



## The Chemistry of van de Moer Test for Cytisine

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### Abstract

Cytisine is an alkaloid with biological activity that is used as medication for smoking cessation treatment. It is a quinolizidine derivative, one ring being 2-pyridinone, and there is a third cycle, a disubstituted piperidine. Thus, the analytical chemistry of this compound is relevant. In this communication we provide the chemistry involved in the spot test for cytisine identification by means of ferric chloride/hydrogen peroxide. The electron flow, step by step, is given from the alkaloid to the final colored products. The sequence is based on the chemical properties of the substrate and reagents, disclosing the series of reactions occurring in this assay. The process is in accordance with the experimental results. We also give the mechanism of cytisine degradation, from a quinolizidine system to 6,8-dimethyl quinoline, which involves an interesting rearrangement.

### 1. Introduction

Cytisine,  $C_{11}H_{14}N_2O$ , is a three ringed alkaloid, featuring a quinolizidine system that includes a 2-pyridinone moiety; the third ring is a disubstituted piperidine. The importance of this compound is due to its biological properties which help to stop smoking in about 25 days. This action was discovered in Eastern Europe, and afterwards cytisine was also used in Central Europe and recently in Canada and Australia, [1].

The alkaloid comes from *Cytisus laburnum* and it is found mainly in the seeds. The tree is also called Golden Rain (Acacia), and the leaves were used as false tobacco. In the

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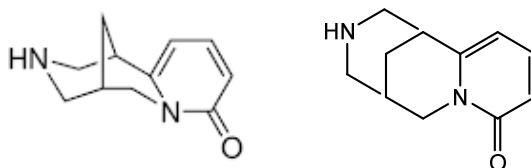
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test under study cytosine reacts with ferric chloride and hydrogen peroxide giving a red colour that changes to blue. In this communication we provide the mechanism of the reactions that occur in this assay. This communication is a follow up of our studies on the chemistry of colour tests, [2-6].

## 2. Antecedents

The more relevant studies about cytosine, a quinolizidine alkaloid, Figure 1, are pointed out. Gray in Edinburgh made extracts containing cytosine and studied the physiological action on animals and man, [7, 8]. The alkaloid was isolated in Germany by Husemann and Marmé, [9]. After several steps and via the well crystallizing nitrate, cytosine was obtained as colorless radiant crystals. It has a strong alkaline reaction, neutralizes the strongest acids and can be sublimated between watch glasses.



**Figure 1.** Cytosine structures.

Ewins established the constitution of two bases previously obtained by the action of hydriodic acid and phosphorus on cytosine at 225-230°C. One of them afforded a third product by reduction with sodium in alcohol. These products were identified by synthesis and are related to 6,8-dimethyl quinoline, a nitrogen being lost as ammonia, [10]. This result is very interesting but it is misleading since the product comes from a rearrangement. The reaction mechanism of this degradation is provided in the 'Discussion'.

Other studies were made in Austria by Späth [11], and the structure elucidation was achieved in England by Ing, [12, 13]. The synthesis of cytosine was carried out by Bohlman and collaborators, [14]. The absolute configuration was studied in Japan [15], and the crystal structure was reported in England, [16]. There is other synthesis of cytosine, [17].

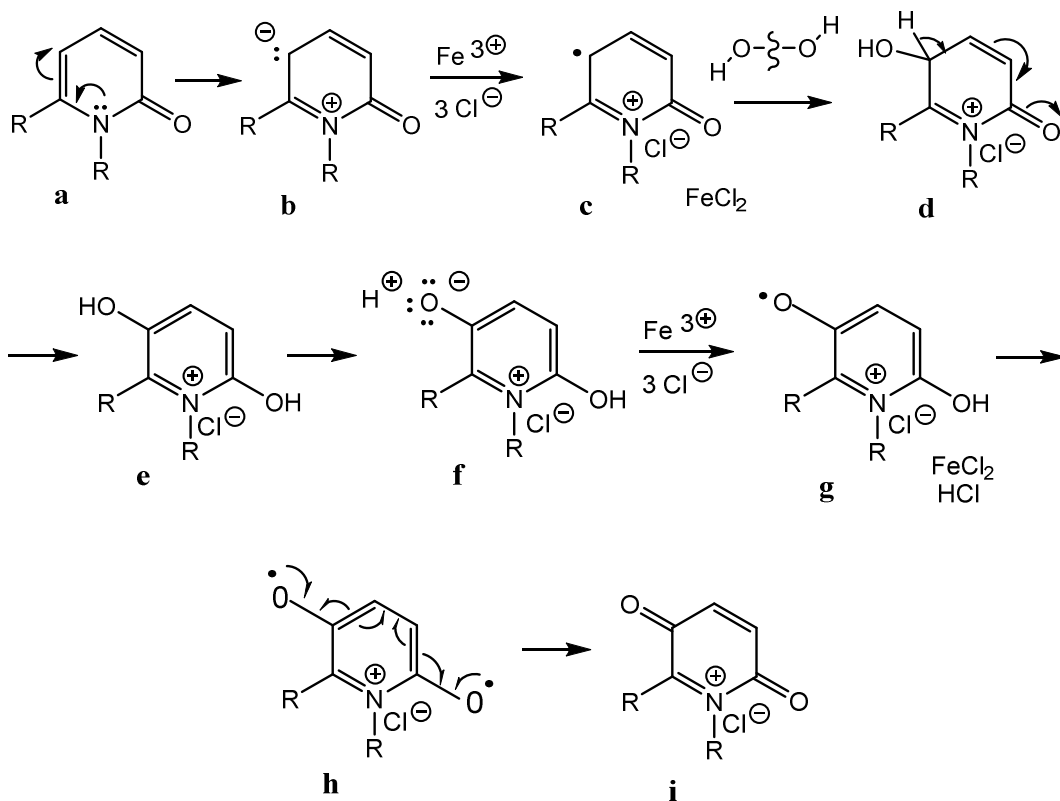
There are two recent patents for cytosine isolation, [18, 19]. This indicates the importance of the compound. The colour reaction for cytosine studied in this communication is due to J. van de Moer, who published it in the Netherlands, was reviewed in England, [20], and registered in the United States, [21]. Since the chemistry

of this test has not been described, we provide the reaction course, step by step, in the next section.

### 3. Discussion

Let us see the reaction sites in cytosine, especially in the 2-pyridinone ring. Besides the tautomeric phenol structure, 2-hydroxypyridine, there are contributing dipolar structures. We consider that the 2-pyridinone ring contains two halves: one with an electron donor enamine, and the other having an electron withdrawing system, an  $\alpha,\beta$ -unsaturated carbonyl group. Polarization of these two groups indicates the pertinent reaction sites (C-H functionalization). This is confirmed experimentally by the bromination of 2-pyridinone [22], and recently by the halogenation of cytosine, [23, 24].

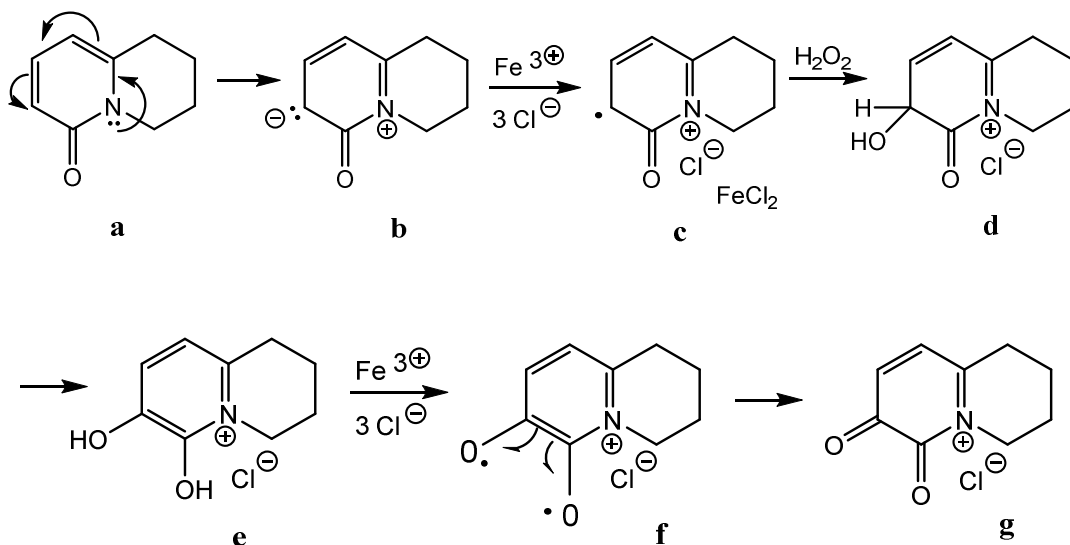
Thus, the colour reaction of cytosine with ferric chloride/hydrogen peroxide goes as follows, Figure 2.



**Figure 2.** Reaction of cytosine at five position.

Ferric chloride takes out one electron from C-5; besides the resulting free radical, ferrous chloride and the iminium chloride are formed, **a**, **b**, **c**. The free radical at C-5 induces homolytic fission of hydrogen peroxide. Ferric chloride can also activate or decompose hydrogen peroxide, [25, 26]. Combination of the resulting free radicals gives a hydroxy group at C-5, **d**. The hydrogen at C-5 is  $\gamma$ -to the  $\alpha,\beta$ -unsaturated system, thus prototropy occurs, affording a quinol, 2,5-dihydroxyiminium chloride, **e**. The acidic phenol groups are electron rich substrates for reaction with ferric chloride, **f**, **g**. So, oxygen radicals can be formed and a p-quinone is obtained, **h**, **i**.

Reaction at C-3 can be explained by the electrodotic [27] properties of the conjugated enamine, providing a negative charge  $\alpha$  to carbonyl, Figure 3.



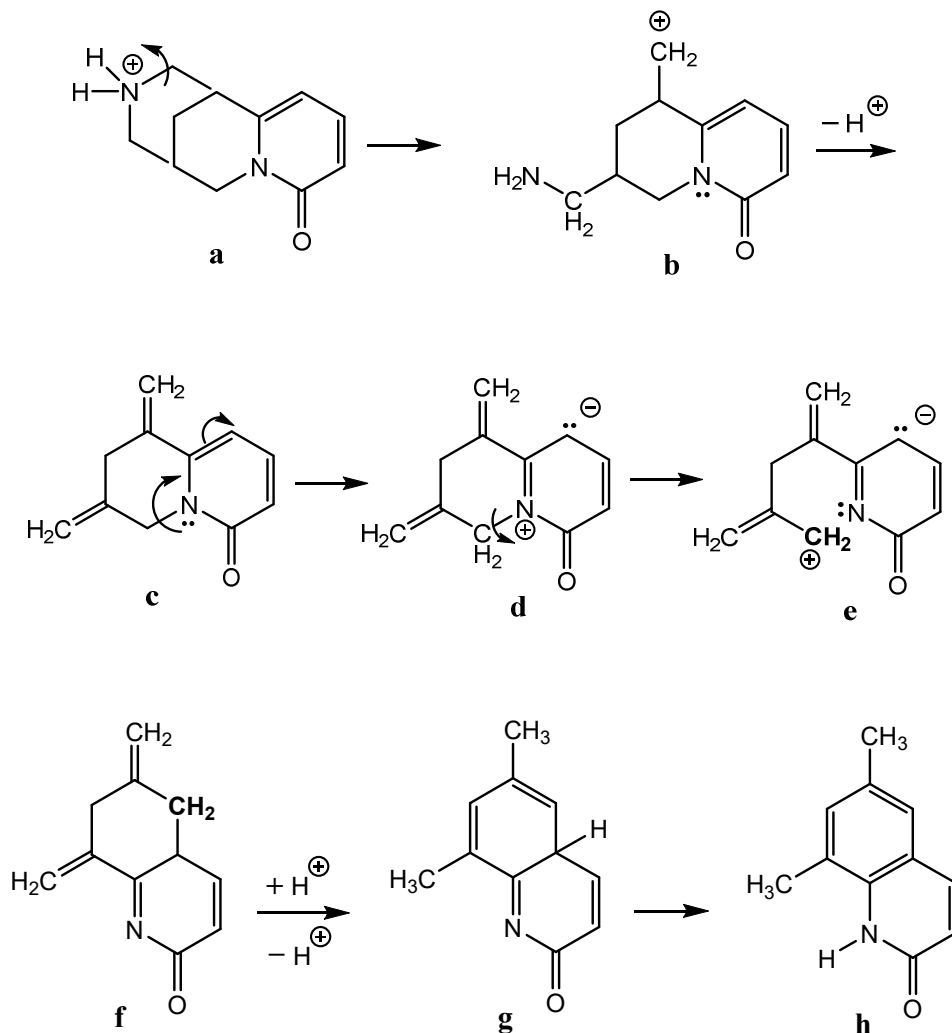
**Figure 3.** Reaction of cytosine at 3 position.

Reaction with ferric chloride affords the free electron that reacts with hydrogen peroxide, **b**, **c**, **d**. The transient alcohol isomerizes to an ortho diphenol, **e**. The electron shift (carbon acid) is favored besides by aromatization. Further oxidation via free radicals at oxygen gives an ortho quinone, **f**, **g**. This is the key to the red colour produced in the assay, since ortho quinones are red; for instance, o-benzoquinone [28], 1,2-naphthoquinone [29], o-toluquinone [30], and others.

Finally, the change from red to blue is explained by formation of a quinhydrone-type complex, that is, a hydrogen bonded complex with a network  $\text{C}=\text{O}\cdots\text{HO}$  hydrogen bonds, [31]. The 0.5% solution of ferric chloride and 0.05% hydrogen peroxide solution

are insufficient for complete quinone formation. If quinol remains, the corresponding green- blue or blue quinhydrone is formed.

The other point of interest is the degradation of cytisine to 6,8-dimethylquinoline since this involves a rearrangement from a quinolizidine structure, with a nitrogen common to both rings, to quinoline, with the nitrogen only in one ring, Figure 4.



**Figure 4.** Cytisine degradation by means of hydriodic acid.

First occurs the acid/base reaction of hydriodic acid with the piperidine nitrogen; the salt is broken by heat, a  $\text{CH}_2$  cation is formed and an exocyclic methylene results by

neutralization, **a**, **b**, **c**. The primary amine reacts similarly, ammonia is released, and a second exocyclic methylene is formed, **d**.

Polarization of the enamine in the 2-pyridone ring and high temperature produces ring opening and 1-3 migration via 180° rotation of the C6-R bond and a new ring closure, **e**, **f**. Acid catalyzed isomerization of the methylene groups gives two methyl groups and two double bonds, **g**. Protonation at nitrogen induces aromatization of ring B, **h**.

Finally, reduction of the carbonyl group by hydriodic acid affords 6,8-dimethylquinoline. The phosphorus reacts with the iodine formed in the oxidation of iodide ions.

#### 4. Conclusion

The chemistry of the test for cytosine due to J. van de Moer has been cleared up. It involves ferric chloride promoted free radical reactions which in combination with hydrogen peroxide produce quinols, a p-quinone and an o-quinone. The last one brings about the red colour observed in the test.

The colour change to blue comes from a quinhydrone-type complex. If there is incomplete oxidation, quinol remains and the hydrogen bonded complex is formed.

Finally, the reaction mechanism of cytosine degradation is provided. It involves ammonia elimination from the piperidine ring, exocyclic methylene groups and a 1-3-migration via ring opening and ring closure at different reaction site. Prototropy and reduction affords the final product, 6,8-dimethylquinoline.

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