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(RESEARCH ARTICLE)

The chemistry of Crismer's test for glucose in urine

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Abstract

The study of what is going on in a chemical test is important, the more if the involved substance is glucose in urine. In the Crismer's test the blood-red colour of safranin solution in alkaline medium is discharged if the glucose concentration is higher than 100 mg/dl. The reagent is not affected by uric acid or creatinine as is the case with the Fehling solution. The safranin test detects sugar in saliva when other reagents failed. Since the reaction mechanism of this test has not been advanced, we provide the electron flow, step by step.

Keywords: Glucose; Oxido-reduction; Phenazinium; Reaction mechanism; Safranin; Urine test

1. Introduction

Glucose is an analyte, thus the Crismer's test for glucose in urine is also important. The test is based on the reduction of the safranin red dye and the oxidation of glucose to the osone, as we will see, not to gluconic acid as in other tests. The blood-red colour of the reagent fades completely if there are higher levels of glucose than the normal range. The test is very sensitive and specific.

Safranin is a biological stain used in histology and cytology microscopy. It stains Gram negative bacteria, chromosomes, cartilage and mast cells.

This communication is a follow up of our studies on reaction mechanism, [1-5].

2. Antecedents

The test under study is due to the Belgian chemist Leon Crismer (1858-1944). He published his test in German journals [6] and was reviewed in Paris [7] and in America [8-10]. His test for glucose in urine is as follows: Heat to 60-65°C 5 ml of a 1:1000 aqueous solution of safranin with 1 ml urine and 2 ml of a 10% sodium hydroxide solution. The red blood solution is decolorized if urine contains more than 100 mg/dl of glucose. Uric acid and creatinine do not affect safranin as they do to Fehling test. The Crismer's test can be applied to saliva whereas other tests failed, [11].

The safranine colorants are bis-amino derivatives of N-phenylphenazonium salts and are used for dyeing cotton and silk, [12]. Phenazine is a dibenzo annulated pyrazine and the parent substance of various dyestuffs. 3,7-Diamino-2,8-dimethyl-5-phenylphenazin-5-ium is safranin O, is common in commerce, Figure 1.

There is a novel synthesis of dibenzophenazine derivatives using lead dichloride as catalyst, [13]. For general information on azine dyes see ref. [14]. There is a biographical sketch on Crismer, [15].

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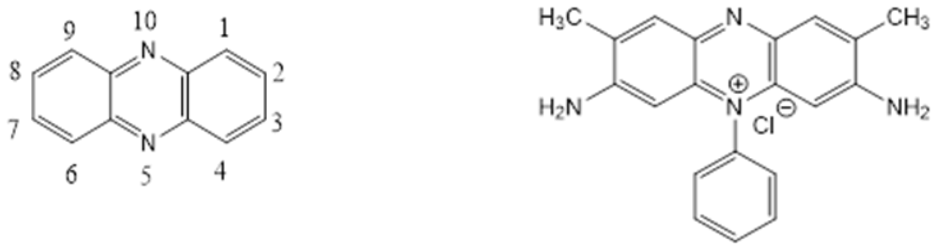

Figure 1 Phenazine and safranin O

3. Discussion

The acidic hydrogen at C-2 in glucose affords a carbanion in alkaline medium and is stabilized by resonance to the enolate (ambident ion). The reactive site in safranin is the electrophilic center at C-5a. Due to steric hindrance, addition of the terminal glucose-enolate to that position is the first oxido-reduction step. The branched carbanion does not react notwithstanding being more instable and by the same usually more reactive. Figure 2.

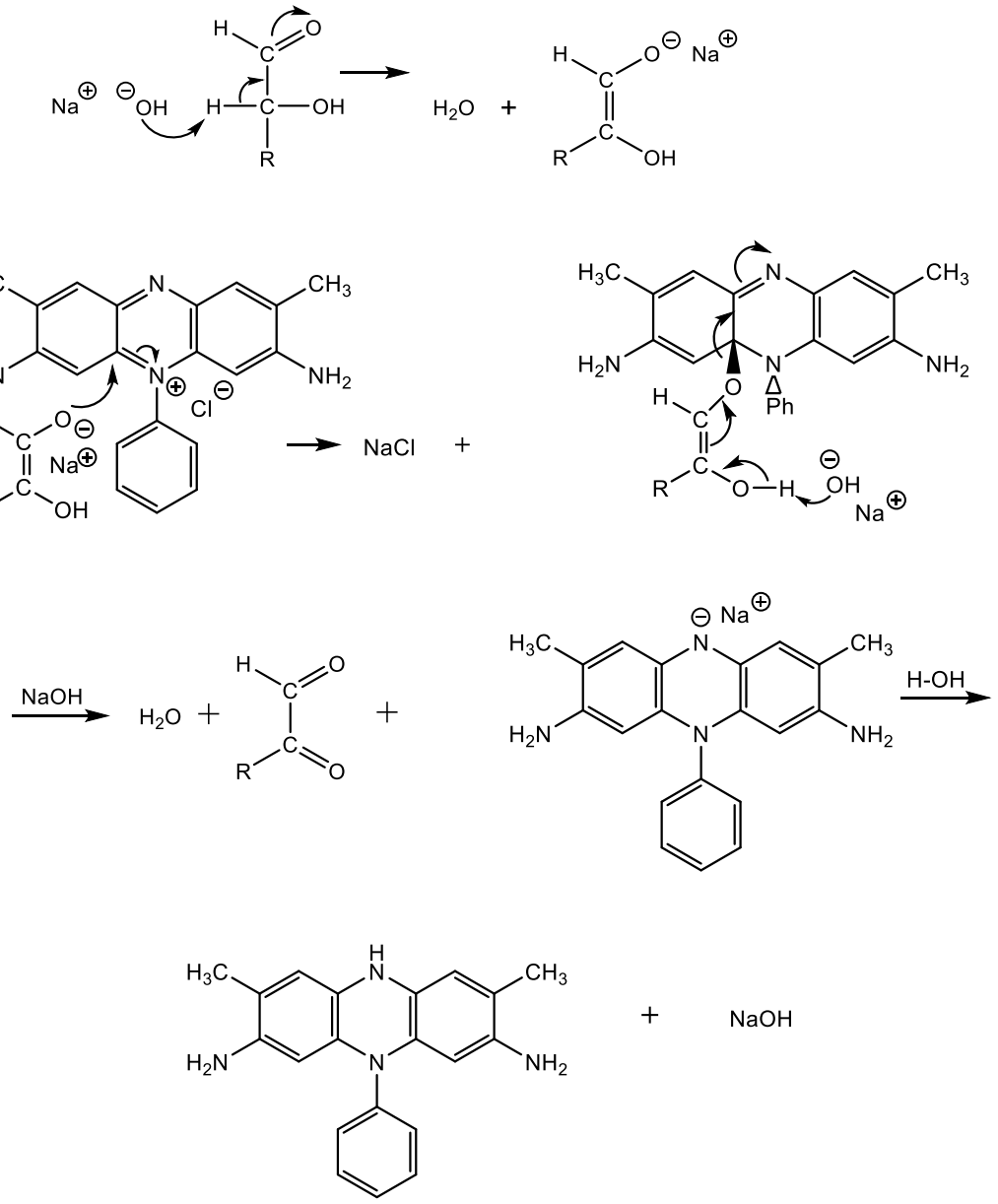

Figure 2 Mechanism of safranin reduction by glucose

As it can be seen, a second enol is present. The respective enolate gives rise to a concerted mechanism that yields a keto aldehyde, an aromatic ring and a nitrogen negatively charged (amidylidene group) which is neutralized by water. This second redox step eliminates the conjugation existing between the three rings and the red colour of safranin is discharged due to leucobase formation. The resulting 2-keto-D-glucose (D-glucosone) is readily hydrated.

The red colour returns on shaking the liquid due to air oxidation, thus abstain do this during testing. The mechanism of leucosafranin oxidation is due to the electrodotic effect [16] of the nitrogen at 5-position, transmitted through the vinylene bridge, and reaction with oxygen as hydride scavenger. This way the rings are connected by conjugation in an o-quinoneimine structure. Figure 3.

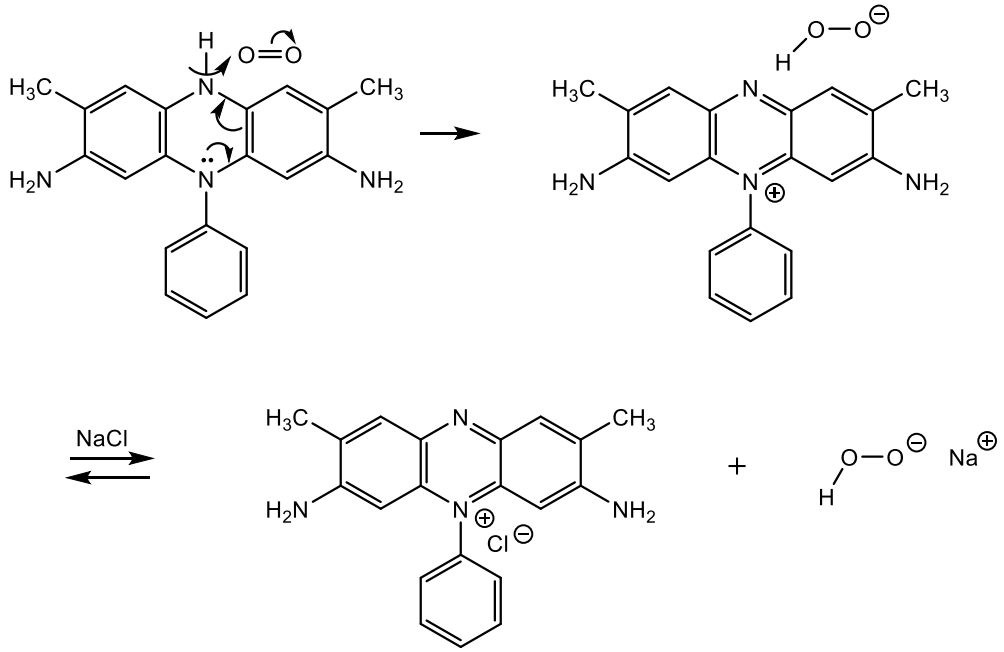

Figure 3 Mechanism of air oxidation of leucosafranin

Finally, a Friedmann reaction [17] occurs, which is explained as follows. The hydration of the osone precludes nucleophilic reaction at C-1. Thus, the hydroperoxide anion formed in the previous oxidation reacts with the keto group at C-2. The resulting alkoxide subtracts a proton from the near hydroxyl group at C-1. The new alkoxide could eliminate the ipso hydroxy group and form aldehyde, but the δ+ charge at the hydroperoxide is strong enough for deviate the reaction course. A concerted mechanism occurs and two carboxylic acids are formed via a C-C fission and OH ion elimination from the hydroperoxide. Figure 4.

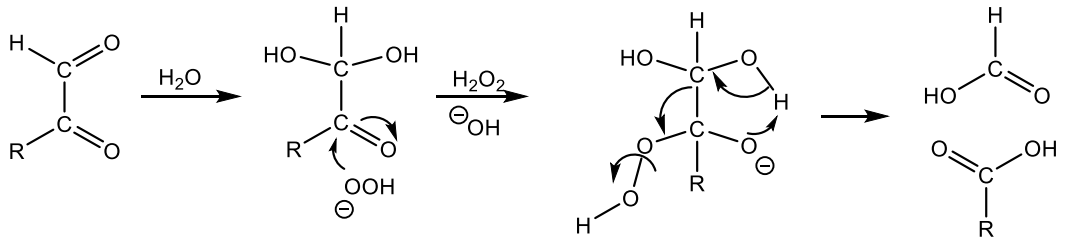

Figure 4 Friedmann degradation of D-glucosone

This reaction gives evidence of the electron repulsion of the unshared electrons in the two oxygen atoms of the H–O–O– group, causing some separation of the oxygen atoms and creating partial electric charges capable of contribute to C-C fission.

4. Conclusion

The red colour of safranin solution in alkaline medium is discharged due to reaction with glucose enolate yielding a condensation product (adduct), first redox step, followed by a second oxido-reduction which produces the leuco derivative.

Air oxidation can restore the red colour, so the test tube must not be shaken. The mechanism of both reactions has been provided.

If the red colour is restored on purpose, a Friedmann degradation takes place due to reaction of a hydroperoxide anion, formed in the oxidation, with glucosone from the previous reduction. This fission is explained by a concerted mechanism leading to formic and arabinonic acids.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There is no conflict of interest among the authors or any other person.

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