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A novel transamination reaction in a murexide-like sequence for caffeine detection

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Abstract

This communication is a theoretical organic chemistry study on the Hammarsten test for caffeine. He used chlorine water and ammonium hydroxide; a violet colour indicates presence of caffeine. Since a derivative of ammonium purpurate is formed, the assay has been considered a murexide test. However, there are several important variants. The original murexide test for uric acid employs diluted nitric acid; the five-member ring in uric acid molecule is an imidazolone whereas in caffeine it is an imidazole. This difference alters the reaction starting site. Uric acid has no substituents, caffeine presents three methyl groups. The methyl al N-7 is an impediment for purpuric acid formation since a primary amine is required in order to react with a carbonyl group and form a double bond. So, assisted ammonolysis is invoked since ammonium purpurate is formed. This chemical deportment is explained by reaction of the methylamine at N-7 with the very reactive central carbonyl group in alloxan. A concerted mechanism takes place: ammonia displaces the nitrogen of the hemiaminal, a nitrogen-carbon double bond is formed with concomitant separation of hydroxyl ion. The methylimino group at alloxan is hydrated and protonation of the carbinolamine restores alloxan molecule and separation of methylamine.

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1. Introduction

Caffeine is the active alkaloid found in coffee and black tea. Caffeine increases the activity of the brain and nervous system. It is found also in soft and energy drinks. A dose of 400 mg per day is acceptable. Caffeine levels per 250 ml are: instant coffee, 80-120 mg; black tea, 65-105 mg; cola drinks, 40-49 mg; energy drink, 160 mg; [1].

Caffeine is 1,3,7-trimethyl-2,6-dioxopurine or 1,3,7-trimethylxanthine. It exists in several crystalline forms (crystallographic polymorphism): it appears as white crystalline powder, white glistening needles, hexagonal prisms, or long flexible silky crystals, [2].

The coffee plant is an evergreen small tree. Its botanical name is Coffea (Genus), Rubiacea (Family). Coffee is original from Ethiopia and is now cultivated in various tropical regions. The coffee bean is the seed of the coffee fruit (coffee cherry). Most cherries contain two beans, [3].

In this communication we study the reaction route as well as the mechanism of Hammarsten test for caffeine.

This paper is a follow up of our studies on reaction mechanism, [4-8].

2. Antecedents

Caffeine has been identified as follows: dissolve 10 mg in 1 ml of concentrated hydrochloric acid, 100 mg of potassium chlorate and evaporate to dryness; a reddish-brown spot is formed which changes to purple with ammonia vapor, [9].

A close test is that of Hammarsten, he used chlorine water and ammonia; a violet colour indicates presence of caffeine, [10].

These tests are experimental variants of the murexide reaction [11], in which a few centigrams of uric acid and some drops of slightly diluted nitric acid are evaporated to dryness in a small porcelain basin. When ammonia is added to the residue an intense purple colour is produced (ammonium purpurate).

There is a mixed procedure for alloxanthin preparation that uses uric acid (murexide reaction) and hydrochloric acid/potassium chlorate (as in the test for caffeine), instead of diluted nitric acid. After sulfhydration, elemental sulphur is separated, and alloxanthin is obtained as white crystals, [12]. This preparation has an extra step, the redox reaction with hydrogen sulphide, and is used for the partial reduction of alloxan to dialuric acid.

These tests for caffeine have not been studied previously, and since there are different oxidizers and different substrate it is pertinent to look at how these differences alter the chemical deportment. In uric acid the five-member ring is an imidazolone, whereas in caffeine it is an imidazole. Uric acid has not substituents, in the other hand caffeine has three methyl groups. The methyl group at seven-position will give a methylamino group after ring opening, but for the synthesis of purpuric acid a primary amine is required, that is, formation of uramil (5-aminobarbituric acid). So, this reactive intermediate must be formed in a very different way, as we shall see. Figure 1.

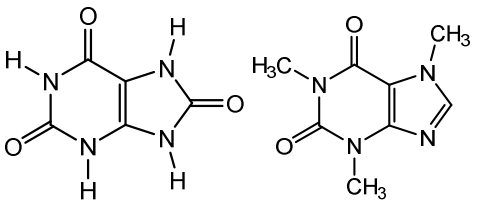


Figure 1. Uric acid and caffeine structures.

Finally, the obtained purpuric acid will have four extra methyl groups, and the added ammonia will not only form an ammonium salt, but it is a key reagent as is disclosed in the next section.

3. Discussion

Purpuric acid can be formed by condensation of uramil (5-aminobarbituric acid) with alloxan (5-oxobarbituric acid). Thus, the two barbituric acid moieties are connected by a nitrogen atom, and its name derives from it: 5,5'-nitrilo-dibarbituric acid, [13, 14]. Figure 2. The purple or violet colour observed is due to ammonium purpurate formation.

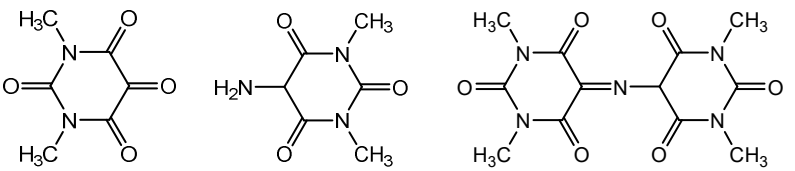


Figure 2. Formation reaction of tetramethyl purpuric acid.

Now let us see the reactions that occur during Hammarsten test for caffeine. Acid catalyzed hydration of the imino group in the five-member ring forms a carbinolamine whose acidolysis produces ring opening and a substituted formamido chain.

Hydration of the C=C double bond in the pyrimidine ring affords a hemiaminal that losses ammonia by acidolysis, and a carbonyl group is formed at C-4, Figure 3.

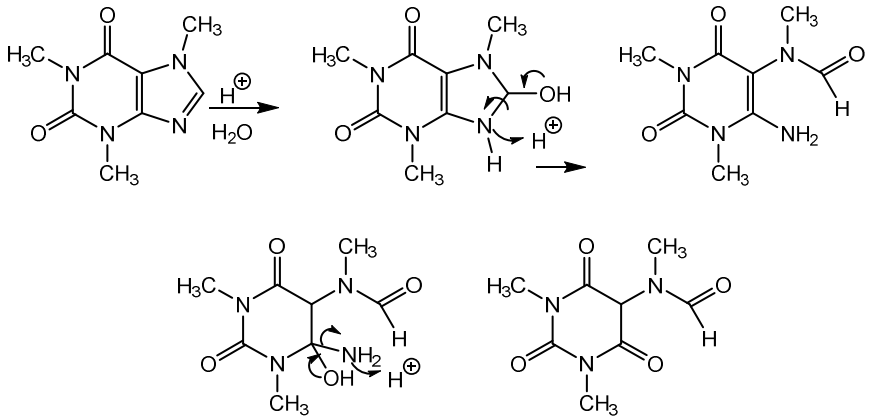


Figure 3. Ring opening and formation of a trioxo-pyrimidine.

Subsequent hydrolysis of the amide occurs by protonation of the dipolar form, water addition, and hydrion shift to nitrogen, yields a methylamino group, that is, 1,3-dimethyl-5-methylaminobarbituric acid, and formic acid. Compare [15, 16]. Figure 4.

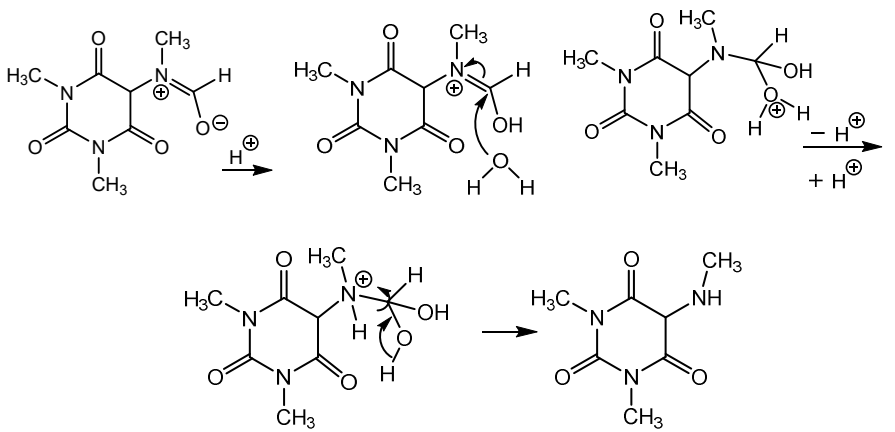


Figure 4. Hydrolysis of the formamido chain via zwitter ion and hydrion shift.

This sequence is different to that occurring in the murexide reaction, [17].

1,3-Dimethylalloxan is obtained by oxidation of the 5-methylamino group in the above barbiturate by means of chlorine water. The N-chloramine is formed by reaction with protonated hypochlorous acid, a positive-halogen releasing species, [18-20],

followed by elimination of a negative chloride ion (oxidation step), in an assisted dehydrohalogenation. The methylimine is hydrated and elimination of methylamine originates a carbonyl at C-5, (1,3-dimethylalloxan). Figure 5.

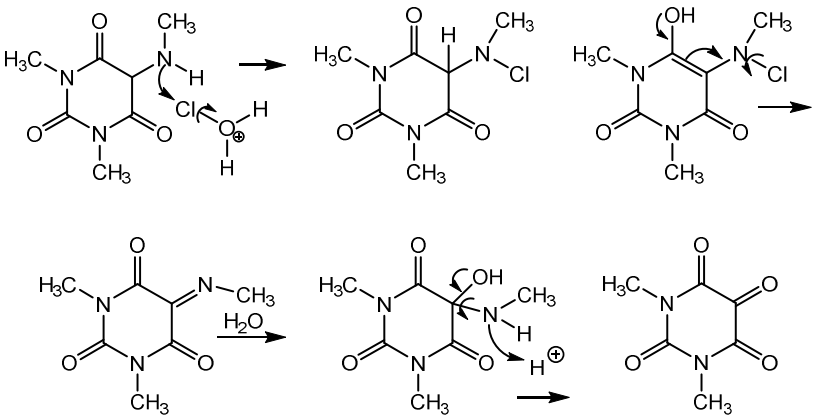
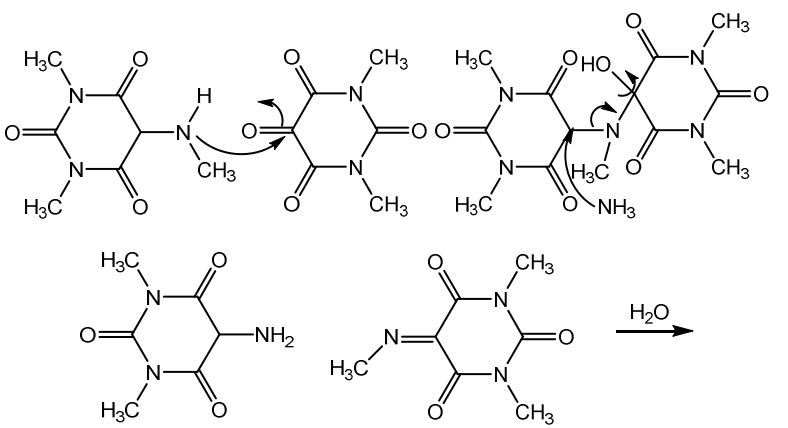


Figure 5. N-chlorination, dehydrohalogenation, imine hydrolysis and deamination.

Finally comes the indispensable formation of 5-amino-1,3-dimethylbarbituric acid (1,3-dimethyluramil). Reaction of the above cited 1,3-dimethyl-5-methylamino-barbituric acid with alloxan yields a hemiaminal that favors an ammonolysis via a concerted mechanism: 5-aminobarbituric acid is formed, and a nitrogen-carbon double bond results in the other ring, whose hydrolysis and elimination of methylamine restores the carbonyl system in alloxan molecule. There has been assisted ammonolysis with concomitant transamination. Figure 6.



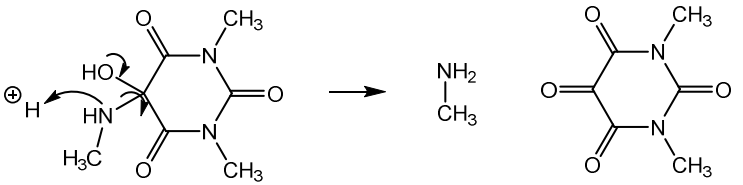


Figure 6. Ammonolysis and transamination. Amine elimination.

This novel transamination reaction makes use of the very reactive central carbonyl in alloxan, instead of an α-keto acid and an enzyme.

This is a direct route to 1,3,1',3'-tetramethylpurpuric acid whose ammonium salt derives from the enolic form. The C=C double bond is conjugated with the double bond in the 5,5'-nitrilo, and with cross conjugation with other three carbonyl groups.

4. Conclusion

The primary amine in uramil (5-aminobarbituric acid) is indispensable in order to synthesize purpuric acid. However, the degradation of the imidazole ring in caffeine yields a methylamine at C-5 which is an impediment for the formation of the 5,5'-nitrilo in di-barbituric acid. Since ammonium purpurate is formed, there must be a way to uramil or a derivative substituted in the ring. It has been found that reaction of 5-methylamino-barbituric acid with alloxan yields a hemiaminal that favors ammonolysis and a transamination, producing 1,3-dimethyl-uramil.

5. Conflicts of Interest

There are no conflicts to declare.

6. Acknowledgement

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