



## The effect of benzene ring substituents on the mechanism of Duquenois Levine (DL) test for cannabinoid detection

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### ABSTRACT

*Tetrahydrocannabinol is detected by the presumptive Duquenois-Levine Test (DL), a chemical spot test based on the aldol condensation reaction. However, the chemistry and mechanism of the reaction is not fully understood. In the present study several substituted benzene compounds were tested to better comprehend the mechanism of the reaction. Compounds containing different substituents on the benzene ring gave differential results when tested with the Duquenois-Levine reagents. While activating substituents containing a lone pair of electrons resulted in a positive color change, the presence of substituents with no lone pair resulted in no change in color. These studies suggest that the activating groups contribute to the conjugation of the resultant chromophore product resulting in a color change. These studies provide a better understanding of the mechanism of the presumptive cannabinoid color test.*

**Key words:** Duquenois Levine test, cannabinoid, benzene ring substituents

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### INTRODUCTION

The chemical spot test, Duquenois-Levine test is a presumptive color test for the presence of cannabinoids [1, 2]. The Duquenois reagent (aldehyde and vanillin) mixed with hydrochloric acid yields a bluish-violet hue when the cannabinoids delta-9-tetrahydrocannabinol, delta-8-tetrahydrocannabinol, cannabinol, or cannabidiol is present. Mixing the solution with petroleum ether or chloroform will extract the organic purple layer isolating the bluish-violet solution. In a forensic laboratory the Duquenois-Levine test is not used by itself as a final identifier for the presence of cannabinoids, often times it is combined with botanical examination i.e. the microscopic morphology of cystolithic hairs [3].

Though the Duquenois Levine chemical test has been utilized for a number of years, the chemistry supporting the presumptive test is not fully understood. The reaction is believed to involve an aldol condensation between the reagents (vanillin and ethanal) followed by an electrophilic aromatic substitution with the cannabinoid resulting in a purple chromophore [4]. Recently the chromophore structure proposed was detected and confirmed using the AccuTOF-DART system[5].

Studies suggest that resorcinol, a derivative present in all cannabinoids, is a significant factor resulting in the color change [6]. In the present study we investigate the effect of substituted benzenes on the color change of the Duquenois-Levine Test. Specifically, substituents on the benzene ring which donate electrons (activating) vs electron withdrawing substituents (deactivating) were tested with the Duquenois-Levine test.

## EXPERIMENTAL SECTION

*Chemicals*

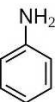

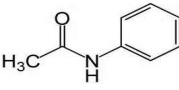
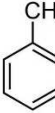
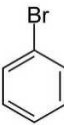
All chemical compounds were HPLC grade and purchased from Sigma-Aldrich Co. St Louis, MO. Leaves were collected from native and cultivated plants.

*Duquenois Levine chemical Test*

The Duquenois Levine reagent was prepared by mixing 2.5 mL of Acetaldehyde with 2.0g of Vanillin in 100mL of 95% Ethanol. Chemical compounds were mixed with 1 volume of the Duquenois Levine solution prepared. After three minutes one volume of 37% concentrated hydrochloric acid were added. Any observed color change was recorded immediately. A hue of purple was considered a positive result.

## RESULTS AND DISCUSSION

Table.1: Effect of activating and deactivating substituents on the DL test color change

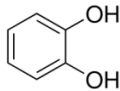
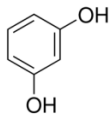
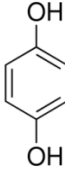
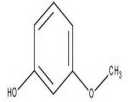
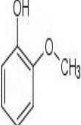
Name of compound	Structure	Color	Directing effects
Aniline		Red	Strongly activating
Phenol		Pink	Strongly activating
Acetanilide		Yellow	Moderately activating
Toluene		Clear	Weakly activating
Bromobenzene		Clear	Weakly deactivating

**Effect of Activating and Deactivating substituents on the benzene ring**

To further understand the reaction and mechanism of the DL test reaction, the effect of activating and deactivating substituents on the benzene ring was investigated. It is well known that the substituents already present on the benzene ring strongly influence the regioselectivity and the speed of the reaction. When the substituent is an activating group such as an amine or a hydroxyl group, the atom of these groups directly bonded to the ring (N or O) will have lone pairs of electrons. Electron-donating groups are known to donate their electrons to the pi system, stabilizing the carbocation intermediate resulting in a faster reaction. In contrast, deactivating groups withdraw electrons from the ring. As shown in Table 1, strongly activating substituents (Aniline and Phenol) reacted with the

DL reagent to give a red or pink color. However, moderately activating (Acetanilide), weakly activating (Toluene) and weakly deactivating (Bromobenzene) did not generate a significant color change. The activating substituents (Aniline and Phenol) contain Nitrogen and Oxygen, both with electron donating capabilities which can contribute to the conjugation of electrons. In addition, the more conjugation in the system, the longer the wavelength is absorbed resulting in a darker color.

**Table 2: Effect of hydroxy substituents on the benzene ring**

Name of Compound	Structure	Color Change
1,2 dihydroxy benzene		No color change
1,3 dihydroxy benzene		Pink
1,4 dihydroxy benzene		No color change
3 methoxyphenol		Dark red
2 methoxyphenol		clear

#### Effect of additional substituents on the benzene ring

To investigate additional substituents attached to the benzene rings that might contribute to the color change, substituents of phenol were investigated (Table 2). As shown in Table 2, when the hydroxyl group was present at the ortho or para position, no color change was observed. However, when the hydroxyl group was present at the

meta position a pink color was observed, indicating that the substitution reaction is occurring at the ortho/para positions. This was further confirmed by the DL reaction with 2 methoxy phenol and 3 methoxy phenol.

### CONCLUSION

The specificity of the Duquenois Levine presumptive color test was tested using several chemical compounds. Strongly activating groups with a lone pair of electrons on a benzene ring will suffice to cause a color change in the Duquenois Levine chemical test. These results support the proposed mechanism of the reaction involving an aldol condensation followed by an electrophilic aromatic substitution resulting in the proposed chromophore structure.

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