Summary

1. The action of ferrous salts on pyrimidines is described under conditions similar to those found in biological processes.

2. The reagents used were: (a) $FeSO_4.7H_2O + NaHCO_3 +$ air; (b) $[Fe(CN)_5OH_2]Na_3 + O_2$ or air; (c) $FeSO_4.7H_2O + O_2$; (d) $FeSO_4.7H_2O + H_2O_2$.

3. The cleavage may be considered to take place in two steps: (a) partial hydrolysis and oxidation with the formation of intermediate compounds; (b) complete hydrolysis of the intermediate compounds.

The extent of cleavage is determined by a quantitative estimation of the urea formed. In the case of thymine, acetol and pyruvic acid could be identified as reaction products, a fact which confirms the work of Johnson and Baudisch.[4]

4. It was shown experimentally that the reactions described are brought about by means of auxiliary valences of the ferrous compounds and pyrimidines. The proof was obtained by: (a) occupation (poisoning) of the auxiliary valences of the iron nucleus by sodium hydroxide, potassium cyanide, ammonia or pyrimidines; this poisoning in most cases was indicated by accompanying color reactions; (b) hydrogenation of the pyrimidine ring; (c) stabilization of the pyrimidine ring by a methyl group in Position 5.

5. The relatively greater efficiency of the complex ferrous salt as compared with an ordinary ferrous salt ($FeSO_4.7H_2O$) has been shown.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

THE OXIDATION OF URIC ACID WITH FERROUS SALTS. I

BY MIMOSA HORTENSE PFALTZ

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Introduction.—It was desired to determine the behavior of complex iron salts in the presence of oxygen toward uric acid, the most important member of the purine group. Like the pyrimidines, this compound is of great biological significance, being a product of nuclear metabolism, as well as being further oxidized in the animal body. This oxidation, whatever its nature, takes place under mild conditions (weak alkali and body temperature) and if uric acid could be oxidized in the laboratory under similar conditions, the identification of the products of oxidation might throw some light on the mechanism of uric acid metabolism. With this idea in mind the present investigations were undertaken.

As it has already been shown that pyrimidines can be broken down in the presence of these same complex salts,[1] these investigations were carried out in a manner analogous to those on the pyrimidines.

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[1] Pfaltz and Baudisch, THIS JOURNAL, **45**, 2972 (1923).

The Action of Ferrous Sulfate Plus Sodium Bicarbonate Plus Air on Uric Acid.—The system, ferrous sulfate plus sodium bicarbonate plus air, effects a partial decomposition of the purine molecule and it was found as with pyrimidines[1] that it is necessary to heat the oxidized solution on the water-bath before the formation of urea takes place. To a solution of 0.5 g. uric acid in 1000 cc. of water containing 36 g. of sodium bicarbonate (12 molecular equivalents) was added 10 g. of ferrous sulfate and the reaction mixture shaken until the ferrous carbonate peroxide was oxidized to ferric hydroxide. A determination of urea directly after oxidation gave no trace of urea, but after the heating on the water-bath, 29% of urea was formed.

The Action of Sodium Pentacyano-aquo-ferroate Plus Oxygen or Air on Uric Acid.—The experiments for determining the action of the system sodium pentacyano-aquo-ferroate plus oxygen or air were carried out in exactly the same way as those with pyrimidines,[1] with the exception that the uric acid was added to the oxidation mixture as a solid instead of as a solution. As the oxidation proceeds the solution of aquo salt gradually turns red, thereby indicating that a loose combination is formed between the auto-oxidizing agent and the uric acid. This shows that an activation of uric acid takes place, and as it has been shown already that activated oxygen is present in a solution of aquo salt,[2] it is safe to assume here also, that the oxidation is brought about by the interaction of activated oxygen and activated uric acid.

At the same time it was observed that the ammine salt brings about a greater cleavage of uric acid than the aquo salt when both are used under the same conditions. The reason undoubtedly is that the liberated ammonia reacts with the suspended uric acid to form an ammonium salt of uric acid, thereby increasing the concentration of uric acid in solution, thus accelerating the oxidation. These results are indicated by the fact that when 0.100 g. of uric acid and 0.30 g. of auto-oxidizing agent were used, and the solutions oxidized by means of a stream of oxygen for 21 hours, the aquo salt gave a break of 57.7% and an ammine salt of 60.6%.

Very little speculation can be made concerning the mechanism of the reaction between the aquo or ammine salt and uric acid, except the supposition that again an intermediate product is formed before cleavage to urea takes place. However, this intermediate compound is considerably more unstable than the corresponding intermediate product formed when uracil or thymine are oxidized. This is shown by the fact that evaporation of the neutral oxidized mixture brings already a cleavage with a yield of about 50% of urea, while heating after the addition of sodium hydrogen carbonate brings about a yield of urea which is in the neighborhood of 75%.

When 0.100 g. of uric acid and 0.30 g. of aquo salt were subjected to a

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[2] Baudisch, *Ber.*, **54,** 413 (1921).

stream of oxygen for 86 hours, direct evaporation of the water gave 46.9% of urea and evaporation in presence of sodium bicarbonate gave 71.5% of urea.

The great susceptibility of the uric acid molecule to the influence of the aquo and ammine salts is shown in two ways. First, uric acid can be extensively oxidized by atmospheric oxygen alone, provided the aquo or ammine salts are used as the auto-oxidizing agents and second, the large yields of urea obtained indicate that the uric acid molecule was more completely decomposed than was the case with the pyrimidines. However, the exact role of the ammine and aquo salt is not known, since the yields of urea are in no way proportional to the amount of salt used.

When 0.75 molecular equivalent of aquo salt is used per equivalent of uric acid (namely 0.30 g. of aquo salt per 0.10 g. of uric acid) all of the uric acid ultimately goes into solution during oxidation, and a yield of approximately 75% of urea is obtained. By increasing the relative amount of auto-oxidizing agent, or by increasing the time of oxidation, it was hoped that the yields of urea could be increased. However, by neither of these means was this possible; in fact, it was observed that a great excess of aquo salt tends to decrease the yield of urea.

In each experiment 0.100 g. of uric acid and 200 cc. of water were used and the solutions oxidized by shaking with oxygen under pressure.

Table I

Percentage Decomposition with Different Amounts of Aquo Salt

Aquo salt, g.	0.30	0.40	0.50	1.00
Time of oxidation	23 h.	48 h.	7.5 h.	44.5 h.
Urea formed, %	73.3	75.9	75.1	53.3

These results indicate that the reaction may be catalytic in nature. In this case very much smaller amounts of aquo salt should bring about a relatively large cleavage of uric acid, and this is indeed found to be the case. When amounts of aquo salt are used which correspond to $^1/_4$ and $^1/_{40}$ the amount necessary for a reaction taking place molecule per molecule, values of urea are obtained which are much greater than can result from a reaction taking place in equimolecular proportions. In fact, no stoichiometric relationship whatsoever can be seen between the amounts of aquo salt used and the urea formed.

Nevertheless, all of the uric acid cannot be brought into solution by these small amounts of aquo salt, and thus the completion of reaction is still dependent to some extent on the amounts of auto-oxidizing agent used.

These results find no analogy in the behavior of pyrimidines. For instance, if uracil is oxidized with amounts of aquo salt which correspond to $^1/_6$ and $^1/_{60}$ the amount necessary for a reaction taking place in equimolecular proportions, the respective amounts of urea obtained are considerably less than $^1/_6$ and $^1/_{60}$ of the calculated yield.

TABLE II

COMPARISON OF OXIDATION OF URIC ACID AND URACIL WITH SMALL QUANTITIES OF AQUO SALT

In each experiment 0.200 g. of uracil or 0.100 g. of uric acid and 200 cc. of water were used and the solutions oxidized by shaking with oxygen under pressure for 21.5 hours.

	1	2	3	4
Material used	0.2 g. uracil	0.2 g. uracil	0.1 g. uric acid	0.1 g. uric acid
Amt. of aquo salt, g.	0.10	0.01	0.10	0.01
Frac. of calc. amt.	$^1/_6$	$^1/_{60}$	$^1/_4$	$^1/_{40}$
Urea formed, %	6.4	0.28	47.3	16.7

The behavior of uric acid and aquo salt indicates the possibility that we are dealing with a case of true catalysis, that is, a molecule of aquo salt is able to function more than once in the activation of oxygen and uric acid. Ultimately, however, the delicate aquo salt molecule must break down under the strong oxidizing influence of pure oxygen under pressure, thus preventing its catalytic effect from continuing.

The Action of the System, Ferrous Sulfate Plus Oxygen.—It was found that the system, ferrous sulfate plus oxygen, is not able to bring about a cleavage of the uric acid molecule.

Experimental Part

Apparatus.—The same types of apparatus were used as have been previously described.[1]

Analytical Methods.—The urea was determined quantitatively by the method of Fosse[3] using the precautions mentioned in a preceding article.[1] Calculations are based on the assumption that each molecule of uric acid can break down to form two molecules of urea.

Experiments with Ferrous Carbonate Peroxide.—The uric acid was dissolved in aqueous sodium bicarbonate solution, to which was then added solid ferrous sulfate. No attempts were made to precipitate the ferrous carbonate in the absence of air. Urea determinations were made on aliquot portions of the clear solution.

Experiments with Sodium Pentacyano-aquo-ferroate.—A weighed portion of uric acid was added to an aqueous solution of the complex salt and the resulting suspension oxidized either by a stream of oxygen or by oxygen under a pressure slightly greater than one atmosphere. When oxidation was discontinued the suspended uric acid had always completely dissolved with the exception of Expts. 3 and 4, Table II.

Experiments with Ferrous Sulfate Plus Oxygen.—A weighed amount of uric acid was added to an aqueous solution of ferrous sulfate containing two molecular equivalents of ferrous sulfate per equivalent of uric acid. Oxidation was attempted by using oxygen under a pressure slightly greater than one atmosphere.

[3] Fosse, *Ann. chim.*, [9] **6**, 13 (1916).

The author wishes to express her appreciation to Professor Treat B. Johnson and to Dr. Oskar Baudisch for their suggestions and criticisms during the progress of this work.

Summary

1. The mild conditions under which uric acid is oxidized are emphasized.
2. The extent of cleavage of uric acid by means of complex iron salts is shown.
3. No mechanism is suggested, but it is demonstrated that an intermediate compound must be formed because the very sensitive xanthydrol test is obtained only after the oxidation mixture has been heated on the water-bath.
4. Experiments show that the oxidation of uric acid by means of $[Fe(CN)_5H_2O]Na_3$ may be catalytic in nature, and brought about by means of auxiliary valences. The latter is proved by the formation of a brilliant red solution during oxidation.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF PRESIDENCY COLLEGE, CALCUTTA]

CONDENSATIONS OF ALDEHYDES WITH RESORCINOL AND SOME OTHER AROMATIC HYDROXY COMPOUNDS

BY RAJENDRA NATH SEN AND NRIPENDRA NATH SINHA

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The condensation of aldehydes with various aromatic hydroxy compounds has been previously studied by several investigators.[1] A close study of these condensations brings out certain facts of great importance, as follows.

1. Though the modes in which these various condensations took place and the natures of the resulting products were different in many cases, yet in a few cases only two molecules of hydroxy compounds reacted with one molecule of the aldehyde, forming the pyrone ring with the elimination of water by the interaction of two hydroxyl groups in the *ortho* position to the reacting hydrogen atoms. The mode in which these condensations were carried out by the previous workers may be generally represented either as Type 1[1f] or Type 2[1e] (R is a phenyl residue).

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[1] (a) Michael, *Am. Chem. J.*, **5**, 339 (1883). (b) Causse, *Bull. soc. chim.*, [3] **3**, 861 (1890). (c) Hewitt and Pope, *Ber.*, **29**, 2824 (1896). (d) Michael, *J. prakt. Chem.*, **165**, 334 (1898). (e) Weidel and Wenzel, *Monatsh.*, **21**, 61 (1900). (f) Pope and Howard, *J. Chem. Soc.*, **78**, 1023 (1910). (g) Michael and Comey, *Am. Chem. J.*, **5**, 349 (1883). (h) Liebermann and Lindenbaum, *Ber.*, **37**, 2728 (1904). (i) Fischer and Jennings, *Ber.*, **27**, 1355 (1894). (j) Hofmann, *Ber.*, **26**, 1139 (1893). (k) Zenoni, *Gazz. chim. ital.*, **23**, II, 215 (1893). (e) Trzcinski, *Ber.*, **17**, 499 (1884). (m) Möhlau and Koch, *Ber.*, **27**, 2887 (1894).