Communications to the Editor

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INHIBITORY EFFECT OF GALLOTANNINS ON THE RESPIRATION OF RAT LIVER MITOCHONDRIA

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Inhibitory effects of galloylglucoses having 3 to 10 galloyl groups isolated from crude drugs were examined on the State 3 respiration of rat liver mitochondria. The degree of the inhibition of respiration by these gallotannins depended on the number of galloyl groups per glucose.

KEYWORDS ——— mitochondria respiration; succinate oxidation; gallotannin; Chinese gallotannin; Turkish gallotannin; Paeoniae Radix; Uvae-ursi Folium

Tannic acid, a mixture of gallotannins (galloylglucoses) obtained from Chinese galls and Turkish galls, was previously known to inhibit succinate oxidation in rat liver mitochondria[1,2] and interact with mechanisms mediating inorganic phosphate and porassium ion fluxes in the mitochondrial membrane.[3] However, the structure-activity relationship has not yet been clarified due to the difficulty of the isolation of individual galloylglucoses.

We have recently reported a method of the separation of galloylglucoses according to the degree of galloylation (number of galloyl groups per glucose) by a combination of Sephadex LH-20 chromatography and normal phase high performance liquid chromatography.[4,5,6,7] The inhibitory effects of galloylglucoses separated according to the degree of galloylation on State 3 respiration of rat liver mitochondria were determined in order to clarify the relation between the degree of galloylation and the degree of inhibition.

Rat liver mitochondria were isolated by conventional methods using a medium of 0.25 M sucrose and 5 mM Tris-HCl(pH 7.4). State 3 respiration was measured with a Clark-type oxygen electrode.[8] The reaction mixture contained 0.25 M sucrose, 10 mM KCl, 10 mM Tris-HCl(pH 7.4), 5 mM potassium phosphate buffer(pH 7.4) and 0.2 mM EDTA. Rate of oxygen uptake was continuously recorded after the addition of mitochondrial suspension(1 mg of mitochondrial protein), 5 mM sodium succinate, 0.8 mM ADP and various concentrations of gallotannins. Penta- to octagalloylglucose isolated from Chinese gallotannin, tri- to octagalloylglucose from Turkish gallotannin, penta- to decagalloylglucose from Paeoniae Radix (*Paeonia albiflora* Pall. var. *trichocarpa* Bunge., Paeoniaceae) and tri- to pentagalloylglucose from Uvae-ursi Folium (*Arctostaphylos uva-ursi* L., Ericaceae) were tested. All gallotannins were mixture of isomers having same molecular weight, except for pentagalloylglucose from Chinese gallotannin and Paeoniae Radix, and tetragalloylglucose from Turkish gallotannin. The

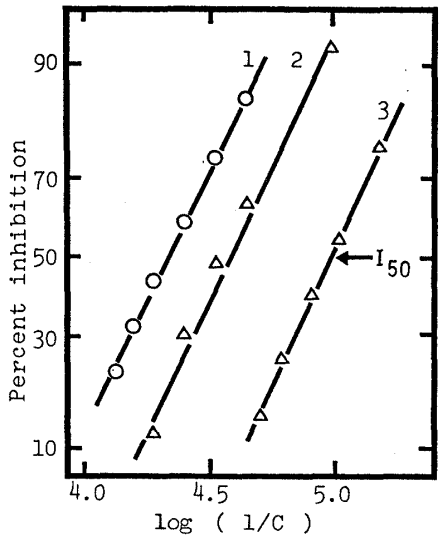

Fig. 1. Effects of Pentagalloylglucose and Nonagalloylglucose on State 3 Respiration of Mitochondria

1: a mixture of 1,2,3,4,6-penta-O-galloyl-β-D-glucose and 6-O-digalloyl-1,2,3-tri-O-galloyl-β-D-glucose from Turkish gallotannin.
2: 1,2,3,4,6-penta-O-galloyl-β-D-glucose from Paeoniae Radix.
3: a mixture of nonagalloylglucoses from Paeoniae Radix.

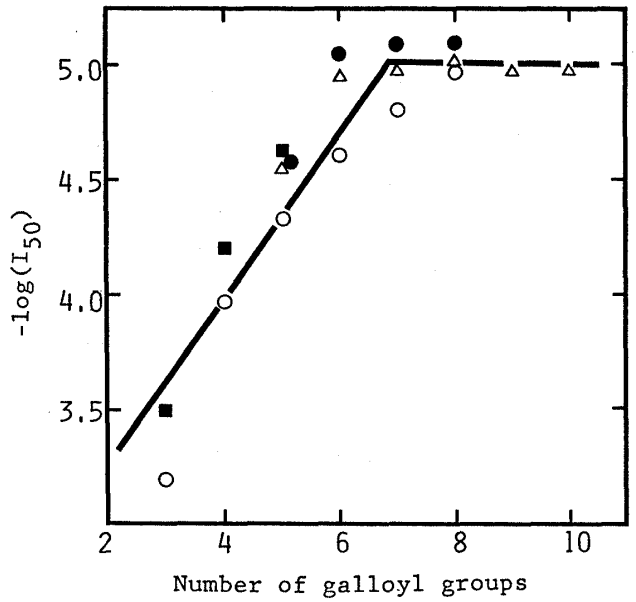

Fig. 2. Relation Between Number of Galloyl Groups of Gallotannins and Their Concentrations Giving 50%-Inhibition(I_{50}) for State 3 Respiration

: Chinese gallotannin.[5]
: Turkish gallotannin.[6]
: gallotannins from Paeoniae Radix.[4,7]
: gallotannins from Uvae-ursi Folium.

gallotannins were dissolved in ethanol.

Typical inhibition of State 3 respiration of mitochondria by pentagalloylglucose separated from Turkish gallotannin and Paeoniae Radix, and nonagalloylglucose from Paeoniae Radix, are shown in Fig. 1. The concentration of each gallotannin which gave 50% inhibition (I_{50}) was calculated in probit scale. A slight difference in I_{50} value between pentagalloylglucose separated from Turkish gallotannin and that from Paeoniae Radix was observed. This was presumably due to the presence of different isomers of galloylglucose. Logarithms of I_{50} value plotted against the number of galloyl groups of gallotannin are shown in Fig. 2. As the number of galloyl groups increased from 3 to 7, a linear relation was observed between the $\log(I_{50})$ and the number of galloyl groups. On the other hand, in the galloyl number above 7, it appeared that the degree of the inhibition was almost the same in the order of 10^{-5} M. The potencies of inhibition by gallotannins having more than 7 galloyl groups were as same as those of 2-thenoyltrifluoroacetone[9] and malonic acid,[10] which are well-known inhibitors for succinate oxidation by mitochondria.

A linear relation between the logarithm of partition coefficients of DDT ana-

logues and the logarithm of I_{50} value of State 3 respiration of rat liver mitochondria was observed.[11] A similar tendency was reported by Ohyama[12] in the inhibition of State 3 respiration by phthalate esters according to the length of the alkyl chain of the phthalate. Furthermore, participation of hydrophobic groups in the formation of tannin-protein complex was indicated by several investigators.[13,14,15] This effect might be dependent on the hydrophobic nature of galloyl groups of gallotannins. The potent inhibitory effect of gallotannins having more than 7 galloyl groups might be explained in terms of specific interaction between the inhibitory sites and galloyl groups, while weaker interaction might be also responsible for the effect of gallotannins having below 7 galloyl groups.

Further experiments are required for the elucidation of the mechanism of this inhibitory effect. However, our present study is the first to show the correlation between biological activities and the structure of gallotannin.

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