INHIBITORY EFFECT OF KOJIC ACID UPON OXIDATIONS MEDIATED BY LIVER AND KIDNEY*

BY J. RAYMOND KLEIN AND NORMAN S. OLSEN†

(From the Departments of Psychiatry and Biological Chemistry, University of Illinois College of Medicine, Illinois Neuropsychiatric Institute, Chicago)

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Administration of increasing doses of kojic acid, 5-hydroxy-2-(hydroxymethyl)-1,4-pyrone, a product of the metabolism of a number of molds and bacteria, produces gastrointestinal disturbances, ataxia, excitement, and convulsions (1). The pattern of electrical activity of brain accompanying the convulsions indicates that they are of the clonic-tonic variety.1

In the present work it was found that the rates of oxidation of L-phenylalanine, L-methionine, xanthine, and a number of D-amino acids by rat liver and of L-phenylalanine and D-amino acids by kidney are inhibited in vitro by relatively low concentrations of kojic acid.

EXPERIMENTAL

Liver, kidney, and brain were blended briefly with 1 ml. of 0.05 M potassium phosphate, pH 7.8, per gm. of tissue and then squeezed through muslin. Muscle was ground in a Latapie mincer with the same proportion of buffer; 8 volumes of buffer were then added to 10 volumes of ground tissue.

The rates of oxygen uptake of 1.8 ml. aliquots of the tissue preparations, to which was added 0.2 ml. of buffer or buffer containing substrate, inhibitor, or both, were measured at 37° with the usual Warburg apparatus. The pH of the final mixtures was about 7.4. The difference between the oxygen uptake in the presence of substrate and that of its suitable control was considered a measure of the oxidation of the substrate.

The data in Fig. 1 indicate that 0.01 M kojic acid2 had little effect on the rate of oxygen consumption of skeletal muscle and decreased the rate of oxygen consumption of brain, liver, and kidney considerably. The oxygen uptake of heart, like that of skeletal muscle, was not appreciably affected by the acid. The uptake by liver was inhibited to the greatest extent.

The convulsant dose of kojic acid, when given by intravenous injection,
is about 0.3 gm. per kilo of body weight, which corresponds to a blood and total tissue concentration of about 0.05 and 0.004 M respectively. Thus, the possible range of concentration attained \textit{in vivo} with the convulsant dose is of the order of concentration, \textit{e.g.} 0.001 M, which appreciably inhibits the oxygen uptake of brain \textit{in vitro}. A somewhat similar relation between the convulsant dose of picrotoxin and the concentrations which \textit{in vitro} inhibit the oxygen uptake of brain has been pointed out previously (2).

Whether a depression of cerebral oxygen uptake plays a rôle in seizures produced by convulsant drugs is of course not certain. Since kojic acid has no considerable effect on the oxygen consumption of heart muscle \textit{in vitro}, it is of interest that the electrical activity of heart is little affected by the convulsant dose.\footnote{Kindly supplied by Dr. Clarence P. Berg.}

The data in Table I indicate that kojic acid inhibits the rate of oxidation of L-methionine, L-phenylalanine, L-tyrosine, D-phenylalanine,\footnote{Kindly supplied by Dr. Clarence P. Berg.} DL alanine,
and xanthine by liver. Compared on the basis of concentration of acid producing an inhibition of 0.5, the oxidation of methionine was most sensitive to the presence of the acid and that of tyrosine the least. The rates of oxidation of L-phenylalanine, D-phenylalanine, and DL-alanine by kidney were inhibited by kojic acid to about the same extent as with liver. The rates of oxidation by kidney and liver of DL-leucine and DL-isoleucine, the L forms of which, like L-alanine, were not oxidized by the kidney and liver preparations, were inhibited by the acid to about the same degree as the rate of oxidation of DL-alanine and n-phenylalanine.

Two of the enzymes inhibited by kojic acid, namely the xanthine and D-amino acid oxidases, are flavoproteins. The L-amino acid oxidase of kidney and liver is also a flavoprotein (3). Whether the oxidations of the L forms of methionine, tyrosine, and phenylalanine by the liver and kidney preparations used in the present work are mediated by the L-amino acid oxidase is not clear; for oxidation of L-leucine, estimated from oxygen uptake and ammonia formation, by the liver and kidney preparations was negligible, whereas L-leucine is of all L-amino acids most rapidly oxidized by the purified L-amino acid oxidase (3). However, it is possible that the inhibitory effect of kojic acid on the oxidation of the L forms of methionine, phenylalanine, and tyrosine found in the present work also represents inhibition of a flavoprotein.

The degree of inhibition produced by the acid in the case of each substrate considered above did not depend upon the sequence of addition of substrate and inhibitor to the tissue. Also, the degree of inhibition was not a linear

### Table I

Inhibitory Effect of Kojic Acid upon Oxidations Effected by Liver

The rates of oxidation, expressed in micromoles of oxygen per 100 minutes, are the differences in oxygen uptake of the liver preparation in the presence and absence of the substrates. The concentration of kojic acid giving an inhibition of 0.5 was determined by extrapolation from concentrations producing inhibitions less and greater than 0.5. The concentration of DL-alanine was 0.06 M; the concentration of the other substrates was 0.03 M.

| Substrate          | Rate of oxidation | Concentration of kojic acid producing inhibition of 0.5
<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>micromoles O₂ per 100 min</td>
<td>M × 10⁻⁴</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>5.0</td>
<td>4</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>7.6</td>
<td>12</td>
</tr>
<tr>
<td>D-Phenylalanine</td>
<td>11.2</td>
<td>12</td>
</tr>
<tr>
<td>DL-Alanine</td>
<td>10.1</td>
<td>16</td>
</tr>
<tr>
<td>Xanthine</td>
<td>10.9</td>
<td>70</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>6.0</td>
<td>800</td>
</tr>
</tbody>
</table>
function of the concentration of inhibitor. These facts suggest that the inhibitions are of the competitive type.

Further test of the kind of inhibition was made in the case of the D-amino acid oxidase. As indicated by the data in Fig. 2, in which the reciprocal of the rate of oxidation at various concentrations of substrate is plotted against the reciprocal of the concentrations of substrate, the ordinate intercepts are about the same for each concentration of kojic acid. This indicates that the inhibition is of the competitive type.

![Fig. 2. Effect of kojic acid on the D-amino acid oxidase.](image)

Tests with reconstructed D-amino acid preparations (4) containing rate-limiting concentrations of flavin-adenine dinucleotide indicated that the degree of inhibition of the oxidase obtained with a given ratio of kojic acid to amino acid was not a function of the concentration of nucleotide. This indicates that kojic acid competes with the oxidase for the substrate and not with the flavin for the specific protein. In this respect the inhibition produced by kojic acid is like that produced by benzoic acid (5) and is in contrast with the inhibition produced by atabrine and quinine, which compete with the flavin for the specific protein (6). The degrees of inhibition of a preparation of D-amino acid oxidase made from dried kidney (cf. the
Kojic acid is therefore a more effective inhibitor of the oxidase than the other substances. It has been stated (6) that competitive inhibition of the d-amino acid oxidase is exhibited to some extent by compounds containing system (I). However, fumarate does not inhibit the oxidase in reasonable concentration, e.g. 0.005 M, and the ion of kojic acid (II) is not obviously related to (I). Thus, to attribute special significance to a relation between (I) and the presumed characteristic configuration of the enzyme which permits activity and reversible inhibition seems without more than casual merit.

The rates of oxidation of the following substances by the tissues indicated were not inhibited by 0.005 M kojic acid: succinate by muscle, liver, kidney, and brain; tyramine and isoamylamine by brain, kidney, and liver; L-proline, hydroxy-L-proline, choline, and uric acid by liver.

In the case of the substances whose oxidation by brain, liver, and kidney
was not inhibited by kojic acid, increases in rate of oxidation were consistently produced by the presence of kojic acid. The oxidation of succinate by muscle was not so affected. The oxidation of succinate by liver, depicted in Fig. 3, illustrates this effect. It is possible that the increase in rate is attributable to depression by kojic acid of the activity of systems competing in some way with the oxidative systems in question. This possibility was tested in the case of succinate.

The usual preparations of liver, kidney, and brain were centrifuged, and the precipitates washed in the centrifuge with 0.05 M potassium phosphate, pH 7.4, and finally suspended in enough buffer to restore the original volumes. The effect of this procedure on the oxidation of succinate by liver, which is representative of the effects on the other tissues, is indicated in Fig. 3. The pertinent points are that kojic acid still inhibited the oxygen uptake of the washed control, and the increase in rate of oxidation of succinate produced by kojic acid, while decreased by washing, still persisted. It seems, therefore, that the increase in rate of oxidation of succinate produced by the acid depends upon depression of uptake of the control. These points, coupled with the fact that the oxygen uptake of muscle and its oxidation of succinate were not appreciably affected by kojic acid, suggest that in the case of succinate, and presumably the other substrates, the increase in rate of oxidation effected by the acid is consequent upon depression of competing systems.

**SUMMARY**

Kojic acid in relatively low concentrations, *in vitro*, inhibits the oxidation of D-amino acids, xanthine, L-phenylalanine, and L-methionine by liver and the oxidation of D-amino acids and L-phenylalanine by kidney. Oxidation of L-tyrosine by liver is inhibited by relatively high concentrations of the acid. The acid competes reversibly with the substrate for the D-amino acid oxidase. The inhibition of the other oxidations also appear to be of the competitive type.
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J. Raymond Klein and Norman S. Olsen


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