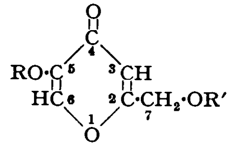
758. *The Mechanism of Alkaline Cleavage of Some γ-Pyrones.*

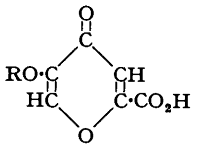
By H. R. V. Arnstein and Ronald Bentley.

The alkaline cleavage of the γ-pyrones, dimethylkojic acid and methylcomenic acid, has been investigated by the isotopic tracer technique. Dimethylkojic acid, labelled with ^{14}C in the methoxymethyl group, was cleaved either between carbon atoms 2 and 3 or between carbon atoms 3 and 4, although statistically the $C_{(2)}$–$C_{(3)}$ bond was broken more frequently. The hydrolysis products, methoxyacetone and methoxyacetic acid, can therefore arise from different carbon atoms. The hydrolysis of methylcomenic acid on the other hand did not give rise to any pyruvic acid and the cleavage in this case took place only between carbon atoms 2 and 3.

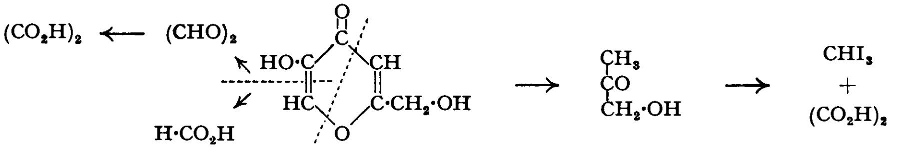
During an investigation of the biosynthesis of kojic acid (I; R = R′ = H) from ^{14}C-labelled precursors by various *Aspergilli* (Arnstein and Bentley, *Nature*, 1950, **166**, 948) an unequivocal method of degradation was required in order to locate the distribution of radioactive carbon in the molecule. According to Yabuta (*J. Chem. Soc. Japan*, 1916, **37**, 1185, 1234; *Chem. Abstr.*, 1923, **17**, 1475), the dimethyl derivative of (I) (R = R′ = Me) is cleaved by barium hydroxide to give formic acid, methoxyacetic acid, and methoxyacetone. Similarly, kojic acid (I; R = R′ = H) is cleaved by alkaline hypoiodite to give formic acid, oxalic acid, iodoform, and glycollic acid as final products (Birkinshaw and Raistrick *Phil. Trans.*, *B*, 1931, **220**, 139). The mechanism of this breakdown of kojic acid was represented by Birkinshaw and Raistrick (*loc. cit.*) as follows:

(I)

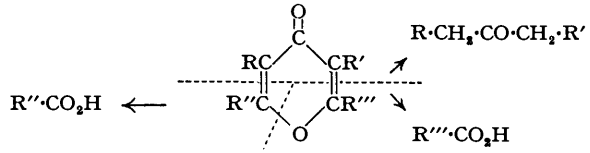




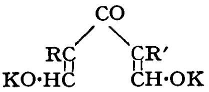
(II)



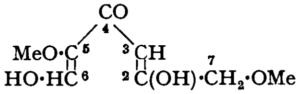
However, a comparison of the alkaline hypoiodite degradation of kojic acid with the alkaline hydrolysis of other γ-pyrones such as (II; R = Me), which is hydrolysed to oxalic acid, methoxyacetone, and formic acid, led us to believe that the alkaline hydrolysis of such γ-pyrones takes place by the following mechanism which differs from that postulated by Birkinshaw and Raistrick (*loc. cit.*) for the hypoiodite degradation:



This conclusion appeared to be supported by the isolation of methoxyacetone from the hydrolysis products of 5-*O*-methylkojic acid (I; R = Me, R′ = H). On the basis of these observations we concluded that dimethylkojic acid yields formic acid arising from $C_{(6)}$, methoxyacetic acid from $C_{(2)}$ and $C_{(7)}$, and methoxyacetone from the remaining three carbon atoms of the molecule. However, in many experiments using different ^{14}C-labelled precursors it was observed that the radioactivity of the methoxyacetic acid arising from $C_{(2)}$ and $C_{(7)}$ of kojic acid was always almost exactly equal to the radioactivity of the methoxyacetic acid obtained by further degradation of the methoxyacetone with sodium hypoiodite. We therefore considered the possibility that the mechanism of hydrolysis of kojic acid resulted in randomisation of the label originally located in $C_{(4)}$, $C_{(5)}$, $C_{(2)}$, and $C_{(7)}$. Many γ-pyrones are split by alkali under mild conditions to give bishydroxymethylene compounds, usually as the potassium derivatives (III), which are reconverted into γ-pyrones by acid. Dimethylkojic acid would similarly give (IV), which would be converted into the symmetrical diketone (V) by loss of the hydroxymethylene group (carbon atom 6). Further hydrolysis of (V) would yield methoxyacetic acid, arising from $C_{(4)}$ and $C_{(5)}$, or $C_{(2)}$ and $C_{(7)}$, and methoxyacetone arising from $C_{(3)}$, $C_{(2)}$, and $C_{(7)}$, or $C_{(3)}$, $C_{(4)}$, and $C_{(5)}$.



(III)



(IV)

$$MeO\cdot\overset{5}{C}H_2\cdot\overset{4}{C}O\cdot\overset{3}{C}H_2\cdot\overset{2}{C}O\cdot\overset{7}{C}H_2\cdot OMe$$

(V)

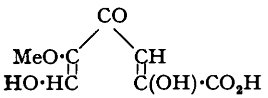
If such a mechanism existed, dimethylkojic acid labelled with ^{14}C in one of the two methoxyl groups should give rise to both methoxyacetic acid and methoxyacetone containing ^{14}C in the methoxyl group. Kojic acid 5 : 7[$^{14}C_1$]-dimethyl ether * (I; $R = CH_3$, $R' = {}^{14}CH_3$) was therefore prepared by methylating 5-*O*-methylkojic acid (I; R = Me, R′ = H) with a slight excess of [^{14}C]methyl iodide. The possibility of exchange of the methoxyl group of 5-*O*-methylkojic acid with the [^{14}C]methyl iodide was excluded by showing that the specific radioactivity of the recovered [^{14}C]methyl iodide was identical with that of the starting material. Degradation of (I; $R = CH_3$, $R' = {}^{14}CH_3$) with barium hydroxide, essentially as described by Yabuta (*loc. cit.*), afforded methoxyacetic acid, methoxyacetone, and formic acid. The formic acid was completely non-radioactive and the methoxyacetic acid and methoxyacetone together accounted for the whole of the radioactivity in the kojic acid dimethyl ether. However, the radioactivity in the methoxyacetic acid was about 1·2 times that in the methoxyacetone, as shown below, indicating a more frequent rupture of the $C_{(2)}$–$C_{(3)}$ bond than of the $C_{(3)}$–$C_{(4)}$ bond. Since cleavage of (V) would result in equal radioactivity in the methoxyacetic acid and the methoxyacetone, it is clear that (IV) is at least partly cleaved at the $C_{(2)}$–$C_{(3)}$ bond while the hydroxymethylene group is still intact.

Distribution of ^{14}C *in degradation products of kojic acid* 5 : 7[$^{14}C_1$]-*dimethyl ether.*

	Radioactivity *	
	counts/min.	counts/min./micromol.
Kojic acid 5 : 7[$^{14}C_1$]-dimethyl ether	500·6	85·2
Methoxyacetone 2 : 4-dinitrophenylhydrazone	138·8	37·2
,, ,, ,, ,, recryst.	142·3	38·2
Silver methoxyacetate, *ex* kojic acid dimethyl ether	239·2	47·1
Silver methoxyacetate, *ex* methoxyacetone	194·8	38·4

* See Experimental section.

In view of the small but definite effect of the hydroxymethylene group on the cleavage of (IV) it was of interest to investigate the cleavage of *O*-methylcomenic acid (II; R = Me). A preliminary experiment showed that hydrolysis of this with barium hydroxide yielded an almost equimolecular quantity of barium oxalate. Nevertheless, it seemed possible that, by analogy with the cleavage of (IV), hydrolysis of a hypothetical intermediate (VI) might yield also some pyruvic acid. In the preliminary experiment, no pyruvic acid 2 : 4-dinitrophenylhydrazone could be isolated, but the possibility existed that under the conditions of the experiment the pyruvic acid might be destroyed.



(VI)

The hydrolysis of *O*-methylcomenic acid was therefore repeated in the presence of a small excess of ethyl pyruvate. Although both methoxyacetone and pyruvic acid were easily isolated as the 2 : 4-dinitrophenylhydrazones, the latter was obtained only in poor yield, especially when the reaction mixture had been steam-distilled, indicating that under the conditions of the hydrolysis substantial destruction of pyruvic acid had occurred. As the amount of pyruvic acid expected from the hydrolysis of *O*-methylcomenic acid would be very small it seemed possible that owing to destruction during the reaction this hydrolysis product might not be detected by the above method. It was decided therefore to use the isotope dilution technique in order to detect the possible formation of pyruvic acid in this reaction. Since this method is based on dilution of radioactivity of pyruvic acid present during the entire course of the

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* The symbol 5 : 7[$^{14}C_1$] indicates the position of labelling for $R = CH_3$, $R' = {}^{14}CH_3$. The compound in which $R = {}^{14}CH_3$ and $R' = CH_3$ would have the symbol 5[$^{14}C_1$] : 7. The compound in which both R and R′ are $^{14}CH_3$ would have the symbol 5 : 7[$^{14}C_2$]. Ed.

hydrolysis by any pyruvic acid which may be formed, it is independent of the subsequent destruction of part of this compound. When labelled sodium pyruvate, $CH_3{\cdot}CO{\cdot}^{14}CO_2Na$, was added to the reaction mixture the specific radioactivity of the pyruvic acid 2 : 4-dinitrophenylhydrazone isolated at the end of the reaction was identical with that of the original pyruvic acid. No pyruvic acid had therefore been formed during the hydrolysis and the cleavage of *O*-methylcomenic acid must have occurred exclusively at the $C_{(2)}$–$C_{(3)}$ bond.

It seems likely, therefore, that substitution of γ-pyrones by an electron-attracting group at position 2, such as the carboxyl group in *O*-methylcomenic acid, greatly enhances the tendency to split preferentially at the $C_{(2)}$–$C_{(3)}$ bond.

EXPERIMENTAL.

Radioactivity Determinations.—All measurements were carried out on " infinite thickness " samples, *i.e.*, 25 mg. or more of material/sq. cm., mounted on 1 sq. cm. polythene discs, as described by Popják (*Biochem. J.*, 1950, **46**, 560). Samples were counted with a helium-filled bell-shaped Geiger–Müller counter which had a thin mica window. The " background " of the instrument was 8—10 counts/minute and a sample containing 10^{-3} μc of ^{14}C/mg. gave a counting rate of approx. 1100 counts/minute under the above conditions.

[^{14}C]*Methyl Iodide.*—[^{14}C]Methanol (obtained from the Radiochemical Centre, Amersham) was diluted with carrier methanol and converted into methyl iodide according to Tolbert (*J. Amer. Chem. Soc.*, 1947, **69**, 1529). A portion of this material was converted into the methylthiuronium picrate, which had 287·7 counts/minute. After recrystallisation from acetone the picrate had m. p. 224—225° and gave 262·1 counts/minute.

Kojic Acid 5 : 7[$^{14}C_1$]-*Dimethyl Ether.*—5-*O*-Methylkojic acid (1 g.), prepared from kojic acid as described by Campbell, Ackerman, and Campbell (*J. Org. Chem.*, 1950, **15**, 221), in boiling methanol (25 c.c.) was treated with silver oxide (4 g.) and [^{14}C]methyl iodide (5 c.c.) in two portions (Armit and Nolan, *J.*, 1931, 3023). The solution was filtered and evaporated to dryness. After being washed with ether the crude product (0·87 g.; 407 counts/minute) was recrystallised from chloroform by addition of ether and light petroleum (b. p. 60—80°), to give kojic acid dimethyl ether, m. p. 84—85° (417 counts/minute). Sublimation *in vacuo* afforded pure material, m. p. 88—89° (500·6 counts/minute).

Hydrolysis. To the above material (0·25 g.) in water (25 c.c.) was added barium hydroxide octahydrate (0·47 g.). After 30 minutes' heating at 100° the solution was kept at room temperature for 16 hours and then steam-distilled, 170 c.c. of distillate being collected. 60 C.c. of the distillate were treated with 2 : 4-dinitrophenylhydrazine in 6N-hydrochloric acid; methoxyacetone 2 : 4-dinitrophenylhydrazone (138·8 counts/minute) was filtered off and, recrystallised from ethanol, had m. p. 155° (142·3 counts/minute). The remainder of the steam-distillate was treated with excess of sodium hypoiodite. The iodoform, which was completely non-radioactive, was filtered off. The filtrate was acidified, iodine was removed by extraction with a small volume of ether, and the solution was continuously extracted with ether for 18 hours. The ethereal extract was evaporated to dryness and the residual methoxyacetic acid was dissolved in water (10 c.c.) and treated with finely ground silver carbonate. After filtration, the aqueous solution was concentrated *in vacuo* below 50° almost to dryness; on addition of ethanol, silver methoxyacetate was obtained as a flocculent precipitate (194·8 counts/minute).

The residue from the steam-distillation was acidified with sulphuric acid (1·5 c.c.) in water (10 c.c.). The barium sulphate was removed by centrifugation; the solution was refluxed with mercuric oxide (3 g.) in order to oxidise the formic acid to carbon dioxide, which was collected as barium carbonate and counted. The carbonate was completely non-radioactive. Excess of mercuric oxide was removed by filtration and methoxyacetic acid was isolated from the filtrate by continuous ether-extraction. The silver salt prepared as described above had 239·2 counts/minute.

Hydrolysis of O-*Methylcomenic Acid.*—Barium hydroxide octahydrate (2·33 g.) was added to a suspension of methylcomenic acid (0·85 g.) in hot water (50 c.c.). After 20 minutes at 100° the solution was filtered to remove barium oxalate (0·94 g., 84%). The filtrate was divided into two equal portions. One portion was acidified with concentrated hydrochloric acid and treated with 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid. The 2 : 4-dinitrophenylhydrazone (0·35 g.) had m. p. 156—157° after chromatography on alumina; the mixed m. p. with authentic methoxyacetone 2 : 4-dinitrophenylhydrazone was not depressed. The second portion, which was neutral, was steam-distilled. One half of the distillate was converted into the 2 : 4-dinitrophenylhydrazone (0·118 g., 70·5%), m. p. and mixed m. p. with methoxyacetone 2 : 4-dinitrophenylhydrazone, 154—155° after recrystallisation from ethanol. The residue from the steam-distillation gave no precipitate with 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid. When the hydrolysis was repeated with methylcomenic acid (0·283 g.) and ethyl pyruvate (0·232 g.), the residue from the steam-distillation of one half of the reaction mixture gave a 2 : 4-dinitrophenylhydrazone (84 mg., 32%), m. p. 217—218° alone or mixed with authentic pyruvic acid 2 : 4-dinitrophenylhydrazone. The remainder of the reaction mixture was acidified with concentrated hydrochloric acid and treated with excess of 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid. The precipitate was centrifuged and washed with water. An aqueous solution of sodium hydrogen carbonate was added and the insoluble portion of the precipitate was filtered off. The filtrate was acidified with 2N-hydrochloric acid, to give pyruvic acid 2 : 4-dinitrophenylhydrazone (125 mg., 47%), m. p. 213—214° alone or mixed with authentic material.

Hydrolysis of O-*Methylcomenic Acid in Presence of* $CH_3{\cdot}CO{\cdot}^{14}CO_2H$.—Pyruvamide (2·4 mg.), labelled with ^{14}C in the carbamyl group, was hydrolysed by heating at 100° for 3 hours with 0·03 c.c. of N-hydrochloric acid in water (0·2 c.c.). Carrier sodium pyruvate (0·5 g.) was then added and the volume made up to 30 c.c. A portion (10 c.c.) of this solution was converted into the 2 : 4-dinitrophenylhydrazone, m. p. 217—218°, which gave 974 counts/minute. The remainder of the above solution was added to methylcomenic acid (0·85 g.) suspended in water (20 c.c.). The hydrolysis was carried out with barium hydroxide octahydrate (2·23 g.) as described above. At the end of the hydrolysis pyruvic acid 2 : 4-dinitrophenylhydrazone was isolated from the reaction mixture. It had m. p. 217—218° and gave **973** counts/minute.

We thank Dr. G. Hunter for the ^{14}C-labelled pyruvamide and Dr. D. Herbert for a sample of pure sodium pyruvate. We are indebted to Dr. J. A. Barltrop for a helpful discussion concerning the mechanism of hydrolysis of kojic acid dimethyl ether.

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