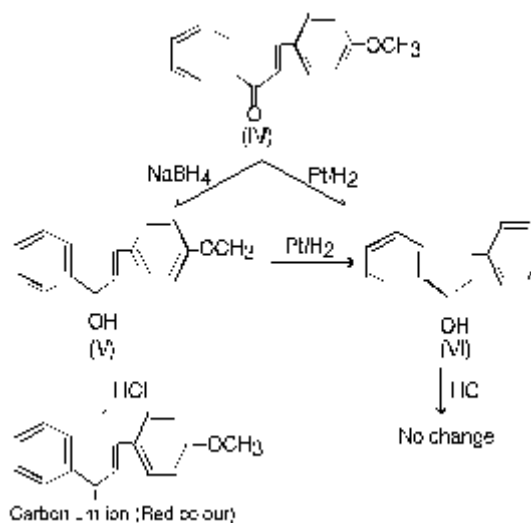
Krishnamurthy<sup>10</sup> *et al.*, observed that the positive response given by flavanones is due to the formation of traces of chalcones formed during the reaction as a resulting of ring opening and their immediate conversion into coloured product. They also introduced new reagents like  $\text{HClO}_4$  and  $\text{ZnCl}_2$  (Lewis acid) which are capable of generating acetylium ions from  $\text{Ac}_2\text{O}$  serve equally well in the place of  $\text{H}_2\text{SO}_4$  acid.

#### Sodium borohydride and hcl acid

Transient colours are developed when chalcones after reduction with sodium boro hydride, are treated with conc. HCl acid. This colour test can therefore be utilized for their identification. The mechanism of this colour reaction can be illustrated with reference to 2'-hydroxy-3, 4, 4', 6-tetramethoxychalcone (I). Krishnamurthy and Seshadri<sup>10</sup> noted that when chalcone (I) treated with sodium borohydride-HCl acid reagent it gave purple colour. This observation provided evidence to demonstrate that carbonium ion (III) arising in accordance with Geissman's scheme is involved in the colour test.



More satisfying evidence in support of the above scheme was provided by the following experiment. Selective reduction of 4-methoxychalcone (IV) with sodium borohydride gave a colourless unsaturated alcohol (V) (m.p. 48°). Its IR, spectrum showed the presence of hydroxyl and no carbonyl. It was different from the propanol (VI). It was different from the propanol (VI) prepared from 4-methoxy chalcone (IV) by  $\text{Pt}/\text{H}_2$  reduction and it also gave the propanol (VI) by further catalytic reduction. It was very sensitive to acid and gave an immediate red colour with ethanolic HCl while the propanol (VI) showed no change.



**Table 2: Colours obtained with various Chalcones in the SbCl<sub>5</sub> test**

Chalcone	Colour
2-Hydroxy-2-methoxy	Blood red
2-Hydroxy-4, 4', 5-trimethoxy	Blood red
2-Hydroxy-4,5-dimethoxy-3', 4'-methylenedioxy	Cherry red
2-Hydroxy-3, 4, 4', 5-tetramethoxy	Brick red
2, 2'-Dihydroxy-3',4', 5, 6, 6'-pentamethoxy	Violet red
4-Methoxy	Red
3', 4'-Dimethoxy	Cherry red
3', 4'-Methylenedioxy	Violet red
2', 4, 5-Trimethoxy	Dark red
2, 3, 4, 4', 5-Pentamethoxy	Red
2, 3, 4-Trimethoxy-3', 4'-methylenedioxy	Cherry red

**Wilson's boric test**

Chalcones having an *ortho*-hydroxy or methoxy group give a positive colour reaction with borocitric acid reagent<sup>11</sup> as for example, 2', 4, 4', 6'-tetrahydroxychalcone. The reagent is prepared by mixing equal parts of two separate solution, one composed of 100 ml of absolute acetone saturated with boric acid, and the other 100 ml of absolute acetone containing 10 gm of anhydrous citric acid (made by allowing crystalline acid to efflorescence completely in air at 30-40°, then heating it in a thin

layer to 100° for 2 hours. For determining the reactivity, approximately 5 mg of chalcone derivative is dissolved in about 1ml of dry acetone and the solution divided into two equal portions. To one portion is added 2ml of boric acid-citric acid-acetone reagent, and the other portion is diluted to an equal volume with a mixture of equal parts of the citric-acetone solution. The colours of the two tubes are compared at the end of a few minutes and any definitely stronger colour in the boric acid containing tube is regarded as a positive reaction.

This test is also very specific for partially methylated hesperatin<sup>12</sup>, 5-hydroxy and methoxy chalcone<sup>13</sup>.

**Antimony pentachloride**

Various chalcones treated with SbCl<sub>5</sub> in CCl<sub>4</sub> yields intense red or violet precipitates, which are characteristically different from the yellow or orange precipitates from flavones, flavanones and flavonols.

In this test 5–10 mg of the compound is dissolved in 5ml of anhydrous CCl<sub>4</sub> and 1ml of 2% anhydrous CCl<sub>4</sub> solution of SbCl<sub>5</sub> is added.

**Mechanism**

The results indicate that the formation of chalcone complex, which, as, with the complexes from stannic chloride<sup>14</sup> is characterised by strong

**Table 3: Colours given by Various Chalcones with Different Reagents**

Chalcone	MeOH	Alc. FeCl <sub>3</sub>	Conc. H <sub>2</sub> SO <sub>4</sub>	Ac <sub>2</sub> O–H <sub>2</sub> SO <sub>4</sub>
4, 4'-Dihydroxy	Yellow	Green	Reddish orange	Dark yellow
4, 4'-Dihydroxy-3-methoxy	Yellow	Light green	Cherry red	Dark yellow
2-Chloro-4'-hydroxy	Colourless	Light green	Yellow	Yellow
4-Hydroxy-4'-nitro	Yellow	Blackish green	Red	Wine red
4-Hydroxy-3-methoxy-4'-nitro	Yellow	Blackish green	Purple-orange	Violet
4-Dimethylamino-4'-nitro	Yellow	No change	Reddish orange	Greenish yellow
4'-Amino-4-hydroxy	Orange	Blackish green	Red	Dark orange
4'-Amino-4-hydroxy-3-methoxy	Yellow	Blackish green	Violet- blood red	Brownish yellow
4'-Amino-4-dimethylamine	Orange	No change	Reddish orange	Dark orange
4'-Amino-2-chloro	Pale yellow	No change	Yellow	Yellow
4-Hydroxy-3'-nitro	Brown	Blackish brown	Brick red	Greenish yellow
4-Hydroxy-3-methoxy-3'-nitro	Brown	Blackish brown	Reddish orange	Greenish brown
4-Dimethylamino-3'-nitro	Brown	No change	Brick red	Greenish yellow

halochromism, depends upon the  $>C=O$  group activated by the double bond, and the colours, which differ only within a limited range of wavelengths in accordance with the number of substituents<sup>15</sup> indicate the presence of the  $-COCH=CH-$  group united to two benzene rings.

The  $SbCl_5$  test makes it possible to distinguish chalcone from flavonic pigments and to ascertain the purity of flavones, flavonols and flavanones synthesized from the respective hydroxy chalcones, which remain as impurities difficult to eliminate even by repeated crystallisation. The  $SbCl_5$  reaction is extremely sensitive<sup>16</sup> e.g. a positive test for 2-hydroxy-3-,4,4'-trimethoxychalcone is obtained at a concentration of 1 in 1000000 (1 PPM). A positive test is obtained with the chalcones listed in table 2.

In present work we have also studied the halochromy of some new chalcones synthesized by Claisen-Schmidt condensation of substituted acetophenone and substituted benzaldehydes. These chalcones gave deep colours (due the bathochromic shift in  $\lambda_{max}$  ranges from +12 to +90) with alcoholic  $FeCl_3$ , Conc.  $H_2SO_4$  and Conc.  $Ac_2O-H_2SO_4$  listed in table 3.

The above discussion about the colour reactions of chalcones shows that alcoholic  $FeCl_3$  gives colour reaction only with hydroxy chalcones while conc.  $H_2SO_4$ -Conc.  $HNO_3$  reagent provides colours due to the nitration of A-ring of chalcone molecule at 3'-position.  $NaBH_4-HCl$  and  $Ac_2O-H_2SO_4$  acid tests for chalcone show that the chromogen involved is  $Ar-CO-CH=CH-Ar$  and the reaction involves the attack by acetylium ions at the carbonyl of the above chromogen giving  $C^+-(OAc)-CH=CH-$ .

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