Resolution of N²-Phenacyl-N⁶-benzoyl-DL-lysine (II) by the Metabolism of KT-313——KT-313 was inoculated into 100 cc. of the culture medium mentioned above containing 1.84 g. of N²-phenacyl-N⁶-benzoyl-DL-lysine and incubated at 25° for 5 days.   The culture medium was treated according to the resolution procedure of di-N²,N⁶-benzoyl-DL-lysine and afforded 0.33 g. (52.8%) of N⁶-benzoyl-L-lysine (III) as colorless leaves, m.p. 270°(decomp.); $[\alpha]_D^{20}$: +19°(c=2, 5N HCl) (*Anal.* Calcd. for C₁₃H₁₈-O₃N₂: C, 62.38; H, 7.25.   Found: C, 62.53; H, 6.44.), 0.85 g. (93%) of N²-phenacyl-N⁶-benzoyl-D-lysine (V) as colorless leaves, m.p. 134~135°; $[\alpha]_D^8$: −3.3°(c=3, EtOH) (*Anal.* Calcd. for C₂₁H₂₄O₄N₂: C, 63.73; H, 7.55.   Found: C, 63.93; H, 7.78), and 0.04 g. (10%) of phenylacetic acid, m.p. 74~76°. (III) was characterized as its phenylacetate (III P), m.p. 134~135°; $[\alpha]_D^8$: +3.3 (c=3, EtOH), obtained in 67.9% yield after recrystallization from Me₂CO and benzene.   The mixture of (V) and (III P) melted at ca. 149°.   Incidentally, N²-Phenacyl-N⁶-benzoyl-DL-lysine melts at 150~151°.

Summary

A certain strain (KT-313) isolated from soil had the metabolic activity shown in Table I and could metabolize di-N²,N⁶-benzoyl-DL-lysine (I) and N²-phenacyl-N⁶-benzoyl-DL-lysine (II) to produce N⁶-benzoyl-L-lysine (III), di-N²,N⁶-benzoyl-D-lysine (IV) and N²-phenacyl-N⁶-benzoyl-D-lysine (V), respectively.   KT-313 seemingly belongs to the *Pseudomonas* group.   It is of interest that KT-313 hydrolyzed benzoyl (IIIB) and phenacyl (IIIP) derivatives of N⁶-benzoyl-L-lysine (III), while KT-301 hydrolyzed (IIIB), but not (IIIP), and KT-311 hydrolyzed (IIIP), but not (IIIB).

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133. Tchan Gi Bak[*1] and Shigeaki Kuwano : Reversible and Irreversible Inhibitions of Glutamic and Arginine Decarboxylase Activities of *Escherichia coli* by Gallic Acid and *d*-Catechin.

(*Kotaro Institute for Physiological Chemistry of Crude Drugs*[*2])

Kimura, *et al.* have found that tannins and related compounds inhibit the decomposition of amino acids to form diamines [1], hydrogen sulfide[2] and indole[3] by pyridoxal phosphate-dependent enzymes of *Escherichia coli*.   It has also been shown that gallic acid and *d*-catechin, characteristic components of pyrogallol- and catechol-tannins respectively, inhibit the enzyme reactions through somewhat different mechanisms.

The present paper deals with the kinetic studies on the inhibition of two pyridoxal phosphate-requiring enzymes of *E. coli*, i.e. glutamic and arginine decarboxylases, by gallic acid and *d*-catechin.   It was especially aimed to elucidate in details the differences in inhibition mechanisms due to gallic acid and *d*-catechin.   Efforts were also made to show that these inhibitions are caused by both reversible and irreversible

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1) K. Kimura, S. Kuwano, H. Hikino : Yakugaku Zasshi, **78**, 236 (1958).
2) K. Kimura, K. Yamauchi, S. Kuwano : This Bulletin, **7**, 426 (1959).
3) *Idem* : *Ibid.*, **7**, 531 (1959).

types of mechanism as suggested by Vallee, *et al.*,[4] Margoliash, *et al.*,[5] and Kuwano, *et al.*[6] for other enzyme–inhibitor systems.

Materials and Methods

Acetone-dried Cells——Strains ML 32400 and 7020 (E. F. Gale) of *E. coli*, kindly supplied from the Research Institute for Microbial Diseases, Osaka University, were used as the sources of glutamic and arginine decarboxylases, respectively.   They were grown at 37° for 18 hr. in a medium containing 2% each of casein tryptic digest,*3 glucose, and $NaH_2PO_4 \cdot 2H_2O$, 0.5% of yeast extract, 0.1% of NaCl, and 0.05% of $MgSO_4 \cdot 7H_2O$. The pH of this medium was adjusted to 5.5 with NaOH. After cultivation, the cells were harvested, washed twice with distilled water, and suspended in distilled water. Me_2CO-dried cells were prepared by treating the suspension with 20 vol. of Me_2CO at −35°. The dried cells thus prepared were used as enzyme preparations.   The Me_2CO-dried cells of *E. coli* ML 32400 have been shown to decarboxylate L-glutamic acid immediately at pH 3.6 to 5.0, but not arginine, histidine, lysine, etc.   According to Gale,[7] on the other hand, L-arginine is the only substrate which is decarboxylated by the dried cells of *E. coli* 7020.

Assay of Enzyme Activities——The decarboxylase activities were measured at 37° by the conventional manometric technique using air as the gas phase.   The manometric vessel contained 0.3 mg. (ML 32400) or 0.5 mg. (7020) of dried cells, 1.0 cc. of 0.1*M* acetate buffer of pH 4.8 (for glutamic enzyme) or pH 5.2 (for arginine enzyme) containing, if necessary, inhibitor,*4 0.5 cc. of $4.8 \times 10^{-2}M$ of sodium glutamate or $3.6 \times 10^{-2}M$ of arginine hydrochloride, and distilled water to make the final volume to 3.0 cc.   The initial velocity v was estimated from the tangents at zero time of the CO_2 evolution–time curves and expressed in terms of μl. CO_2 evolved per 10 min. per mg. of dried cells. The manometric readings were taken at 10 min. intervals for 60 min.   Gallic acid and *d*-catechin showed neither O_2 uptake nor CO_2 output when added to the enzyme preparations in the absence of substrates.

Other Methods——A Horiba model T pH titrator was used for pH measurements.   The inhibitors used were prepared as described by Kimura, *et al.*[1,2]

Results

Inhibitions by Gallic Acid and *d*-Catechin——The time courses of two decarboxylase reactions in the presence and absence of gallic acid and *d*-catechin are shown in Fig. 1 in which the logarithms of remaining substrate concentrations (μmoles per reaction vessel) are plotted against reaction time.   It will be seen that glutamic decarboxylase was inhibited by both inhibitors and the inhibitions increased with time.   The inhibited reactions proceeded according to the first-order kinetics during the initial 20 minutes, but was considerably slowed down when the reaction time exceeded over 30 minutes. It was suggested that the inhibition mechanism had changed during the reaction. Arginine decarboxylase, on the other hand, was only slightly inhibited by the inhibitors, but even in this case the inhibition seemed to increase as the reaction proceeded.   The time-dependent nature of the inhibitions were more clearly demonstrated by the experiments shown in Fig. 2 in which the dried cells were preincubated with gallic acid or *d*-catechin prior to the initiation of the reaction.   It will be clear that the inhibitions increased with increasing preincubation time up to 60 minutes.

Reversibility and Irreversibility of Inhibitions——The reversibility of the inhibitions was examined by both dilution and dialysis methods.   The dilution experiments were

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*3 Commercial preparation of Wako Pure Chemical Industries, Ltd., Osaka.

*4 Adjusted to the required pH by 0.1*M* sodium acetate or *N* NaOH in the case of gallic acid and other acids.

4) F. L. Hoch, R. J. P. Williams, B. L. Vallee : J. Biol. Chem., **232**, 453, 465 (1958).

5) E. Margoliash, A. Novogrodsky, A. Schejter : Biochem. J., **74**, 339 (1960).

6) S. Kuwano, K. Yamauchi : This Bulletin, **8**, 497 (1960); S. Kuwano, K. Yamauchi, T. G. Bak : *Ibid.*, **9**, 651 (1961).

7) E. F. Gale : "Methods of Biochemical Analysis," **4**, 285 (1957).   Interscience Publishers, Inc., New York.

carried out as follows. The dried cells were mixed with various amounts of gallic acid or *d*-catechin in 0.04M acetate buffer.[*5] The mixture was incubated at 37° for 30 minutes, and then diluted with the buffer until the inhibitor concentration was lowered to a fixed value. The diluted mixture was maintained at 37° for another 30 minutes to allow equilibration and then the enzyme activity was measured. Control experiments were similarly run without the inhibitors. Since the change in cell concentration alone did not alter the degrees of inhibition by gallic acid and *d*-catechin, it could be expected that the decrease in inhibitor concentration by dilution would result in the restoration of the inhibited activity to the level due to the decreased inhibitor concentration if the inhibition is completely reversible. The data shown in Figs. 3 and 4, however, indicate that the inhibitions after preincubation for 30 minutes were only partly reversible (dotted lines) at higher concentrations of the inhibitors. Figs. 5 and 6 further show that the inhibitions became more difficult to be reversed when the preincubation was made for longer period of time.
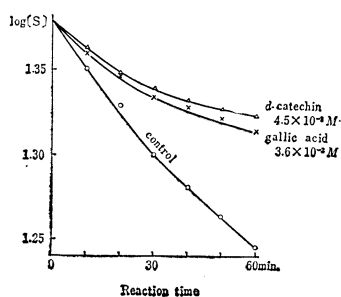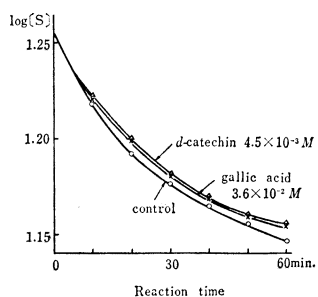
Glutamic decarboxylase system   Arginine decarboxylase system

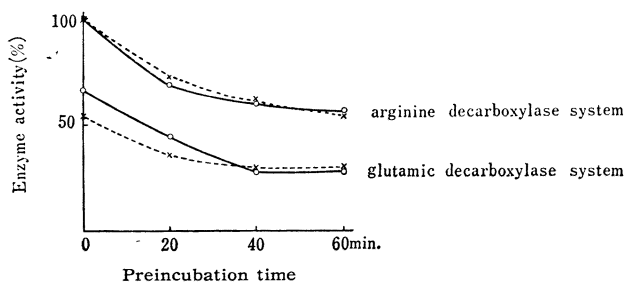Fig. 1.   Inhibitory Action of Gallic Acid and *d*-Catechin

Fig. 2. Effect of Preincubation
on Inhibition

o—o ; Gallic acid $4.3 \times 10^{-2} M$
x····x ; *D*-Catechin $5.4 \times 10^{-3} M$

Ordinate : per cent enzyme
activity to respective
controls

Concentration of gallic acid

o—o, o····o; Glutamic
decarboxylase system
x—x, x····x; Arginine
decarboxylase system

0.3 mg. (glutamic system) or 0.5 mg. (arginine system) of dried cells were preincubated with indicated concentrations of gallic acid in 2.5 cc. of $4 \times 10^{-2} M$ acetate buffer (pH 4.8 for glutamic system or pH 5.2 for arginine system) at 37° for 30 min. The enzyme activity in each vessel was measured immediately after preincubation and is plotted by solid lines (o—o and x—x).

The preincubation mixture was also diluted with $4 \times 10^{-2} M$ acetate buffer to make the final concentrations of gallic acid to $8 \times 10^{-3} M$ (indicated by arrow G) in the glutamic system and to $10^{-2} M$ (indicated by arrow A) in the arginine system. The diluted mixtures were then incubated. The enzyme activity measured after 30 min. incubation is plotted by dotted lines (o····o and x····x).

Ordinate : per cent enzyme activity to respective controls

Fig. 3.   Effect of Concentration of Gallic Acid on Reversibility of Inhibition

[*5] The same pH as the conditions of enzyme reaction, respectively.
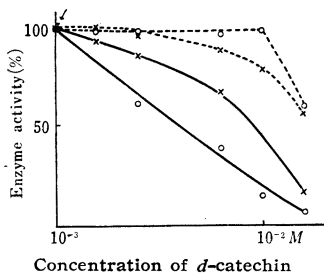
Concentration of d-catechin

Fig. 4. Effect of Concentration of
d-Catechin on Reversibility
of Inhibition

Experiments were carried out exactly
in the same ways as in Fig. 3 except that
d-catechin was employed as inhibitor ins-
tead of gallic acid and the final concent-
ration of d-catechin was made to $10^{-3}M$
(indicated by arrow) after dilution. The
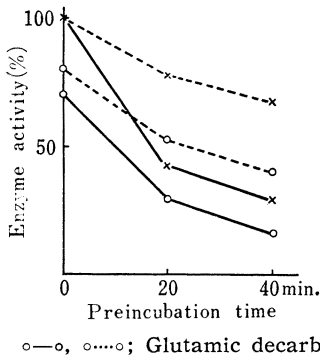other symbols are as in Fig. 3.

Preincubation time

0.3 mg. (glutamic system) or 0.5 mg. (arginine system) of dried
cells were preincubated with $8 \times 10^{-2}M$ gallic acid in 2.5 cc. of
$4 \times 10^{-2}M$ acetate buffer (pH 4.8 or 5.2) at 37° for indicated periods
of time (0, 20 or 40 min.). The enzyme activity in each vessel
was measured immediately after preincubation and is plotted
by solid lines (o—o and x—x).

The mixture was also diluted with $4 \times 10^{-2}M$ acetate buffer to
make the final concentration of gallic acid to $8 \times 10^{-3}M$. The
diluted mixture was further incubated for 60, 40 or 20 min.
depending on the first preincubation time being 0, 20 or 40 min.
The enzyme activity immediately after the second incubation
was measured and is plotted by dotted lines (o····o and x····x).

Ordinate : per cent enzyme activity to respective controls.

o—o, o····o; Glutamic decarboxylase system   x—x, x····x; Arginine decarboxylase system

Fig. 5. Effect of Preincubation Time on Reversibility of Gallic Acid Inhibition
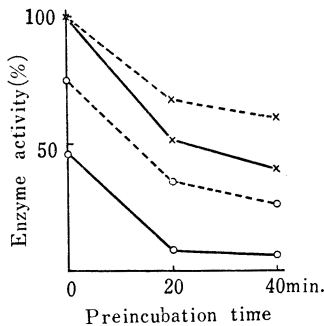

Preincubation time

Fig. 6. Effect of Preincubation Time on
Reversibility of d-Catechin Inhibition

Experiments were carried out in similar
ways to Fig. 5. But, d-catechin (final
concentration $2 \times 10^{-2}M$ for the glutamic
system or $10^{-2}M$ for the arginine system)
was added as inhibitor in the first pre-
incubation mixture, and the concentration
of d-catechin in diluted mixtures was
made to $2 \times 10^{-3}M$ for the glutamic system
or to $10^{-3}M$ for the arginine system.
Symbols are as in Fig. 5.

The dialysis experiments also showed that the inhibitions were only partly rever-
sible.   As will be seen from Table I, it was clearly shown that the activities of the
inhibited enzymes could not be fully restored to those of control experiments even if
the preincubated mixtures were throughly dialyzed until they showed no colorreaction
with ferric chloride.

It will be clear from these observations that the inhibition by gallic acid and
d-catechin increases as a function of both the inhibitor concentration and the duration
of preincubation.   These situations can be best explained, as in the inhibition of tryp-
tophanase by berberine,[6] by assuming the following interactions between enzyme (E)
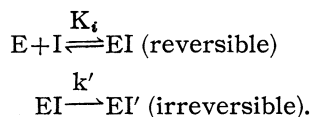and inhibitor (I) :

$$E + I \overset{K_i}{\rightleftharpoons} EI \text{ (reversible)}$$

$$EI \overset{k'}{\longrightarrow} EI' \text{ (irreversible).}$$

TABLE I. Reversal of Inhibition by Means of Dialysis[a]

Glutamic decarboxylase system

| | Control | Preincubated[b] | | | | | |
| | | with gallic acid (M) | | | with d-catechin (M) | | |
		2×10^{-2}	4×10^{-2}	10^{-1}	4×10^{-3}	8×10^{-3}	2×10^{-2}
Activity (v) before dialysis	102	63	46	10	50	30	7
(inhibition %)	—	(38.2)	(54.9)	(90.2)	(51.0)	(70.6)	(93.1)
Activity (v) after dialysis	63	60	56	30	60	33	27
(inhibition %)	—	(4.8)	(11.1)	(52.4)	(4.8)	(47.6)	(57.2)
Pyridoxal phosphate[d] 2 γ added	63	60	60	30	60	36	27
(inhibition %)	—	(4.8)	(4.8)	(52.4)	(4.8)	(42.9)	(57.2)

Arginine decarboxylase system

| | Control | Preincubated[c] | | | | | |
| | | with gallic acid (M) | | | with d-catechin (M) | | |
		2×10^{-2}	5×10^{-2}	10^{-1}	4×10^{-3}	8×10^{-3}	2×10^{-2}
Activity (v) before dialysis	64	44	28	12	42	30	8
(inhibition %)	—	(31.2)	(56.3)	(81.3)	(34.4)	(53.1)	(87.5)
Activity (v) after dialysis	52	40	32	10	46	44	12
(inhibition %)	—	(23.1)	(38.5)	(80.8)	(11.5)	(15.4)	(77.0)
Pyridoxal phosphate[d] 2 γ added	80	70	56	16	58	54	16
(inhibition %)	—	(12.5)	(30.0)	(80.0)	(27.5)	(32.5)	(80.0)

a) At below 5° for 4∼5 hr. through collodion membrane.
b) At 37° and pH 4.8 for 30 min., 0.6 mg. of dried cells plus inhibitor in a total volume of 2.5 cc. of $4 \times 10^{-2} M$ acetate buffer.
c) At 37° and pH 5.2 for 30 min., 1.0 mg. of dried cells plus inhibitor in a total volume of 2.5 cc. of $4 \times 10^{-2} M$ acetate buffer.
d) Disodium salt, product of Sigma Chemical Co.

This formulation implies that when the enzyme is mixed with the inhibitor, a dissociable enzyme–inhibitor complex (EI) is rapidly and reversibly formed (K_i being the dissociation constant of EI) and the complex is then slowly and irreversibly converted to another inactive form disignated as EI′ with a velocity constant k′.

The inhibition data reported above suggest that the complex EI is formed very rapidly in the glutamic decarboxylase system. In the arginine decarboxylase system, on the other hand, the formation of EI seems to be greatly reduced in the presence of substrate and therefore only slight inhibitions could be observed under this condition. It was, however, shown that even in the latter system considerable inhibition becomes observable if the enzyme is preincubated with the inhibitor before the addition of substrate.

The schemes shown above indicate that the formation of EI′ is dependent on the concentration of EI, the duration of preincubation, and k′. The results so far obtained seem to be compatible with these expectations. It is likely that the irreversible formation of EI′ is accompanied by the denaturation of the enzyme protein, and if this is really the case it is expected that the irreversible inhibition by gallic acid or d-catechin is augmented with increasing temperature. As can be seen from Fig. 7, it was in fact found that the inhibition did increase with increasing temperature. The increase in inhibition was especially pronounced in the presence of higher concentrations of the inhibitors as expected from increased irreversible inhibitory actions under these conditions.

Effects of Substrates and Pyridoxal Phosphate——In Figs. 8 and 9 are plotted the kinetic data, obtained at the initial phase of reactions, according to Lineweaver-Burk[8]

8) H. Lineweaver, D. Burk : J. Am. Chem. Soc., **56**, 658 (1934).
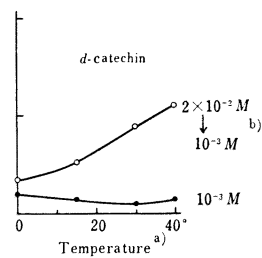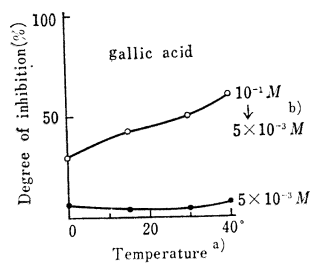
Glutamic decarboxylase system
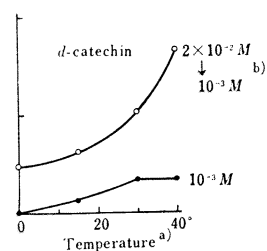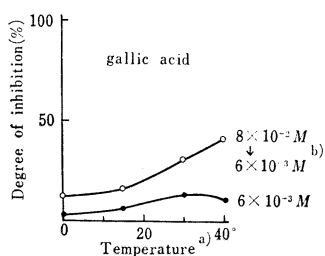
Arginine decarboxylase system

a) Indicates the temperature at which the enzyme and inhibitor were preincubated. After 30 min. preincubation the temperature of the reaction system was adjusted to 37°; the enzyme activity was measured at this temperature.

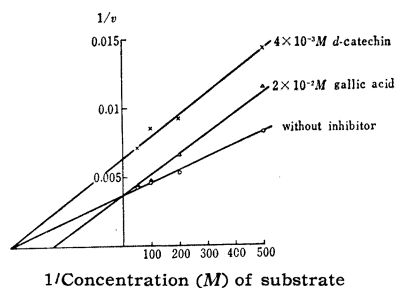b) ↓ indicates the dilution of inhibitor concentration before the measurement of enzyme activity.

Fig. 7. Effect of Temperature on Inhibition by Gallic Acid or d–Catechin

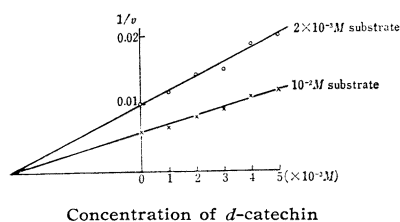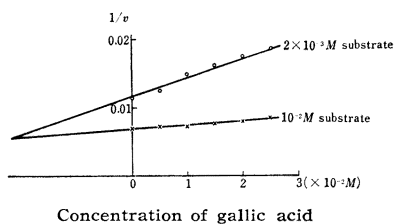Fig. 8. Lineweaver–Burk Plots for Glutamic Decarboxylase System

Fig. 9. Dixon Plots for Glutamic Decarboxylase System

and Dixon[9] methods, respectively. It will be clear that the inhibition of glutamic decarboxylase by gallic acid is competitive with respect to substrate concentration. The inhibition by d-catechin, on the other hand, appears to be of non-competitive type suggesting the difference in the two inhibition mechanisms. Under the experimental conditions employed, both inhibitions were freely reversible. In addition to gallic acid, protocatechuic acid and benzoic acid were also found to inhibit glutamic decarboxylase in competition with the substrate, while the inhibitions by methyl, ethyl and propyl esters of gallic acid were of non-competitive nature. It is likely that a competitive relationship exists between the free carboxylic group of gallic acid (inhibitor) and that of glutamic acid (substrate) in the enzyme reaction. Neither gallic acid nor d-catechin competes with pyridoxal phosphate in the glutamic decarboxylase system.

The inhibition of arginine decarboxylase by either gallic acid or d-catechin was markedly reduced in the coexistence of the substrate as already shown in Fig. 2. As will be seen from Table II, arginine decarboxylase was found to be competitively pro-

TABLE II. Protective Effect of Pyridoxal Phosphate on Inhibition
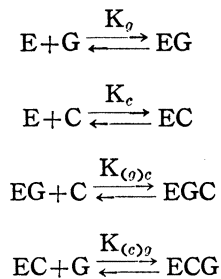of Arginine Decarboxylase by Gallic Acid

Pyridoxal phosphate added[a] (γ/3 cc.)	0		0.2		0.4		0.6		1.0		2.0	
Gallic acid added[a] ($4.3 \times 10^{-2}M$)	−	+	−	+	−	+	−	+	−	+	−	+
v	60	30	68	50	68	56	62	60	66	64	66	64
Inhibition (%)	−	(50.0)	−	(26.5)	−	(17.7)	−	(3.2)	−	(3.0)	−	(3.0)

   a) Pyridoxal phosphate and gallic acid are simultaneously added to 0.5 mg. of dried cells in $4 \times 10^{-2}M$ acetate buffer (pH 5.2) 2.5 cc., and then after preincubating at 37° for 60 min. activity is measured.

tected by the addition of pyridoxal phosphate from the gallic acid inhibition. Such protection by pyridoxal phosphate was also observed when protocatechuic acid and benzoic acid were used as inhibitors, but not with d-catechin, gallic acid esters and pyrogallol as inhibitors. It is therefore probable that gallic acid, protocatechuic acid and benzoic acid inhibit arginine decarboxylase by competing with pyridoxal phosphate, and free carboxylic groups of the inhibitors play a role in this competition.

In any case, it is certain that the tnhibition mechanism by gallic acid clearly differs from that by d-catechin.

Simultaneous Action of Gallic Acid and d-Catechin——The results described in the preceding section suggest that gallic acid and d-catechin attack the enzyme protein at different sites. If this can be admitted, the following equilibria may be formulated for the simultaneous action of both gallic acid (G) and d-catechin (C) toward an enzyme (E):

$$E + G \underset{\longleftarrow}{\overset{K_g}{\longrightarrow}} EG$$

$$E + C \underset{\longleftarrow}{\overset{K_c}{\longrightarrow}} EC$$

$$EG + C \underset{\longleftarrow}{\overset{K_{(g)c}}{\longrightarrow}} EGC$$

$$EC + G \underset{\longleftarrow}{\overset{K_{(c)g}}{\longrightarrow}} ECG$$

where K_g, K_c, $K_{(g)c}$, and $K_{(c)g}$ represent dissociation constants of respective equilibria. If the total concentration of enzyme is e, the reaction velocity in the presence of excess

   9) M. Dixon : Biochem. J., **55**, 170 (1953).

substrate and both inhibitors (v_{gc}) can be calculated according to Takamiya[10] as follows :

$$v_{gc} = \frac{ke}{1 + \dfrac{[G]}{K_g} + \dfrac{[C]}{K_c} + \dfrac{[G][C]}{K_{(c)g}K_c}}$$

where k is the velocity constant of the overall reaction, and [G] and [C] are molar concentrations of gallic acid and d-catechin, respectively. The reaction velocity in the absence of inhibitors (v_o), that in the presence of gallic acid alone (v_g), and that in the presence of d-catechin alone (v_c), on the other hand, can be derived by the usual procedures as follows :

$$v_o = ke$$

$$v_g = \frac{ke}{1 + \dfrac{[G]}{K_g}}$$

$$v_c = \frac{ke}{1 + \dfrac{[C]}{K_c}}$$

Assuming, as is very likely the case, that the inhibitions by gallic acid and d-catechin occur quite independently to each other, we can write :

$$K_g = K_{(c)g} \quad \text{and} \quad K_c = K_{(g)c}.$$

Using these relations it is possible to show that the following equations obtain :

$$v_o/v_g = v_c/v_{gc} \quad \text{and} \quad v_o/v_c = v_g/v_{gc}.$$

The experimental results obtained were found to satisfy these relations as plotted in Fig. 10. It is thus certain that gallic acid and d-catechin independently combine with glutamic decarboxylase at different sites on the enzyme protein.

Fig. 10 Simultanenus Action of Gallic Acid and d-Catechin
toward Glutamic Decarboxylase System
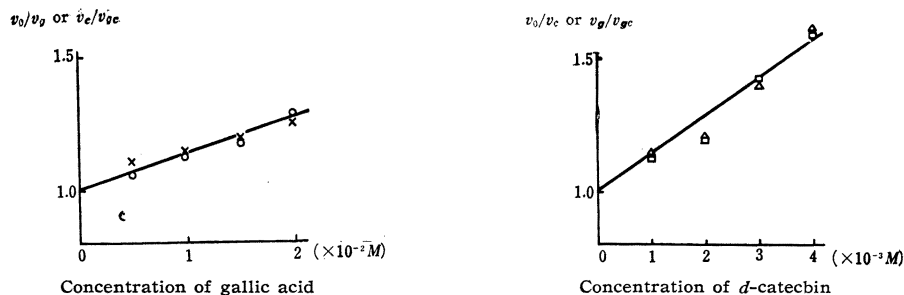
$\circ$—$\circ$, v_0/v_g; $\times$—$\times$, v_c/v_{gc}; $\square$—$\square$, v_0/v_c; $\triangle$—$\triangle$, v_g/v_{gc}
v_c and v_{gc} were measured by keeping the concentration of d-catechin at $4 \times 10^{-3} M$.
v_g and v_{gc} were measured by keeping the concentration of gallic acid at $2 \times 10^{-2} M$.

Discussion

Both gallic acid and d-catechin have been shown to inhibit several enzymes of E. coli which are mainly responsible for the putrefaction phenomena in the intestine. It has also been pointed out that the inhibition mechanisms due to the two compounds

10) A. Takamiya : "Kosokenkyuho," **1**, 255 (1955). Asakura Publ. Co., Tokyo.

are considerably different from each other.   This fact is of interest since gallic acid and d-catechin are characteristic components of pyrogallol- and catechol-tannins respectively.

The present work using two amino acid decarboxylases of E. coli was primarily concerned with the reasons for which these two inhibitors act differently toward the enzymes.   The data reported in this paper now indicate that the enzyme proteins bind gallic acid and d-catechin at evidently different sites.   This conclusion is based largely on the observations that gallic acid but not d-catechin competes with the substrate in glutamic decarboxylase and with pyridoxal phosphate in arginine decarboxylase, and that the inhibitions of glutamic decarboxylase by gallic acid and d-catechin take place quite independently to each other.

The fact that gallic acid competes with pyridoxal phosphate in the arginine decarboxylase system is of considerable interest because of the profound dissimilarity in the chemical structures of the two compounds.   Attempts to prove the coenzymatic role of pyridoxal phosphate in arginine decarboxylase of E. coli 7020 have, however, so far been unsuccessful.

It seems rather difficult to interpret the reasons why gallic acid competes with pyridoxal phosphate only in arginine decarboxylase and not in glutamic decarboxylase. The competition of gallic acid with glutamic acid and not with arginine[*6] is, on the other hand, easier to explain, since it seems likely that the carboxylic group of gallic acid competes with the γ-carboxylic group of glutamic acid.

The second purpose of the present investigation was to examine if the two decarboxylases interact with the inhibitors in both reversible and irreversible manners.   That this is the case was demonstrated by the results reported in this paper.   Thus, it was confirmed that the inhibitors react with the enzymes instantaneously and reversibly at the initial contact, but later a time-dependent and irreversible interaction develops between the two reactants as evidenced by Figs. 3~6 as well as by Table I.   Similar phenomenon has been observed by Vallee, et al.[4] in the case of chelating agent inhibition of alcohol dehydrogenase, but the mechanisms assumed by them are different from those proposed by the authors[6] for berberine inhibition of tryptophanase.

It seems quite probable that the irreversible inhibition is due to the denaturation of enzyme proteins caused by gallic acid or d-catechin.   In the reversible phase of the inhibition, gallic acid seems to compete with the substrate in the glutamic decarboxylase system according to the Lineweaver-Burk equation.   The procedure developed by Takamiya for the simultaneous action of two independent inhibitors is applicable for the inhibition of glutamic decarboxylase by a combined action of both gallic acid and d-catechin as illustrated in Fig. 10.

Summary

Gallic acid and d-catechin inhibitions of glutamic and arginine decarboxylases of Escherichia coli occur in both reversible and irreversible manners.   Gallic acid competes with the substrate in glutamic decarboxylase and with pyridoxal phoshate in arginine decarboxylase, whereas the interactions of d-catechin in both enzymes are of non-competitive nature with respects to both substrates and pyridoxal phosphate.   Gallic acid and d-catechin combine with glutamic decarboxylase protein at different sites.

[*6] Arginine is protective for the inhibition of arginine decarboxylase activity by gallic acid, but not competitively.