The Metabolic Fate of Gallic Acid and Related Compounds

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The discovery in this laboratory that 3,4-dihydroxyphenyl compounds such as caffeic acid and 3-(3,4-dihydroxyphenyl)-\(L\)-alanine undergo \(O\)-methylation as well as dehydroxylation in the animal body (1) has been confirmed by others (2-4). We are not aware of any reports of monohydroxyphenyl compounds undergoing either of these metabolic changes. The question is raised as to what the metabolic fate would be of trihydroxyphenyl compounds such as gallic (2,3,4-trihydroxybenzoeic) and 2,3,4-trihydroxybenzoeic acids.

According to a report by Tomsett (5), the pyrogallol detected in human urine was probably derived from gallic acid by decarboxylation in the alimentary tract. In a subsequent report this worker presented evidence that oral ingestion of tannic acid by man leads to an increased excretion of both 3,4-dihydroxyphenolic and 3-methoxy-4-hydroxyphenolic compounds (4).

Interest in the metabolic fate of gallic acid also stems from its natural occurrence in foods, the use of propyl gallate as an antioxidant in fats and oils, and the earlier use of tannic acid in the treatment of burns (6).

In the present study it will be shown that \(O\)-methylation of gallic acid and 2,3,4-trihydroxybenzoeic acid accounts for the major metabolites in the urine of rats and rabbits ingesting these compounds.

**EXPERIMENTAL**

Methods—The collection of the urine and the paper chromatographic procedures used for the detection of the urinary phenolic metabolites have been previously described (7). The purified diet fed to the rats and the semipurified diet fed to the rabbits have also been described previously (8).

Materials—Gallic acid, pyrogallol, syringic acid, \(n\)-propyl gallate, tannic acid, and 2,3,4-trihydroxybenzoeic acid were purchased from commercial sources. The 4-O-methyl gallic acid was prepared according to the method of Schön and Winterhalder (9). The 2-O-methyl pyrogallol and 3-methoxy-2,4-dihydroxybenzoeic acid were prepared according to Geissman and Mojé (10). The lauryl gallate used in these experiments was kindly supplied by the Animal Fats Laboratory, Eastern Utilization Research and Development Division, Philadelphia, Pennsylvania.

We are indebted to Dr. Leonard Jurd of the Fruit and Vegetable Chemistry Laboratory at Pasadena, California, for a sample of 3-O-methyl gallic acid. 3-Hydroxy-4,5-dimethoxybenzoeic acid was prepared by treating gallic acid with 3 mole equivalents of diazomethane. The product which was extracted from the etheral solution with 4% aqueous sodium hydroxide was hydrolyzed, and the resulting acid recrystallized from water giving a preparation melting at 195-196° (corrected). Fischer et al. (11) reported a value of 197-198°. All compounds were checked chromatographically for purity.

**RESULTS**

Rat Experiments—A diet containing 0.5% of gallic acid was fed to adult albino rats. The urine, collected without the addition of acid in the receiving flask, was normal in color and volume. Two-dimensional paper chromatograms of ether extracts of the acidified urine revealed the presence of one major metabolite in addition to gallic acid itself. The metabolite gave a stable yellow-orange color when sprayed with diazotized sulfanilic acid-sodium carbonate which did not turn black as would be expected if a catechol structure had been involved. Dehydroxylation was ruled out since the \(R_f\) values were not in agreement with those of either 3,4- or 3,5-dihydroxybenzoeic acid. The presence of a carboxyl group was indicated by the fact that the metabolite was extractable with ether from an acid solution but not from an alkaline solution. These observations suggested the possibility that the metabolite was 4-O-methyl gallic acid. When this compound was synthesized its characteristics were identical with those of the urinary metabolite as judged by \(R_f\) values, behavior under ultraviolet light and color after spraying with diazotized sulfanilic acid followed by sodium carbonate (Table I). The isolation of this compound from urine is described under rabbit experiments.

Small amounts of a second metabolite of gallic acid were also detected on the two-dimensional paper chromatogram of the ether extract of the urine. When the ether extracted urine was hydrolyzed (2 N HCl reflux for 1 hour), then extracted into ether and chromatographed, an additional amount of this minor metabolite was obtained. Since gallic acid and 4-O-methyl gallic acid are stable to acid hydrolysis under these conditions, it may be concluded that some of this metabolite was present in the urine as a conjugate. The chromatographic properties of this compound after hydrolysis were identical with those of 2-O-methyl pyrogallol (Table I).

When gallic acid was given to rats by stomach tube (100 mg per rat) the results were similar to those obtained when the compound was ingested in the diet.

In order to bypass the gastrointestinal tract and avoid effects caused by intestinal microorganisms, rats were given gallic acid by intraperitoneal injection (100 mg per rat). Chromatographic examination of the urine revealed the presence of 4-O-methyl gallic acid as well as gallic acid itself. However, an additional metabolite with the same chromatographic behavior as pyrogallol...
was clearly evident (Table I). A trace of 2-O-methyl pyrogallol was also present. Pyrogallol was subsequently isolated from the urine of rabbits receiving gallic acid in the diet.

Additional evidence of O-methylation of a trihydroxyphenyl compound was obtained when rats were given 2,3,4-trihydroxybenzoic acid by stomach tube (100 mg per rat). Some of the administered compound was found in the urine, along with the major metabolite which was identified chromatographically as 3-methoxy-2,4-dihydroxybenzoic acid (Table I).

When rats were given 4-O-methyl gallic acid, the only spots on the chromatogram other than those accounted for in control urine were the compound given, and a conjugate area which on hydrolysis was converted to 4-O-methyl gallic acid. The excretion of a dimethoxy derivative of 4-O-methyl gallic acid by O-methylation of either the number 3 or 5 hydroxyl group was not observed. However, when rats were given 3-O-methyl gallic acid (100 mg per rat by stomach tube), two dimethoxy derivatives were readily detected in the urine. These were identified chromatographically as 3,5-dimethoxy-4-hydroxybenzoic (syringic) acid and 3,4-dimethoxy-5-hydroxybenzoic acid (Table I).

The metabolism in vivo of esters of gallic acid was also studied with the use of propyl and lauryl gallate. Each of these compounds was given to rats by stomach tube (100 mg per rat). The major metabolite in each case was 4-O-methyl gallic acid. In addition, an area on the chromatograms corresponding to gallic acid was clearly evident, indicating cleavage of the ester linkages of propyl and lauryl gallate had taken place after oral ingestion.

The administration of tannic acid to rats by stomach tube (100 mg per rat) yielded the same results as obtained when gallic acid was administered, in that 4-O-methyl gallic acid was the major metabolite excreted. An area corresponding to gallic acid was also located on the same chromatogram. No other metabolites including 3,4-dihydroxyphenolic and 3-methoxy-4-hydroxyphenolic compounds were detected. The sample of tannic acid used was not entirely free from gallic acid as judged by the two-dimensional chromatogram. However, the amount of 4-O-methyl gallic acid excreted could not be accounted for entirely by the small amount of gallic acid present in the tannic acid as an impurity.

When pyrogallol was given to rats by stomach tube (50 mg per rat), there was no evidence of pyrogallol per se or metabolites thereof in the urine.

Gallic acid was incubated with rat and rabbit liver slices in bicarbonate buffer (pH 7). The chromatogram of the ether extracts of the medium contained 4-O-methyl gallic acid. No other metabolites such as vanillic acid or protocatechuic acid were detected. Similarly, 3-methoxy-2,4-dihydroxybenzoic acid was formed from 2,3,4-trihydroxybenzoic acid. Thus the results in vitro and in vivo are in agreement.

Semi-quantitative data on the excretion of 4-O-methyl gallic acid were obtained by a comparison of appropriate dilutions of the hydrolyzed urine of rats receiving gallic acid by stomach tube with known amounts of authentic 4-O-methyl gallic acid. After a 100-mg dose of gallic acid per rat by stomach tube or by intraperitoneal injection, approximately 25% of the gallic acid given (23 mg) was excreted as the methylated derivative. When the amount of gallic acid given was reduced to 10 mg per rat, nearly 60% (1.6 mg) was excreted as 4-O-methyl gallic acid.

**Rabbit Experiments**—After adjustment to the special diet, New Zealand white rabbits weighing approximately 2 kg were fed this diet containing 0.5% gallic acid. Chromatograms of the ether extracts of the acidified urine contained 4-O-methyl gallic acid, pyrogallol, and an additional area on the chromatogram suspected to be a conjugate. When this area was cut from an unsprayed chromatogram and hydrolyzed (2 hr HCl reflux for 1 hour), the ether extract of the hydrolysat was found to contain pyrogallol and 4-O-methyl gallic acid. As mentioned earlier, neither gallic acid nor 4-O-methyl gallic acid are decarboxylated under these conditions of acid hydrolysis. When the aqueous phase of the rabbit urine (urine minus ether solubles) was similarly hydrolyzed, both pyrogallol and traces of 2-O-methyl pyrogallol were readily detected on the chromatogram.

The chromatographic evidence for the identification of 4-O-methyl gallic acid and pyrogallol was confirmed by their isolation from rabbit urine after feeding a diet containing 0.5% gallic acid. The urine was collected in acid (HCl) and extracted with ether in a liquid-liquid extractor for 24 hours. The ether extract was evaporated and the residue dissolved in a small volume of water made slightly alkaline with sodium bicarbonate. This aqueous solution was extracted with ether; pyrogallol was crystallized from this extract. The alkaline aqueous phase was then acidified, extracted exhaustively with carbon tetrachloride and with chloroform. These extracts were discarded. Then the aqueous phase was extracted with ether. This ether extract was chromatographed on several sheets of Whatman No. 1 filter paper.

<table>
<thead>
<tr>
<th>Compound</th>
<th>RF CHCl-acetic- KCl</th>
<th>Appearance under ultraviolet</th>
<th>Color with diazotized sulfanilic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>0.00</td>
<td>Darkens slightly</td>
<td>Blue</td>
</tr>
<tr>
<td>4-O-Methyl gallic acid</td>
<td>0.16</td>
<td>Deep blue</td>
<td>Yellow-orange</td>
</tr>
<tr>
<td>2,3,4-Trihydroxy-benzoic acid</td>
<td>0.13</td>
<td>Absorbs</td>
<td>Red</td>
</tr>
<tr>
<td>3-Methoxy-2,4-dihydroxybenzoic acid</td>
<td>0.65</td>
<td>Deep blue</td>
<td>Yellow-orange</td>
</tr>
<tr>
<td>3-O-Methyl gallic acid</td>
<td>0.20</td>
<td>Blue</td>
<td>Yellow-orange</td>
</tr>
<tr>
<td>3,4-Dimethoxy-5-hydroxybenzoic acid</td>
<td>0.76</td>
<td>Deep blue</td>
<td>Red</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>0.84</td>
<td>Absorbs</td>
<td>Darkens</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>0.14</td>
<td>Deep blue</td>
<td>Darkens slightly</td>
</tr>
<tr>
<td>2-O-Methyl pyrogallol</td>
<td>0.61</td>
<td>Absorbs</td>
<td>Darkens slightly</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>0.00</td>
<td>Absorbs</td>
<td>Absorbs</td>
</tr>
<tr>
<td>Propyl gallate</td>
<td>0.33</td>
<td>Absorbs</td>
<td>Absorbs</td>
</tr>
<tr>
<td>Lauryl gallate</td>
<td>0.52</td>
<td>Deep blue</td>
<td>Green, then darkens</td>
</tr>
</tbody>
</table>

**Table I**

**Chromatographic behavior of trihydroxyphenolic derivatives**

<table>
<thead>
<tr>
<th>Appearance under ultraviolet</th>
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<tr>
<td>Deep blue</td>
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</tr>
<tr>
<td>Deep blue</td>
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<td>Blue</td>
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<td>Blue</td>
<td>Absorbs</td>
</tr>
<tr>
<td>Blue</td>
<td>Absorbs</td>
</tr>
</tbody>
</table>
paper with chloroform-acetic acid-water (2:1:1; lower phase). The appropriate bands corresponding to 4-O-methyl gallic acid were eluted and the eluate rechromatographed on several sheets of paper with 20% KCl. Once again the appropriate bands were cut out and eluted. The eluate was reconstituted in a small volume of water, filtered over charcoal and Celite and crystals of 4-O-methyl gallic acid appeared upon standing. The two isolated metabolites had the same crystallographic measurements as synthetic pyrogallol and 4-O-methyl gallic acid respectively.

**DISCUSSION**

The major urinary metabolites of gallic, 2,3,4-trihydroxybenzoic, and of 3-methoxy-4,5-dihydroxybenzoic acids are shown in Fig. 1. Additional metabolites may eventually be identified, particularly if other methods of detection are used. The results clearly indicate that O-methylation and decarboxylation are the reactions involved in the metabolic conversions of the administered compounds. It is interesting to note that the middle hydroxyl group of both gallic acid and 2,3,4 trihydroxybenzoic acid becomes methylated. This group is para to the carboxyl group in the case of gallic acid and meta in the case of 2,3,4-trihydroxybenzoic acid. This selective O-methylation prevents the formation from either compound of the potential ortho dihydroxy (catechol) configurations. Had O-methylation taken place on either of the outer hydroxyl groups, then a catechol configuration such as 3-O-methyl gallic acid, for example, would have been formed. In this case, further metabolic change leading to the expenditure of another methyl group would be expected, on the basis of previous experience with 3,4-dihydroxyphenolic acids (1). This prompted us to study the metabolic fate of 3-O-methyl gallic acid. As predicted, O-methylation of a second hydroxyl group occurred as evidenced by the appearance in the urine of two dimethoxy derivatives: syringic acid and 3,4-dimethoxy-5-hydroxybenzoic acid. On the other hand, when 4-O-methyl gallic acid, which does not contain a catechol structure, was fed, no further methylation was detected. It is tempting to generalize from these results that O-methylation is a preferred mechanism for the metabolism of acids containing a catechol nucleus.

The observation that as much as 25% of the administered gallic acid is methylated would be expected to bring about an increased dietary requirement for methyl donors. This aspect of the metabolism of gallic acid is currently being investigated.

None of the hydroxyl groups of gallic acid or tannic acid were removed (dehydroxylated). The paper chromatographic system used here would have detected such a reaction if it had occurred. In this respect our results with rats and rabbits differ from those obtained by Tomssett (4) with human beings since dehydroxylation would have to be involved to account for the reported excretion of 3,4-dihydroxy- and 3-methoxy-4-hydroxybenzoic acids after the ingestion of tannic acid. There is agreement concerning the excretion of pyrogallol by man (4) as well as by rats and rabbits. The fact that pyrogallol was found in the urine of rats after the intraperitoneal injection of gallic acid indicates that decarboxylation is not dependent on passage through the gastrointestinal tract.

The use of lauryl and propyl gallate as food additives (antioxidants) is currently being practiced. Studies on the chronic toxicity of lauryl gallate did not indicate any deleterious effects when dietary levels up to 0.5% were fed to rats (12). In view of our finding of a major pathway for the metabolism and excretion of gallic acid by means of O-methylation, it is understandable that symptoms of choline deficiency would not be expected as long as the diet contains an adequate reserve of methyl donors.

**SUMMARY**

O-Methylation resulting in the formation of 4-O-methyl gallic acid accounts for the major metabolite in the urine of rats or rabbits ingesting gallic acid, propyl gallate, lauryl gallate, or tannic acid. Decarboxylation accounts for a second metabolite identified as pyrogallol in the urine of rats receiving gallic acid by intraperitoneal injection or rabbits receiving gallic acid in the diet. 3-Methoxy-2,4-dihydroxybenzoic acid was the major urinary metabolite of rats given 2,3,4-trihydroxybenzoic acid. Syringic acid and 3,4-dimethoxy-5-hydroxybenzoic acid were identified as urinary metabolites of rats receiving 3-O-methyl gallic acid.

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