Metabolism of Gallic Acid and Tea Catechin by Rabbit

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Received July 4, 1964

Metabolism of gallic acid in the rabbit has been studied. Orally administered gallic acid was excreted mostly unchanged and partially as pyrogallol and 4-methoxy-3,5-dihydroxybenzoic acid in the urine. Conjugates could hardly be found. Moreover, evidently by administration of gallic acid three phenolic acids in the urinary excretion were diminished compared with that of fed on the control diet. Effect of tea catechin was also studied.

There are many reports concerning metabolism of phenolic substances in the animal body. Namely, detoxication reactions such as conjugation,¹⁾ hydroxylation,²⁾ methylation,^{3~8)} dehydroxylation,^{9~11)} and benzol nucleus decomposition¹²⁾ have been known. In this reports, the metabolism of gallic acid and gallocatechin, and the effects on the excretion of other phenolic substances in the urine were studied after oral administration of these substances to rabbit. Gallic acid is ingested by man in the free form or as derivatives present in tea, and gallocatechin also constitutes about 5% of the tea catechins.

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MATERIALS

Gallic acid and pyrogallol were commercial products. The following compounds were synthesized for comparison with the metabolites in the urine. Homoprotocatechuic acid was prepared by demethylation of homoveratric acid¹³⁾ by refluxing for three hours with three parts of pyridine hydrochloride.¹⁴⁾ *m*-Hydroxyphenylacetic acid was prepared from *m*-methoxyphenylacetic acid¹⁵⁾ by demethylation with pyridine hydrochloride in the same way. 4-Methoxy-3,5-dihydroxybenzoic acid,¹⁶⁾ syringic acid,¹⁷⁾ 3-methoxy-4,5-dihydroxybenzoic acid,¹⁸⁾ protocatechuic acid,¹⁹⁾ *m*-hydroxybenzoic acid,²⁰⁾ vanillic acid²¹⁾ and homovanillic acid²²⁾ were synthesized by the methods described in the literature cited. Tea catechin was prepared by extraction of dried and powdered tea leaves with ethylacetate and pale brown product consisted of about 35% epigallocatechin and *dl*-gallocatechin and about 55% gallo catechingallate and catechingallate.²³⁾

EXPERIMENTALS

Two groups of 2 male rabbits of about 3 Kg were

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fed for a week with bean-curd leavings* only as the basal diet. To each animal of the group, 0.5 g of gallic acid or tea catechin per day per Kg of body weight was administered orally with the basal diet, respectively. The combined urine, collected in the receiving vessel, was kept acidic by addition of 10% hydrochloric acid to avoid oxidation of phenolic compounds. Over the period of 10 days were collected 6.8 L of urine from the gallic acid-fed, 7.1 l from the tea catechin-fed and 5.0 l from the basal diet-fed group.

Extraction

The collected urine, filtered and saturated with sodium chloride, was extracted three times with an equal volume of ethylacetate. The ethylacetate solution was dried with sodium sulfate and concentrated under vacuum in the nitrogen atmosphere. From the urine of the gallic acid- and the catechin-fed rabbits were obtained 14.5 g and 10.1 g of dark brown residues, respectively. In the same way, from the urine of control group was obtained 6.1 g of brown residue.

Paperchromatography of the Extracts

The components in the extracts were examined by two-dimension paperchromatography using solvent systems of chloroform-acetic acid-water (2:1:1 V/V) and 20% aqueous solution of potassium chloride. Each phenolic compound on the paperchromatogram was detected with the diazotized sulfanilic acid-sodium carbonate aqueous solution²⁴⁾ and compared with the authentic substances (Fig. 1).

The spots A, C and D were found only in the extracts of the urine of the gallic acid-fed rabbits and it was considered that A, C and D were pyrogallol, 4-methoxy-3,5-dihydroxybenzoic acid and gallic acid, respectively.

Identification of A, C and D

1) Separation of A, C and D

Two grams of the extracts from the urine of the gallic acid-fed rabbits was dissolved in 2 ml of ethanol, absorbed in 2 g of cellulose powder (Toyoroshi 200~300 mesh) and deposited on the top of a column of cellulose powder (2.3 cm × 45 cm) and the ethanol of the top layer was removed by evacuating the column from the top. Elution was performed with 1 l of

* Bean-curd leavings is the residue obtained by filtration of soluble proteins of the milk of ground and boiled soy beans through cloth in the process of bean-curd manufacture.

24) H. G. Bray, W. V. Thorpe and K. White, *Biochem. J.*, **46**, 271 (1950).

FIG. 1. Paperchromatogram of Ethylacetate Extracts of the Urine of Gallic Acid-fed Group and Control Group.

Colored with diazotized sulfanilic acid and 20% Na₂CO₃.

●: Spots found only for the gallic acid-fed group.

○: Spots which were found for the control group and not found for the gallic acid-fed group.

A: Pyrogallol.

C: 4-Methoxy-3,5-dihydroxybenzoic acid.

D: Gallic acid

chloroform-acetic acid-water (2:1:1 V/V) at a flow rate of 5 ml per minute and each fraction contained 20 ml of the effluent was collected. Substance C was found in the fractions No. 35~43. The column was washed further with 200 ml of the same solvent and dried completely under vacuum. Elution was restarted with 20% aqueous solution of potassium chloride at a rate of 0.5 ml per minute and each fraction contained 10 ml of the effluent was collected. The substance A was found in the fractions No. 15~18 and the substance D was found in the fractions No. 26~36 by the paperchromatographic examination.

2) Identification

Substance A. Substance A was extracted from the combined corresponding fractions with ethylacetate. The ethylacetate solution was dried with sodium sulfate and evaporated under vacuum. The residue was recrystallized from water to give 50 mg of crystals, m.p. 132°C. Depression of the melting point was not observed when mixed with pyrogallol. *Anal.* Found: C, 57.12; H, 5.65. *Calcd.* for C₆H₆O₃: C, 57.17; H, 5.80%.

Substance C. Substance C was obtained from the

FIG. 2. Urinary Metabolites of Gallic Acid Fed to the Rabbit.

corresponding fractions in the same way. After recrystallization from water 35 mg of crystals was obtained. m.p. 242°C (decomp). The infrared spectra of C and authentic sample 4-methoxy-3,5-dihydroxybenzoic acid showed no difference in absorption. *Anal.* Found: C, 52.08; H, 4.32. Calcd. for $\text{C}_8\text{H}_6\text{O}_5$: C, 52.18; H, 4.38%.

Substance D. In the same way, substance D was extracted from the corresponding fractions with ethylacetate. After evaporation of the solvent, the residue was recrystallized from water with aid of activated charcoal, and 45 mg of white needle crystals were obtained. m.p. 250°C (sintering at 225°C). Its R_F value and color reaction were the same as those of gallic acid. *Anal.* Found: C, 49.35; H, 3.94. Calcd. for $\text{C}_7\text{H}_6\text{O}_5$: C, 49.42; H, 3.55%.

The ethylacetate-extracted urine was treated in 2N H_2SO_4 at 100°C for one hour and extracted with ethylacetate. The components in the extract were examined in the same way and no conjugate of the metabolite of gallic acid was found.

In the case of the administration of tea catechins, the paperchromatogram of the extract of the urine was almost the same as in the case of the administration of gallic acid and the other spot with diazotized sulfanilic acid reagent, was not found.

Substance P, E and K (Fig. 1) were found only in the urine of basal diet-fed group and were presumed to be homovanillic acid, homoprotocatechuic acid and *m*-hydroxyphenylacetic acid respectively in view of the R_F values and color reactions on the paperchromatogram.

DISCUSSION

It was found that gallic acid, fed to the rabbit orally in a large quantity, was excreted mostly unchanged in the urine, and some

part of it was metabolized to pyrogallol and 4-methoxy-3,5-dihydroxybenzoic acid. When gallic acid was fed to the rabbit as the mixture of tea catechins, only the metabolites of gallic acid presumably originating from the gallates of the catechin mixture were found but the other blue or blue black spot that is characteristic of trihydroxyphenyl radical of gallic acid with diazotized sulfanilic acid reagent was not found in the paperchromatogram. Therefore, it was considered that trihydroxyphenyl radical of gallic acid underwent a rapid metabolic decomposition.

The remarkable difference of the urine components of the gallic acid-fed rabbit compared with that of control group was the disappearance of three phenolic acids. These acids are presumably homovanillic acid, homoprotocatechuic acid and *m*-hydroxyphenylacetic acid in view of the R_F values and color reactions on the paperchromatogram. Previously Tompsett²⁵⁾ reported that oral ingestion of tannic acid by man caused an increased excretion of both 3,4-dihydroxy and 3-methoxy-4-hydroxybenzoic acids. Though it has been known that the metabolism of phenolic com-

FIG. 3. Metabolic Transformation of DOPA.

25) S. L. Tompsett, *J. Pharm. and Pharmacol.*, **11**, 32 (1959).

TABLE I. R_F VALUES AND COLOR REACTIONS OF PHENOLIC COMPOUNDS

Phenolic oxycarbonic acid	R_F values		Color with diazotized sulfanilic acid
	CHCl_3 -acetic acid- H_2O	20% KCl	
Gallic acid	0	0.33	Dark violet
3-Methoxy-4,5-dihydroxybenzoic acid	0.18	0.32	Light violet
3,4-Dimethoxy-5-hydroxybenzoic acid	0.85	0.50	Orange-yellow
Syringic acid	0.88	0.33	Red
4-Methoxy-3,5-dihydroxybenzoic acid	0.11	0.51	Brown-yellow
Pyrogallol	0.05	0.61	Pale violet then dark brown
<i>m</i> -Hydroxybenzoic acid	0.41	0.56	Yellow
<i>m</i> -Hydroxyphenylacetic acid	0.47	0.84	Dark yellow
Protocatechuic acid	0.03	0.41	Violet
Homoprotocatechuic acid	0.07	0.80	Violet
Vanillic acid	0.88	0.43	Orange
Homovanillic acid	0.78	0.85	Light violet

pounds differs depending on the species of the animal generally, it is considered that the oral ingestion of gallic acid in a large quantity has some influence upon the metabolism of phenolic compounds such as DOPA,⁵⁾ and could have caused the disappearance of these

phenolic acids from the urine of the rabbit.

Acknowledgements. The authors express their sincere gratitude to Dr. M. Fujisawa and Dr. N. Sugimoto of Osaka Research Laboratory, Tanabe Seiyaku Co., Ltd. for their continuous interest and support.