STUDIES ON KOJIC ACID AND ITS RELATED γ -PYRONE COMPOUNDS—XI

THE OXYGEN EXCHANGE REACTION OF KOJIC ACID AND ITS RELATED COMPOUNDS WITH WATER

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Abstract—The oxygen exchange reactions of kojic acid and γ -pyrone were carried out in aqueous neutral, acidic and basic solutions. The incorporation of ¹⁹O in kojic acid is similar to that of γ -pyrone and the incorporation into the ring oxygen was observed. The exchange reactions of pyromeconic acid, maltol, allo-maltol, 5-methoxykojic acid, 6-methylkojic acid and 6-hydroxymethylkojic acid also proceed in an alkaline medium. The results obtained show that the ring opening is sensitive to the position and nature of a substituent on the γ -pyrone ring. A compound, having an electron-withdrawing group at the 2- or 6-position of the γ -pyrone ring incorporated more ¹⁸O than a compound having an electron-releasing group. The mechanism of oxygen exchange reaction is discussed together with resonance effect of kojiate anion.

THE structure of kojic acid was established by Yabuta as 5-hydroxy-2-hydroxymethyl- γ -pyrone.^{1.2} Since then, reactions of kojic acid³⁻¹⁶ particularly with diazonium salt,¹⁷ hydrazine hydrate,¹⁸ formaldehyde¹⁹ and amines²⁰ have been reported. Electrophilic substitution takes place more easily at the 6- than at the 3- position of the γ pyrone ring. This activity of the 6-position may depend on the existence of the two limiting resonance structures of the kojiate anion (I and II).

This activating effect of the phenolic function should be particularly marked in

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alkaline conditions, but then the opening of the pyrone ring by a nucleophilic attack of a hydroxide ion on the pyrone ring would also take place.²¹⁻²⁵

Compound -	Atom % ¹⁸ O				
	Neutral	Acidic	Basic		
Kojic acid	0.23*	0.28	0.51		
γ-Pyrone	0.25	0.22	0.52		

TABLE 1.	Тне	VALUES	OF	¹⁸ O	INCO	RPORAT	nons	IN	THE	RECOVERED	колс
		ACID AN	D٦	-PYR	ONE	UNDER	VARI	OUS		DITIONS	

Natural abundance of ¹⁸O was 0.204 atom % ¹⁸O.

In this paper, the structure of the kojic acid in aqueous solution, especially in the presence of alkali using ¹⁸O as a tracer is discussed. Kojic acid, dissolved in ¹⁸Oenriched water (1.5 atom % ¹⁸O), was allowed to stand at 40° for 20 hr under neutral, acidic and basic conditions. After neutralization and water removal (red. press.) the resulting precipitates were subjected to ¹⁸O analyses. The values of ¹⁸O incorporation in the recovered kojic acid are shown in Table 1 and compared with the exchange reaction of y-pyrone under similar conditions. Although the tendency of ¹⁸O incorporation in kojic acid is similar to that of the γ -pyrone, one molecule of kojic acid contains four oxygen atoms whereas γ -pyrone contains two. Thus, in order to discuss the position and the ratio of ¹⁸O incorporation, a degradation reaction or substitution of the oxygen atoms in the compound must be achieved. When kojic acid is heated with aqueous ammonia in a sealed tube, the ring oxygen atom is substituted by nitrogen, giving the corresponding γ -pyridone³⁶ and removal of one oxygen atom. As in the presence of alkali and high temperature, the oxygen exchange of carbonyl oxygen occurs this method is not suitable for the degradation of the kojic acid. A complete cleavage reaction with aqueous barium hydroxide, giving formic acid, methoxyacetic and methoxyacetone²⁷ also is not applicable because of the use of water. These objections also apply to γ -pyrone. Although the reaction of γ -pyrone with phosphorous pentasulphide in ligroin or benzene to give 4-thio-y-pyrone²⁸ is free from oxygen exchange, the ¹⁸O analytical results of the compounds containing sulphur did not show a definite value because when the compounds were pyrolysed with mercuric cyanide and mercuric chloride in a vacuum sealed tube at 500°, the resulting

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sulphur dioxide, sulphur monooxide, etc., disturbed the peak of the mass number-46 in a mass-spectrum.*

In a preliminary experiment, it was found that the value of incorporation in 4-thio- γ -pyrone was larger than that in γ -pyrone.²⁹ The oxygen exchange reactions of γ -pyridone, pyromeconic acid, allo-maltol, maltol, 5-methoxykojic acid, 6-methyl-kojic acid and 6-hydroxymethylkojic acid under similar conditions were carried out and the results are shown in Table 2.

Compound	Atom % ¹⁸ O
y-Pyridone	0.20*
Pyromeconic acid	0.28
Allo-maltol	0.49
Maltol	0.26
5-Methoxykojic acid	0.28
6-Methylkojic acid	0.32
6-Hydroxymethylkojic acid	0.64

 TABLE 2. THE VALUES OF ¹⁸O INCORPORATION IN THE RECOVERED COMPOUNDS RELATED TO KOJIC ACID

* Natural abundance of ¹⁸O was 0.204 atom %.

All the compounds were recovered from 18 O-enriched 0.8% NaOH solution.

The value for γ -pyridone proves that the oxygen exchange reaction had not taken place. In view of this result, the large ¹⁸O incorporation in γ -pyrone must mainly be due to the cleavage of the γ -pyrone ring. In fact, when 4-thio- γ -pyrone from ¹⁸Oenriched γ -pyrone was subjected to ¹⁸O-analysis, an increased ¹⁸O incorporation was observed. This evidence indicates that the ¹⁸O incorporation in the ring oxygen is larger than that in the carbonyl oxygen. The 4-thio- γ -pyrone derived from the γ pyrone which was recovered from acidic or neutral solution, also showed an excess of ¹⁸O over the natural. These results with 4-thio- γ -pyrones show that incorporation takes place in ring oxygen, even in acidic or neutral solution.



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* The precise procedure of analyzing the sulphur-compounds is now being studied in this laboratory. The purification of the evolved carbon dioxide using lead acetate powder showed rather good results. However, this analytical procedure of oxygen-18 has as yet many problems to be solved.
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Recently, Beak and Carls³⁰ reported ¹⁸O incorporation under acidic conditions in the ring oxygen of γ -pyrone and the following mechanism was suggested.

Our results also indicate that the oxygen exchange occurs through an open structure which is hypothetically predicted, even in acidic or neutral condition.

According to this evidence, the following mechanism can be suggested.



The process A involves ring opening and attack by nucleophiles such as water, hydroxide anion, ammonia and amines on the 2- or 6-position of the pyrone ring while process B is the exchange of the carbonyl oxygen through an intermediate (VI). The ratio of A to B depends on experimental conditions, the reactivity of attacking reagents and the particular pyrone derivative. Under alkaline conditions, the ring-opening is followed by decomposition and the yield of recovered γ -pyrone is very small.

When a strong nucleophile is used, the equilibrium between a closed form and an open form is in favour of the latter and γ -pyrone decomposes. When aqueous ammonia reacts with γ -pyrone, the corresponding pyridone is formed through an intermediate similar to IV. As the nucleophilicity of the reagents is of importance, the exchange conditions of γ -pyrone in the presence of aqueous alkali was determined by considering the recovery yield of the starting material and finally 0.2% sodium hydroxide solution was used.

If this proposal could be adapted to kojic acid and related compounds, a comparison could be made. When the compound, having a methyl instead of hydroxymethyl group at the 2-position was used, a value similar to that for kojic acid was obtained. (See allo-maltol in Table 2.) However, maltol having a methyl group at the 6-position of γ -pyrone ring, showed a lower ¹⁸O incorporation. Similarly 5methoxykojic acid showed a ¹⁸O value just greater than the natural ¹⁸O abundance. On the other hand, 6-hydroxymethylkojic acid showed more incorporation than kojic acid itself. The ¹⁸O incorporation is in decreasing order in the following compounds: 6-CH₂OH Kojic acid > Kojic acid > Allo-maltol > 6-CH₃ Kojic acid > 5-CH₃O Kojic acid > Maltol.

If the ring opening is initiated by a nucleophilic attack of the hydroxide anion on

the 2- or 6-position in γ -pyrone, then substitution by an electron-withdrawing group in these positions will accelerate the oxygen exchange reaction of the ring oxygen. Although the difference between allo-maltol and maltol is only in the position of the methyl group, the 6-position of 5-hydroxy- γ -pyrones is more electronegative than the 2-position owing to the resonance effect of the 5-hydroxyl group. The substitution of an electron-releasing group such as methyl on the 6-position suppresses the nucleophilic attack on this position and the ring opening is exclusively initiated by an attack of the hydroxide ion on the 2-position. If the incorporation of ¹⁸O in kojic acid occurs exclusively on the carbonyl³¹ or phenolic oxygen, the large difference between the values for 5-methoxykojic acid or maltol and kojic acid can not be explained even on considering the stereochemical effect of the methoxy group or the electrostatic and electromeric effects of the substituents. The difference between the ¹⁸O value for allo-maltol and maltol can be explained only by ring opening, providing that the contribution of the substituent at the 2- or the 6-position to the carbonyl oxygen exchange through resonance is similar and the electrostatic effect is small. The lower value for 5-methoxykojic acid may be due to difficult ring opening.

Kojic acid, allo-maltol and 6-hydroxymethylkojic acid are all easily oxidized with Tillman's reagent (2,6-dichlorophenol-indophenol) while 5-methoxykojic acid and maltol are not. As these observations are in keeping with the ¹⁸O exchange reactions, the low value for ¹⁸O incorporation in maltol and 5-methoxykojic acid may be due to difficult opening of the γ -pyrone ring in alkaline solutions. The ring opening seems to be more sensitive to the substituents than the oxygen exchange reaction at the carbonyl oxygen. If the opening of the γ -pyrone ring is initiated by an attack of hydroxide anion, the attacking site in kojic acid will be at the 2-position because the 6-position is more electronegative, owing to a resonance effect of the 5-hydroxy group while the electron-withdrawing effect of hydroxymethyl group gives electron deficiency on the 2-position. When a molecule of water is removed from the resulting endiol (VIII), the kojiate anion is regenerated and at the same time the oxygen exchange reaction is accomplished.



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EXPERIMENTAL

Materials. Kojic acid (5-hydroxy-2-hydroxymethyl-y-pyrone)³¹ was isolated from the culture fluid in which Aspergillus oryzae var. globosus AA2 had grown. The sample for exchange reaction was recrystallized twice from EtOH, m.p. 152-153°. 5-Methoxykojic acid (5-methoxy-2-hydroxymethyl- γ -pyrone)¹⁸ was obtained by reacting kojic acid with dimethyl sulphate in 10% KOHaq. The sample was recrystallized from MeOH, m.p. 158°. Allo-maltol (5-hydroxy-2-methyl-y-pyrone)33 on catalytic hydrogenation of kojic acid over PtO2 yielded both a solid and a viscous material. The solid product, allo-maltol recrystallized from ethyl acetate as colourless crystals, m.p. 151-152°. 6-Hydroxymethylkojic acid (5-hydroxy-2, 6-dihydroxymethyl-y-pyrone)¹⁹ was prepared by the reaction of kojic acid with formaldehyde in aqueous alkaline solution. Recrystallization from EtOH gave white needles, m.p. 158–159°. 6-Methylkojic acid (6-methyl-5-hydroxy-2-hydroxymethyl- γ -pyrone)¹⁹ was obtained by reducing 6-hydroxymethylkojic acid with SnCl₂ under acitic conditions. The crystalline product was recrystallized from EtOH, m.p. 148°. Maltol (3-hydroxy-2-methyl-y-pyrone)¹⁹ was obtained by the following procedure. Kojic acid was oxidized to comenic acid (5-hydroxy-2-carboxy-y-pyrone)⁸⁴ by a microbiological method. The comenic acid reacted with formaldehyde to give 6-hydroxymethylcomenic acid which was reduced to 6-methylcomenic acid. The decarboxylation of 6-methylcomenic acid with KC-400 (tetrachloro-diphenyl) gave maltol, m.p. 160-161°. When comenic acid, obtained in the synthesis of maltol from kojic acid, was heated with KC-400 at 300°, pyromeconic acid (3-hydroxy- γ -pyrone), was obtained, m.p. 118°. γ -Pyrone was prepared by the decarboxylation of chelidonic acid.³⁰ y-Pyridone³⁵ was synthesized by reacting y-pyrone with aqueous ammonia in a sealed tube. Recrystallization from EtOH gave white crystals, m.p. 148°.

Oxygen exchange reaction

Alkaline conditions. The sample (0.5 g) was dissolved in 10 ml ¹⁸O-enriched 0.8% NaOH solution (about 1.5 atom% ¹⁸O) and allowed to stand at 40° for 20 hr. After neutralization with HCl, the solution was concentrated under red. press., and the residue recrystallized. A purified sample was used for the ¹⁸O analysis. In the case of γ -pyrone,³⁹ as the yield of recovery was poor, it was treated with 0.2% NaOH solution (1.5 atom% ¹⁸O) at 30° for 20 hr. After removal of solvent, the product was recovered by distillation and purified by VPC.

Acidic conditions. The sample (0.5 g) was dissolved in 10 ml of ¹⁸O-enriched acidic solution containing HCl (pH = 1.0, 1.5 atom % ¹⁸O) and allowed to stand for 40° for 20 hr. After removal of solvent the resulting solid was recrystallized or redistilled. A purified sample was subjected to ¹⁸O analysis.

Neutral conditions. The term "neutral" means no addition of acid or base to the ¹⁸O-enriched water (1.5 atom % ¹⁸O). In the case of γ -pyrone, the solution was neutral while kojic acid is a weak acid, owing to the phenolic hydroxyl group at the 5-position in γ -pyrone ring. The sample was treated as described.

Reaction of kojic acid and its related compounds with indophenol

Kojic acid (0.5 g) was dissolved in 0.8% NaOH solution at room temp. A solution of indophenol (0.01% aqueous solution) was added dropwise the colour of indophenol disappearing within 30 sec. This quick decolourization was also observed in the case of allo-maltol, pyromeconic acid and 6-hydroxymethylkojic acid. In the case of 6-methylkojic acid, the colour of indophenol disappeared slowly but with 5-methoxykojic acid and maltol the decolourization of indophenol did not occur under similar conditions.

Isotope analysis. The content of ¹⁶O in the compound was determined by Rittenberg's method. The compound was vacuum sealed in a tube together with $Hg(CN)_a$ and $HgCl_a$, and heated at 500° for 5 hr. After cooling, the sealed tube was broken and the CO₂ evolved trapped in a liquid N₂-bath and the uncondensed materials pumped out. The CO₂ was redistilled 3 times in the vacuum line. If the compound contained a sulphur atom, the evolved CO₂ was passed through a column of sodium acetate. The ¹⁶O content was determined by using a Hitachi model RMU-5G mass-spectrometer.

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