REACTION OF KÖJIC ACID WITH SILVER OXIDE

by

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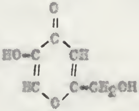
INTRODUCTION

Köjic acid, 2-hydroxymethyl-5-hydroxy-γ-pyrone, according to Barham and Smits (1), is a crystalline compound which may be prepared by fermentation methods from polysaccharides, disaccharides, pentoses, hexoses, sugar alcohols, and organic acids. These authors state that various species of molds, chiefly those of the Aspergillus-oryzae-flavus group, have been used for this purpose. Glucose and xylose give good yields of the compound, and since these substances can be produced in large quantities from farm wastes, a cheap source for the preparation of köjic acid is assured. At present, it cannot be stated that either köjic acid or its derivatives have definite uses, but an examination of its structure suggests that a compound containing so many reactive groups might be expected to find uses in the future.



Köjic acid

Yabuta (2), in determining the structure of köjic acid, attempted to convert it into comenic acid by direct oxidation but failed because of the instability of the pyrone nucleus. His efforts to oxidize monobenzoyl köjic acid with potassium permanganate in acetone solution caused fragmentation. The monoethyl ether of köjic acid and the corresponding pyridone he found likewise could not be oxidized to give comenic acid derivatives. Yabuta (3) noted that with potassium permanganate in alkaline or alkali carbonate solution, köjic acid is fragmented, giving oxalic acid as one of the products. Birkinshaw and Raistrick (4) found that an alkaline iodine solution reacts quantitatively with one mole of köjic acid, forming one mole each of iodoform, oxalic acid, formic acid, and glycollic acid.

Some derivatives of köjic acid have been successfully oxidized to give derivatives of comenic acid. Yabuta and Kambe (5) oxidized 2-hydroxymethyl-5-methoxy-γ-pyrone in acetone solution with potassium permanganate to form the monomethyl ether of comenic acid. They also oxidized 2-hydroxymethyl-5-methoxy-γ-pyridone in aqueous solution with potassium permanganate to yield the methyl ether of comenamic acid. Armit and Nolan (6) were able to accomplish the latter oxidation by the use of nitric acid.

The primary purpose of this investigation is the oxidation of köjic acid in such a manner that the compound is not fragmented, the pyrone nucleus being retained intact.

EXPERIMENTAL WORK

Preparation of Reagents

Silver oxide was selected as the oxidizing agent. This reagent offers certain advantages; namely, (a) ease and uniformity of preparation, (b) ease of removal of silver oxide and precipitated silver at the conclusion of the reaction, (c) weaker oxidizing powers than other reagents. Whitmore (7) states that silver oxide, unlike potassium permanganate, does not attack olefinic linkages. Yabuta (8) noted that köjic acid is oxidized by moist silver oxide but made no study of the reaction involved.

The silver oxide was prepared by a method outlined by Evans et al. (9). A solution of 400 grams of silver nitrate in 1200 c.c. of distilled water was well stirred, and 150 grams of potassium hydroxide or an equivalent weight of sodium hydroxide dissolved in 800 c.c. of water was added at the rate of 100 c.c. per minute. The precipitate was washed by decantation until the wash water was free from silver ions and a 100 c.c. sample required less than 0.3 c.c. of 0.1N HCl to neutralize excess alkali (about ten washings). The silver oxide was used in the moist condition or was dried at 110-115° C. in an electric oven, the color changing from a chocolate brown to a dark purple-brown. The material was passed through a 100-mesh sieve, put in brown bottles, and stored away from light. The activity of the oxide remains unimpaired under these conditions, even after several months of storage.

The acetone, used as a solvent in oxidations conducted in inert solvents, was purified by distillation from over potassium permanganate crystals, drying over potassium carbonate, and by redistillation. Dioxane was dried first over calcium chloride, then over sodium ribbon, and was finally redistilled.

Method of Procedure

Oxidation in Aqueous Solvents. Various amounts of köjic acid were dissolved in distilled water in Erlenmeyer flasks, and one, two, three, or four equivalents of silver oxide added. The solutions were agitated by hand occasionally, or were sealed in bottles and placed in a shaking machine for periods ranging from 1-18 days, at room temperature. The initial action involved a rapid deepening in color of the solution, accompanied by the gradual formation of a silver plate in thirty minutes or less. The deposition of silver apparently reached a maximum at the end of twenty-four hours, but the color of the solutions gradually darkened to a deep red-brown by reflected light and a dark green in transmitted light. The higher concentrations of silver oxide (three and four equivalents) gave a deeper coloration and more silver plate formation than those solutions with a lower silver oxide content, during an equal time interval. Heating the solutions initially to 40-50° C. caused deposition of a silver plate in less than ten minutes and a correspondingly rapid increase in color in the solution. Carbon dioxide was also evolved in measurable amounts at this temperature, as evidenced by reaction with barium hydroxide solution. Moreover, a considerable pressure was released when the sealed bottles mentioned above were opened - apparently due to the escape of an odorless gas. A test was performed in which 3.55

grams of köjic acid were dissolved in CO_2-free water, three equivalents of silver oxide added, and the reaction vessel connected by glass tubing with a tube containing barium hydroxide solution. External air was excluded. At a temperature of 20-25° C., some barium carbonate had been precipitated at the end of twenty-four hours, and this amount increased gradually during a ten-day period. The precipitate effervesced strongly when treated with HCl. No attempt was made to determine the actual amount of carbon dioxide evolved.

All solutions after oxidation were filtered or centrifuged to rid them of undissolved solids, and the filtrates were evaporated isothermally in a current of air. The dark red-brown residues were of a sticky, resinous nature, and were moderately soluble in alcohol, acetone, and dioxane, but scarcely soluble in a number of other organic solvents. By long exposure the residues could be dried to brittle, flaky, brown-to-black materials which either melted over a wide range or decomposed without melting at elevated temperatures.

Extraction of the residues in a Soxhlet apparatus was attempted with ethyl acetate, dioxane, acetone, chloroform and ether, of which the first three gave highly-colored solutions and small amounts of yellow-orange, amorphous solids. These solids did not crystallize readily from the various solvents used. By extraction with ether, köjic acid was recovered and identified by the method of mixed melting points. The residues from the extraction thimbles were amorphous substances which melted roughly at 130° C., but they were too minute in amount to test further.

Various tests conducted on both the original oxidized solutions and the residues remaining after evaporation failed to show the presence of formic acid, oxalic acid, acetic acid, acetone, or any of the products commonly associated with a decomposition of the pyrone nucleus. None of the reactions typical of aldehydes was given.

Since oxidation in aqueous solution had apparently yielded negative results, insofar as the primary purpose of the investigation was concerned, attention was then directed toward oxidation in inert solvents.

Oxidation in Inert Solvents. Köjic acid was dissolved in dioxane or acetone, two or three equivalents of dry silver oxide added, and the solutions agitated mechanically at room temperature for 10-18 days, or heated under reflux for 18-40 hours. Deposition of silver is neither as rapid nor as abundant as with the solvent water, and the color of the solutions does not become so intense. Dioxane solutions appear to give upon refluxing a deeper color than do acetone solutions during the same time interval, possibly because of the higher temperature employed in the former case. Heating the solutions for a period of fifteen hours gives a depth of color greater than that obtained by agitation at room temperature for eighteen days.

The solutions after filtration were evaporated isothermally in a current of air, or the solvent was removed by distillation on a water-bath under reduced pressure to avoid possible thermal decomposition. The granular, orange-to-brown residues obtained were unlike the resinous substances secured by oxidation in aqueous solution, although addition of water to the former appeared to lead to formation of products of the latter type.

Extraction of the granular residues with ether, chloroform, or dioxane gave köjic acid contaminated with traces of another substance which melted in a range of 120-130° C., but could not be purified and secured in amounts sufficiently large for its identification. Unused köjic acid was identified by the method of mixed melting points. The residues, after extraction, were amorphous, dark-brown in color, decomposed at a temperature above the melting point of köjic acid, and did not crystallize from the various solvents tried.

In subsequent work, the expedient was adopted of adding absolute ether to the original dioxane or acetone oxidation mixture after removing the silver oxide-silver precipitate. By this procedure a flocculent, yellow-orange solid is precipitated in small amounts. Seven and one-tenth grams of köjic acid upon oxidation and subsequent extraction with ether yielded 0.6 gram. Chloroform, carbon tetrachloride, or benzene will also cause precipitation to occur. If the agent used is not water-free, the precipitate soon darkens and becomes sticky, adhering to the sides of the vessel.

The solid product is filtered off on a sintered glass crucible with suction. Its direct exposure to air must be prevented, or the still moist solid becomes dark and resinous in nature. Small portions of ether are added to wash the precipitate, which is kept covered with liquid during this stage. The last portion of the wash liquor is removed by placing the crucible in a vacuum desiccator over concentrated sulfuric acid, in an ether atmosphere, for several days. After this treatment the solid is stable unless it comes in contact with water, when a deep red color develops and some resinification occurs.

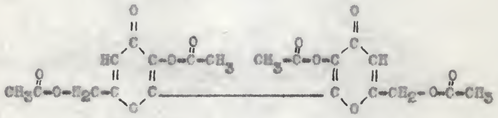
The residues which remain after extraction by ether of the granular, orange-colored substances from oxidations in inert solvents can be redissolved in acetone and dioxane. The resultant solutions then give by addition of ether the same type of unstable substance noted above.

Chemical Nature of the Solid Product. The substance has not been isolated in a crystalline form. It decomposes above 220° C. and is readily soluble in absolute alcohol, dioxane, or acetone, from each of which it can be reprecipitated by ether. In water it dissolves slowly, the solvent action seemingly being retarded by the preliminary resinification which results on contact, to give solutions which are acid to litmus and which impart a dull red color to ferric chloride solution. Strong mineral acids or alkalis give deep red solutions with apparent decomposition.

No coloration results with the unknown and Schiff's aldehyde reagent. With 2, 4-di-nitro-phenylhydrazine, a red-brown amorphous substance is formed which does not crystallize and which decomposes without melting considerably above 200° C. A test for the presence of peroxides by the use of potassium iodide solution acidified with acetic acid liberates no free iodine. An attempt to prepare the acetate by the use of acetic anhydride leads to the formation of resinous substances from which no crystalline product has been isolated.

After filtration of the unstable product, partial evaporation of the ether-dioxane filtrate in one experiment resulted in the precipitation of a scaly, light-brown material. This was filtered, washed with ether, and dried. The yield was 0.6 gram from 7.1 grams of köjic acid originally used. The material melted with decomposition at >220° C., turned deep red in contact with water, and gave a red color with ferric chloride. It seemed to be entirely stable, even in a moist condition, when exposed to air.

The substance reacted with acetic anhydride to give an orange-colored, crystalline compound which, after recrystallization from 95 per cent alcohol, melted at 160.6-162° C. (corr.) and gave no color, either in alcohol or aqueous solution, with ferric chloride. A determination of the acetyl number did not give very clean-cut results, due largely to the fact that refluxing was necessary to cause complete hydrolysis. This action resulted in the formation of orange-colored solutions and thus masked the action of the indicator. The regenerated nuclear hydroxyl groups also were probably reacting with excess alkali and this action would necessitate a correction dependent on the number of such groups present. If the compound is of the type shown below, then calculation gives a value for the acetyl groups, $CH_3-\overset{O}{\overset{\parallel}{C}}-$, of 38.2 per cent. The values actually found on hydrolysis in two determinations were 43.7 and 46 per cent respectively. Rast's camphor method gave a molecular weight for the acetyl derivative of 462 which corresponds roughly with the calculated molecular weight of 450.



Oxidation of Köjic Acid Derivatives. In an attempt to determine at what point or points the köjic acid molecule is attacked by silver oxide, the nuclear hydroxyl and the primary alcohol groups were "protected" by methylation and the resultant derivatives subjected to the action of silver oxide in water and in dioxane.

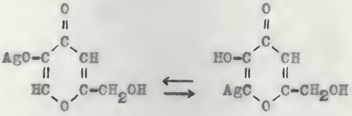
The monomethyl and the dimethyl ethers of köjic acid were prepared from köjic acid in the usual manner by the use of sodium or potassium hydroxide and dimethyl sulfate. The monomethyl ether melted at 160.5-161.5° C. (corr.); the dimethyl ether at 88.6-89.1° C. (corr.).

With two equivalents of silver oxide in aqueous solution, the monomethyl ether had apparently undergone no change after a period of eleven hours at room temperature. The original light-yellow solution had not increased in color, nor was a silver plate formed. (An aqueous solution containing a weight of köjic acid equivalent to that of the ether was exposed to the action of two equivalents of silver oxide and acquired a silver plate, with increased color in the liquid, within ten minutes). The solution of the monomethyl ether was then heated to 60-70° C. for three hours, a silver plate appearing within twenty minutes. The color of the solution had not deepened, but the solid residue was gray in color and very finely divided. The presence of the methyl ether of comenic acid, m. pt. (280-282° C.) was not detected, nor were any products identified except the original ether. Small amounts of some other material were present since before purification the melting point of the residue left by evaporation of the solutions extended over a four or five degree range.

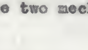
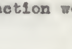
The oxidation of the monomethyl ether in dioxane solution follows similar lines, except that the action is still more retarded.

The dimethyl ether gave no apparent action with silver oxide in aqueous solution after nineteen hours at room temperature. The solution was then heated to 50° C. for five hours with no visible result. When the temperature was raised to 70° C. for three hours, a small amount of silver plate was formed, although the color of the solution had not increased. No product was recovered except the original ether. In dioxane solution the reaction proceeded even more slowly.

Thermal Decomposition of Silver Köjate. In an attempt to formulate a mechanism for the oxidation of köjic acid by silver oxide, attention was turned toward the problem of thermally decomposing the silver salt. If the silver atom could migrate from the nuclear hydroxyl group to the carbon atom in the ring, in the following manner:



I II

as does mercury in mercury phenolates, according to Whitmore (10), then two residues of II could possibly interact, eliminating silver and forming a carbon-to-carbon linkage. This action would constitute an oxidation. If, however, the silver atoms were removed from their customary position without the occurrence of a preliminary migration, then, dependent on whether free silver or silver oxide was formed, a peroxide,  $\begin{matrix} \diagdown C-O-O-C \diagup \\ \| \qquad \| \\ \diagup C \qquad C \diagdown \end{matrix}$ or an ether,  $\begin{matrix} \diagdown C-O-C \diagup \\ \| \quad \| \\ \diagup C \quad C \diagdown \end{matrix}$ linkage might result between the residues. In any case, the isolation and identification of a product analogous to that secured in oxidations of köjic acid would furnish strong evidence that the two mechanisms of reaction were similar.

After many fruitless attempts, a silver salt of köjic acid was prepared as follows: 7.1 grams (1/20 mole) of köjic acid were dissolved in 350 c.c. distilled water and titrated with concentrated ammonia to a faint pink with phenolphthalein. Eight and five-tenths grams (1/20 mole) of silver nitrate were dissolved in 100 c.c. of water and added rapidly with vigorous stirring to the first solution. An orange, flocculent precipitate began to form when about half of the silver nitrate had been added and gradually changed to a cream-yellow color during the addition of the remaining silver nitrate. The precipitate was filtered with suction and washed successively with water, alcohol, and ether. The salt gradually darkened, even when the entire preparation was performed away from light. It was dried in a vacuum desiccator over calcium chloride. The final product was a purplish-brown, amorphous substance which gave by either the ignition or the silver chloride precipitation method 45-46 per cent silver. The theoretical value for silver köjate is 43.3 per cent. If dried to constant weight at 100° C., the salt became flaky and slate-grey in color, and the silver content rose to 49-50 per cent. These changes also occurred to a smaller degree when the salt was dried at temperatures as low as 50-60° C. The silver salt dissolved in strong ammonia to give dark green solutions in which a small amount of black residue remained undissolved even when heat was applied.

The principal observation which can be made from the preparation of the silver salt is its apparent instability, as shown by the change in color and the high silver content. One preparation which had been dried to a fluffy, light-tan powder over calcium chloride was placed in a tightly-stoppered bottle and excluded from light, but within twenty-four hours the salt had acquired the purple-brown color mentioned previously.

When köjic acid was titrated with sodium hydroxide instead of ammonia, and silver nitrate was added, the resultant precipitate darkened much more rapidly and the final product was a dark blue-grey in color, while the silver content rose to values of 70-80 per cent. The higher initial pH probably accounts for the difference since the other conditions of the reaction were unchanged.

Upon ignition, the silver salt fused rather explosively at a temperature greater than 100° C. The charred material was readily burned away, leaving a silvery deposit in which no silver oxide could be detected.

Weighed samples of the silver salt were placed in dry dioxane and heated at 60-70° C., or to gentle ebullition under reflux, for periods of 1-17 hours. The solutions became yellow-to-orange in color, depending upon the length of the heating period. The residues contained some metallic silver and much dark brown material which resembled silver oxide. The silver content was variable in amount.

At low temperatures and during the shorter time intervals, the action on the silver salt is apparently not very pronounced. At the other extreme, the solutions become deep orange in color and give by addition of ether an orange, flocculent material, unstable when moist, and in many respects similar to the product obtained by oxidation of köjic acid in inert solvents. If the silver salt is first extracted with ether before thermal decomposition is attempted, köjic acid can be recovered, for example, 1.5 grams of silver salt yielded 0.285 gram after extraction with ether for twenty hours. It is unlikely that köjic acid forms a double compound with silver köjate as Yabuta (8) found that it does with barium köjate nor is it likely, if this action did occur, that köjic acid could be dissociated from such a complex salt at the low temperature employed in extraction. The köjic acid is probably occluded by the flocculent silver salt during the latter's preparation and is not entirely removed by washing.

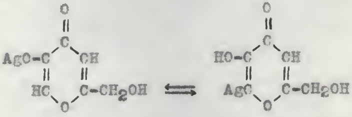
The silver salt, after extraction with ether, has a silver content of 56-57 per cent. When this material is heated in dioxane solution, the unstable substance mentioned previously can then be recovered only in traces. The solutions give upon isothermal evaporation yellow-orange, amorphous residues which are difficult to dry and which resist efforts to recover crystallizable substances from them. They give a decided red coloration with ferric chloride, dissolve slowly in water or alcohol and decompose over a wide range of temperature in a capillary melting point tube.

DISCUSSION OF RESULTS

In any discussion of the nature of the reactions arising in the oxidation of köjic acid by silver oxide, primary consideration must be given to the point or points in the molecule which are most susceptible to attack by the oxidizing agent. Two parallel lines of evidence appear to indicate that the keto-enol configuration is the favored point of entry; namely, (a) oxidation of köjic acid and its derivatives in aqueous and inert solvents and (b) thermal decomposition of silver köjate. These will be reviewed in turn.

From a theoretical standpoint, the nuclear hydroxyl group of köjic acid should be considered as being more sensitive to the action of oxidizing agents. Phenol, for example, oxidizes slowly in the air while polyhydric phenols undergo oxidation very readily with weak oxidizing agents. Alexeyeff (11) concludes that the presence of -OH in the benzene nucleus renders the adjacent C-H group more susceptible to oxidation. He noted that a primary alcohol group, on the other hand, is usually converted to an aldehyde by means of chromic acid mixture, a stronger oxidizing agent than silver oxide. Henrich (12) states that if a primary or secondary alcohol is to be oxidized by silver oxide, an aqueous solution containing caustic alkali is used. Yabuta and Kambe (5) found that when the nuclear hydroxyl group of köjic acid is blocked by methylation, the primary alcohol group can then undergo oxidation. This is another factor which tends to indicate the greater activity of the nuclear hydroxyl group.

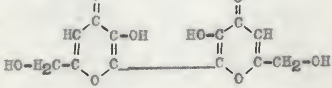
The formation of an aldehyde by the oxidation of köjic acid in either aqueous or non-aqueous solution has not been proved, nor is it considered likely that comenaldehyde is formed. Such a compound, as Yabuta (2) has suggested, might admittedly undergo polymerization if formed, but the results actually found in the present work appear to indicate that oxidation involves the keto-enol configuration, and not the primary alcohol group. The formation of resinous or unstable products can be explained in the light of the possible isomerization resulting upon entry of silver into the molecule, as shown below.



I II

Reaction between two molecules of II at position 6 or between one molecule each of I and II at positions 5 and 6 respectively, with the elimination of silver, should give stable products. If two molecules of I were to react at position 5, eliminating silver, a peroxide linkage would be formed. Such a compound would probably be unstable and in aqueous solutions in particular could undergo secondary reactions of hydration, dissociation, etc., giving rise to products of the type actually found. The particular class of solvent used may influence the relative concentrations of I and II actually formed. Should water as a solvent favor the production of the I-form, subsequent reactions could then give unstable or perhaps resinous products in greater yield than when inert solvents are used; should inert solvents favor the formation of the II-form, more stable products would result. The results actually found indicate that the production of resinous or unstable products is greatest in aqueous solution. It would be unwarrantable to suppose in any case that the oxidation follows one set course to the exclusion of all others, but there should be a favored path of reaction. This latter course, it is believed, may exist in aqueous solutions, but is either concealed or is carried further by secondary reactions. The investigator must turn to oxidation reactions which proceed in inert solvents if any clear picture of the mechanism of reaction is to be drawn.

When non-polar solvents are used as a solution medium in the oxidation of köjic acid, formation of resinous products is reduced. The unstable material first isolated is assumed from its chemical behavior to be of the latter type, possibly a peroxide, and because of its instability cannot furnish a workable explanation of the reaction. It is of interest only in that it may indicate one possible course of reaction. Since the inert solvent should not exert polar effects to any marked degree, the reaction is quite possibly halted at a definite point or is even directed in a certain path. The isolation of a substance whose acetyl derivative has a molecular weight of 462 and which apparently has four acetyl groups would tend to support the latter assumption. The existence of a peroxide or an ether linkage formed between the nuclear hydroxyl groups of two köjic acid molecules is possible, but the peroxide would probably be unstable and would not give a derivative of the type found. The latter consideration would also hold for an ether. We are thus led toward the possibility of a carbon-to-carbon linkage between two köjic acid molecules. Such a compound could have the characteristics shown by the substance actually isolated.



The point of attack of the silver oxide can be visualized more clearly by a consideration of the oxidation of the monomethyl and the dimethyl ethers of köjic acid. When the nuclear hydroxyl group is blocked by methylation, oxidation in aqueous or inert solvents proceeds slowly, only at elevated temperatures, and without the formation of resinous products or highly-colored solutions. This result would appear to indicate that oxidation of the ether is occurring at the primary alcohol group. Since with köjic acid itself and silver oxide at ordinary temperatures, a rapid reaction is noted, one is justified in assuming that oxidation in this case is occurring elsewhere than at the primary alcohol group. With the dimethyl ether of köjic acid oxidation is negligible and may be due to decomposition upon prolonged heating. It has also been noted that if köjic acid be oxidized by silver oxide in aqueous solution in the presence of one equivalent of sodium hydroxide, the reaction proceeds more rapidly than in aqueous solution alone. If two equivalents of sodium hydroxide are used, the reaction appears to be inhibited, no silver plate is formed, and the solutions are not highly-colored, but resemble those which result from oxidation of köjic acid in inert solvents. The residues are granular, not resinous in nature. These observations are clearly indicative of the part which the excess alkali can play by inhibiting the entry of silver into the molecule.

The thermal decomposition of silver köjate has not proved definitely that the course of reaction is the same as that which occurs in the oxidation of köjic acid by silver oxide. The actual migration of the silver atoms, with subsequent elimination of silver from two residues of the salt, cannot be said to have been demonstrated beyond all doubt, nor is such a proof likely to be possible experimentally except insofar as the same products are formed in both types of reaction. Insufficient time remained to make an extended comparison of the products resulting from thermal decomposition with those from oxidations of köjic acid. They seemed to be, however, very similar in such properties as were observed. The results would then seem to indicate that both in the oxidation and in the thermal decomposition reactions the keto-enol configuration of the molecule is largely concerned.

CONCLUSIONS

The actual mechanism of oxidation of köjic acid in aqueous and in non-aqueous solvents is thought to be similar, but secondary reactions in the former instance lead to the difference noted in results.

Oxidation in either type of solvent is assumed to occur largely by a reaction involving the keto-enol configuration of the köjic acid molecule and not the primary alcohol group.

The thermal decomposition of silver köjate follows a course similar to the oxidation of köjic acid and also involves the keto-enol group.

From the oxidation of köjic acid by silver oxide, a compound has been isolated in which apparently the nuclei of two köjic acid molecules are linked together.

ACKNOWLEDGMENT

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