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Epimerization of Tea Catechins and O-Methylated Derivatives of (−)-Epigallocatechin-3-*O*-gallate: Relationship between Epimerization and Chemical Structure

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Epimerization at C-2 of O-methylated catechin derivatives and four major tea catechins were investigated. The epimeric isomers of (−)-epicatechin (I), (−)-epicatechin-3-*O*-gallate (II), (−)-epigallocatechin (III), (−)-epigallocatechin-3-*O*-gallate (IV), and (−)-epigallocatechin-3-*O*-(3-*O*-methyl)-gallate (V) in green tea extracts increased time-dependently at 90 °C. The epimerization rates of authentic tea catechins in distilled water are much lower than those in tea infusion or in pH 6.0 buffer solution. The addition of tea infusion to the authentic catechin solution accelerated the epimerization, and the addition of ethylenediaminetetraacetic acid, disodium salt (Na_2EDTA) decreased the epimerization in the pH 6.0 buffer solution. Therefore, the metal ions in tea infusion may affect the rate of epimerization. The proportions of the epimers to authentic tea catechins [III, IV, V, and (−)-epigallocatechin-3-*O*-(4-*O*-methyl)gallate (VI)] in pH 6.0 buffer solution after heating at 90 °C for 30 min were 42.4%, 37.0%, 41.7%, and 30.4%, respectively. These values were higher than those of I and II (23.5% and 23.6%, respectively). The O-methylated derivatives at the 4′-position on the B ring of IV and VI were hardly epimerized. These results suggest that the hydroxyl moiety on the B ring of catechins plays an important role in the epimerization in the order 3′,4′,5′-triol type > 3′,4′-diol type ≫ 3′,5′-diol type.

KEYWORDS: green tea; catechin; epimers; HPLC-ECD; O-methylated catechin; epimerization

INTRODUCTION

Numerous experimental studies have shown that tea catechins such as (−)-epicatechin (I), (−)-epicatechin-3-*O*-gallate (II), (−)-epigallocatechin (III), and (−)-epigallocatechin-3-*O*-gallate (IV) have various beneficial effects on health (*1*−*5*). Recently, we reported that two O-methylated derivatives of IV, (−)-epigallocatechin-3-*O*-(3-*O*-methyl)gallate (V) and (−)-epigallocatechin-3-*O*-(4-*O*-methyl)gallate (VI), inhibited type I allergy more effectively compared with IV. The O-methylated derivatives of IV were separated from Tong ting oolong tea, a Taiwanese oolong tea product, and Benifuuki cultivar, one of the cultivars used for Japanese black tea (*6*).

Some epimeric isomers of catechins have been detected in tea infusions extracted with hot water (*7*−*9*). Catechin has two asymmetric carbon atoms in the C ring, and therefore four epimerization products are possible in theory. However, epimers of the carbon at the C-2 position exclusively were detected in tea infusion. Analysis of catechins in canned tea drinks has shown that most catechins in tea infusions were decreased by heat processing, but (+)-catechin was remarkably increased, possibly due to the epimerization of (−)-epicatechin (*10*). The decrease of catechins and the epimerization in tea infusion were accelerated at pH values higher than 6.0 and by thermal processing (*7*). Products of tea catechins were individually isolated after heat treatment and identified as their corresponding C-2 epimers (*11*). A recent study has demonstrated that the epimerization of IV to (−)-gallocatechin-3-*O*-gallate (XII) was induced by autoclaving at 120 °C for 20 min, and thus the relatively high amount of XII in commercial tea drinks was most likely produced from IV during autoclaving (*12*). XII has demonstrated potent bioactivity in some bioassays (*13*, *14*). We have shown that the proportion of each epimer at C-2 in tea infusions extracted with hot water was different among the four major catechins (*8*).

Little is known about the relationship between the chemical structure of tea catechins and epimerization. The purpose of this study was to investigate the catechin structure−epimerization relationships. We examined the rate of epimerization of catechins using authentic catechins including the O-methylated derivatives of IV such as V, VI, (−)-4′-*O*-methylepigallocatechin-3-*O*-gallate (VII) and (−)-4′-*O*-methylepigallocatechin-3-*O*-(4-*O*-methyl)gallate (VIII) (**Figure 1**).

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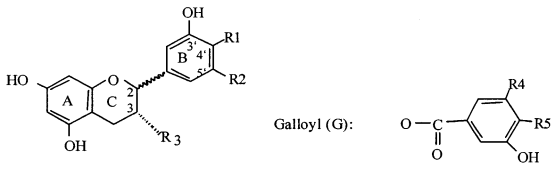

Compounds	2	R_1	R_2	R_3	R_4	R_5
(-)- Epicatechin (I)	*R*	OH	H	OH	-	-
(-)- Epicatechin-3-*O*-gallate (II)	*R*	OH	H	G	OH	OH
(-)- Epigallocatechin (III)	*R*	OH	OH	OH	-	-
(-)- Epigallocatechin-3-*O*-gallate (IV)	*R*	OH	OH	G	OH	OH
(-)- Epigallocatechin-3-*O*-(3-*O*-methyl)gallate (V)	*R*	OH	OH	G	OCH_3	OH
(-)- Epigallocatechin-3-*O*-(4-*O*-methyl)gallate (VI)	*R*	OH	OH	G	OH	OCH_3
(-)- 4′-*O*-methyl epigallocatechin-3-*O*-gallate (VII)	*R*	OCH_3	OH	G	OH	OH
(-)- 4′-*O*-methyl epigallocatechin-3-*O*-(4-*O*-methyl)gallate (VIII)	*R*	OCH_3	OH	G	OH	OCH_3
(±)- Catechin (IX)	*S*	OH	H	OH	-	-
(-)- Catechin-3-*O*-gallate (X)	*S*	OH	H	G	OH	OH
(-)- Gallocatechin (XI)	*S*	OH	OH	OH	-	-
(-)- Gallocatechin-3-*O*-gallate (XII)	*S*	OH	OH	G	OH	OH
(-)- Gallocatechin-3-*O*-(3-*O*-methyl)gallate (XIII)	*S*	OH	OH	G	OCH_3	OH
(-)- Gallocatechin-3-*O*-(4-*O*-methyl)gallate (XIV)	*S*	OH	OH	G	OH	OCH_3

Figure 1. Chemical structures of catechins and their O-methylated derivatives.

MATERIALS AND METHODS

Tea Samples and Authentic Catechins. Tea (Benifuuki and Yabukita cultivars) was cultivated at the plantation of the National Institute of Vegetable and Tea Science in Kanaya, Shizuoka, Japan. Freshly tea leaves picked in June were dried in a microwave oven and stored in a refrigerator before analysis and isolation of catechins. Tong ting oolong tea was obtained from markets. All catechins and their epimers except VII and VIII were isolated from green tea leaves or Tong ting oolong tea according to the method previously reported (*6*). Some catechins (I, II, III, and IV) purchased from Kurita Co. (Tokyo, Japan) were also used. VII and VIII were synthesized from IV by use of methyl iodide in our laboratory (*15*). Water used was purified by the Milli-Q system (Millipore, Bedford, MA). All other chemicals were of reagent grade.

Apparatus. The HPLC system consisted of a pump (Shimadzu, LC-10AD, Kyoto, Japan), an automated sample injector (Shimadzu, SIL-10A), a column oven (Shimadzu, CTO-10AC), a system controller (Shimadzu, SCL-10A), and an electrochemical detector (Shiseido, Nanospace SL-1, Tokyo, Japan). The recording and integrating device was a Chromatopac (Shimadzu, CR-7A plus). The separation column was a TSKgel ODS 80Ts reversed-phase column (250 × 4.6 mm i.d., Tosoh, Tokyo, Japan).

Determination of Catechins and Their Epimers. The determination of catechins was carried out by high-performance liquid chromatography (HPLC) with an electrochemical detector (ECD) as previously described (*8*). In brief, the mobile phase was 0.1 M sodium dihydrogen phosphate buffer (pH 2.5) containing 0.1 mM ethylenediaminetetraacetic acid, disodium salt (Na_2EDTA)/acetonitrile (87:13 v/v) with a flow rate of 1 mL/min. The column was maintained at 30 °C. The catechins were detected electrochemically at an applied potential of 600 mV versus Ag/AgCl.

Extraction of Tea Catechins. Tea catechins were extracted from 50 mg of tea leaf powder with 5 mL of distilled water at several temperatures (30−90 °C) with shaking for 3 min. The extracts were filtered through a membrane filter (pore size 0.45 μm, Ekikurodisk 13, Gelman Science Japan, Tokyo, Japan). The filtrate was diluted with the mobile phase and injected into the HPLC column.

Epimerization of Catechins. Tea infusions used in epimerization studies were obtained from 100 mg of Benifuuki or Yabukita tea leaf powder with 10 mL of distilled water at 90 °C with shaking for 3 min. The infusion was passed through a 0.45 μm membrane filter to remove the tea solids. The concentrations of I, II, III, IV, and V in Benifuuki tea infusion were 348, 205, 918, 694, and 56 μM, respectively. Each authentic catechin was dissolved in distilled water or in 0.1 M McIlvaine buffer solution (pH 5.0−6.0). The tea infusions and catechin solutions were maintained in a temperature-controlled water bath at 30−90 °C for the epimerization studies. The effects of tea infusion on the epimerization were investigated by use of V and a tea infusion prepared from cultivar Yabukita, which does not contain V (*6*).

Effect of Na_2EDTA on Epimerization of Catechins. Tea infusion was obtained from 100 mg of Benifuuki tea leaf powder with 10 mL of 0.1 M McIlvaine buffer solution (pH 6.0) at 90 °C with shaking for 3 min. The infusion was passed through a 0.45 μm membrane filter to remove the tea solids. The tea infusions were maintained in a temperature-controlled water bath at 90 °C for 30 min with or without 10 mM Na_2EDTA.

Analysis of Metal Ions in Tea Infusions. Tea infusion was obtained from 50 mg of Benifuuki tea leaf powder with 5 mL of distilled water at 90 °C with shaking for 3 min. The infusion was passed through a 0.45 μm membrane filter to remove the tea solids. The concentrations of metal ions in the tea infusion were analyzed by a high-resolution inductively coupled plasma mass spectrometer (HR-ICP-MS, Element, Finnigan MAT, Bremen, Germany).

Epimerization of Catechins and Their O-Methylated Derivatives. Authentic catechins (I, II, III, and IV) and O-methylated derivatives of IV (V, VI, VII, and VIII) were dissolved separately in 0.1 M McIlvaine buffer solution (pH 6.0) and maintained in a temperature-controlled water bath at 90 °C for 30−60 min.

RESULTS AND DISCUSSION

Green tea for drinking is generally brewed in hot water for 2−3 min. **Figure 2** shows the concentrations of tea catechins and the epimers at C-2 in infusions obtained by extraction of green tea of the Benifuuki cultivar at various temperatures for 3 min. Catechin levels in the infusion increased with increasing extraction temperature. In particular, the extraction of III at low temperature was higher than the other catechins. An appreciable increase of epimers at C-2 corresponding to catechin was observed at 90 °C. The proportions of the epimers to catechins (IX/I, X/II, XI/III, XII/IV, and XIII/V) were 10.9%, 2.8%, 28.4%, 11.9%, and 4.9%, respectively.

These results suggest that large amounts of epimers are produced easily even in the extraction conditions that is used for everyday tea brewing. **Table 1** shows time-dependent

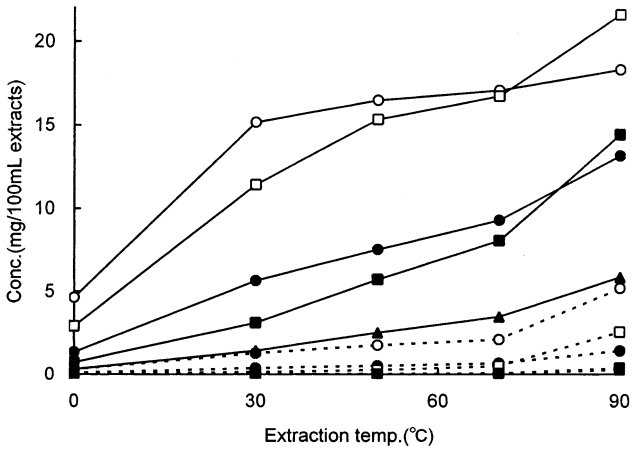

Figure 2. Extraction of tea catechins from Benifuuki tea leaf. Tea catechins were extracted with distilled water at various temperatures for 3 min. (●, —) I; (■, —) II; (○, —) III; (□, —) IV; (▲, —) V; (●, ---) IX; (■, ---) X; (○, ---) XI; (□, ---) XII; (▲, ---) XIII.

Table 1. Epimerization of Tea Catechins in Tea Filtrate[a]

	percent epimerization[b]				
catechin	5 min	10 min	20 min	30 min	60 min
			30 °C		
IX / I	0.7	1.2	0.3	0.3	1.6
X / II	0.2	0.9	0.3	0.0	1.6
XI / III	0.0	0.1	0.0	0.0	0.1
XII / IV	1.4	1.6	1.5	1.3	2.3
XIII / V	0.7	1.5	1.3	0.7	3.8
			90 °C		
IX / I	2.1	5.4	7.6	9.2	19.4
X / II	2.2	4.2	7.1	9.4	18.1
XI / III	4.1	8.0	12.0	15.4	28.4
XII / IV	3.2	6.8	11.6	15.0	27.3
XIII / V	3.8	6.1	10.5	16.8	25.9

[a] Tea extract prepared from Benifuuki tea leaf powder at 90 °C for 3 min was passed through a 0.45 μm membrane filter, and the filtrate was incubated at 30 or 90 °C for 5–60 min. Values are means of duplicate determinations. [b] The percent values were calculated from the catechin concentration obtained by subtracting the initial catechin concentration in tea filtrate as the blank.

changes of catechin levels in the filtrate passed through membrane filters to remove tea solids after extraction at 90 °C for 3 min. The transformation of catechins to the corresponding epimers at 90 °C increased with time, but only slight increases occurred at 30 °C. The epimerization of catechins such as III, IV, and V is greater than that of I and II. The result suggests that, of the catechin structures, the triol type of the B ring, such as III, IV, and V, was epimerized more easily than the diol type, such as I and II. On the other hand, the contribution of the galloyl moiety to the epimerization at the C-2 position is thought to be minor from the comparison of III to IV or I to II. Additionally, the fact that the conversion rate of V to XIII at 90 °C was almost the same as that of IV suggests that the triol structures of the galloyl moiety do not take part in the epimerization reaction.

Table 2 shows the epimerization of authentic catechins in distilled water at 90 °C. The concentration of catechins in the solution was adjusted to 500 μM for each catechin. Epimerizations in distilled water were very low compared with those in tea infusion. The proportions of the epimer to catechins produced by heating for 60 min were all below 5%, but the epimerization of catechins was more accelerated in tea infusion, as shown in **Table 1**. These results suggest that tea infusions

Table 2. Epimerization of Authentic Tea Catechins in Distilled Water[a]

	percent epimerization				
catechin	5 min	10 min	20 min	30 min	60 min
IX / I	1.3	1.5	2.2	2.7	3.7
X / II	0.7	0.6	1.2	1.9	2.5
XI / III	1.2	1.7	2.5	3.1	4.5
XII / IV	1.0	1.3	2.1	2.7	4.0
XIII / V	1.4	1.2	2.1	3.1	4.1

[a] Catechins (500 μM) dissolved in distilled water were incubated at 90 °C for 5–60 min. Values are means of duplicate determinations.

Table 3. Effects of pH and the Addition of Tea Infusion on Epimerization of Authentic (–)-Epigallocatechin-3-*O*-(3-*O*-methyl)gallate[a]

		percent epimerization		
pH	tea infusion	15 min	30 min	60 min
5.0	none			7.8
	added			8.5
5.5	none		12.3	17.8
	added		12.7	20.3
6.0	none	17.2	25.3	
	added	20.3	28.9	

[a] Tea infusion prepared from Yabukita was added to authentic (–)-epigallocatechin-3-*O*-(3-*O*-methyl)gallate buffer solution (1:9, v/v). The reaction mixture was incubated at 90 °C for 15–60 min. Values are means of duplicate determinations.

have a specific role, for example, as a pH balancer or a catalyst for the epimerization of a catechin.

When IV is incubated with blood plasma, intestinal fluids, or solutions over pH 7.4, some dimerization products of IV are recognized in the solution (*16*, *17*). Tea catechins in alkaline solutions (pH > 8) are extremely unstable and degraded almost completely in a few minutes, whereas they are stable in acidic solutions (pH < 4) (*18*−*20*). The epimerization of I in thermal processing was accelerated at pH values higher than 6.0 and inhibited at pH values lower than 5.0 (*7*). The pH of Benifuuki tea infusion used in this study was 6.0−6.2. **Table 3** shows the effect of tea infusion on epimerization of catechins in buffer solutions (pH 5.0−6.0). In this experiment, V as the authentic catechin and tea infusion prepared from tea leaves of Yabukita cultivar were used. Since Yabukita includes very little V (*6*), the epimerization of authentic V by tea infusion can only be estimated. Epimerization of V was accelerated by increasing pH (pH 5.0 < 5.5 < 6.0). At pH 6.0 for 60 min, other degradation products were detected, so we could not examine the rate of epimerization for this condition. The addition of tea infusion to pH 6.0 buffer solution (1:9 v/v) increased epimerization of V by 14.2−18.0% compared to that without tea infusion. This result supports the report that isomerization was accelerated in solutions pH 6.0 and higher (*7*). It is supposed that the increase of pH in tea infusions is caused by ingredients eluted from tea leaf. The fact that the addition of tea infusion to a pH-controlled solution (pH 6.0) accelerated the epimerization of V suggests that the any substance promoting the epimerization exists in the tea infusion.

Table 4 shows the epimerization of catechins in tea infusion extracted with 0.1 M buffer solution (pH 6.0). The tea infusion was maintained at 90 °C with or without 10 mM Na_2EDTA. The pH of the buffer solution was not changed by adding Na_2-EDTA (pH 5.8−6.0), but the addition of Na_2EDTA decreased epimerization of catechins by 22.8−26.6% compared to that without Na_2EDTA. The concentrations of metal ions in the Benifuuki tea infusion were analyzed by HR-ICP-MS. The

Table 4. Effect of Na_2EDTA on Epimerization of Tea Catechins in Tea Filtrate[a]

	percent epimerization[b]	
catechin	–Na_2EDTA	+Na_2EDTA
IX / I	32.8	22.3
X / II	28.9	20.3
XI / III	57.2	40.3
XII / IV	41.8	30.4
XIII / V	42.6	29.5

[a] Tea extract prepared from Benifuuki tea leaf powder with pH 6.0 buffer solution at 90 °C for 3 min was passed through a 0.45 μm membrane filter, and the filtrate was incubated with or without 10 mM Na_2EDTA at 90 °C for 30 min. Values are means of duplicate determinations. [b] The percent values were calculated from the catechin concentration obtained by subtracting the initial catechin concentration in tea filtrate as the blank.

Table 5. Epimerization of Authentic Tea Catechins and O-Methylated Derivatives of (–)-Epigallocatechin-3-*O*-gallate[a]

	percent epimerization	
catechin	15 min	30 min
IX / I	11.4	23.5
X / II	11.3	23.6
XI / III	20.8	42.4
XII / IV	18.7	37.0
XIII / V	19.1	41.7
XIV / VI	14.2	30.4

[a] Catechins (500 μM) dissolved in pH 6.0 buffer solution were incubated at 90 °C for 30 min. Values are means of duplicate determinations.

concentrations of metal ions such as Fe, Cu, Zn, Mg, Ca, Mn, and Al were 3, 10, 18, 10 000, 2000, 1700, and 300 ppb, respectively. The metal ions extracted from tea leaf might have some relevance to epimerization of tea catechins.

The chemical structure of catechins, especially of the B ring, may also be relevant to the epimerization. We further examined epimerization of tea catechins using authentic catechins including I, II, III, IV, and O-methylated derivatives of IV (V, VI, VII, and VIII) in buffer solution (pH 6.0). Table 5 shows that the proportions of the epimer to catechins produced by heating III, IV, V, and VI for 30 min (42.4%, 37.0%, 41.7%, and 30.4%, respectively) were higher than those of I and II (23.5% and 23.6%, respectively). This trend was close to the results obtained by tea infusion shown in Table 1. It should be noted that the value of VI (30.4%) was lower than that of V (41.7%). The presence of a galloyl moiety of the meta-diol type, such as VI, might decrease the epimerization. The epimerization of O-methylated derivatives at the 4′-position on the B ring of IV and VI, VII, and VIII were tested. The epimerization of VII and VIII was estimated from a decrease of VII and VIII in buffer solution (pH 6.0) after incubation because standards of epimers of VII and VIII were not available. Small decreases of VII and VIII were observed compared to IV and VI (**Figure 3**). In particular, the concentration of VII in buffer solution (pH 6.0) was slightly reduced during heating at 90 °C for 60 min. From these results, we speculate that the hydroxyl moiety on the B ring is important in the epimerization of catechins in the order 3′,4′,5′-triol type > 3′,4′-diol type ≫ 3′,5′-diol type.

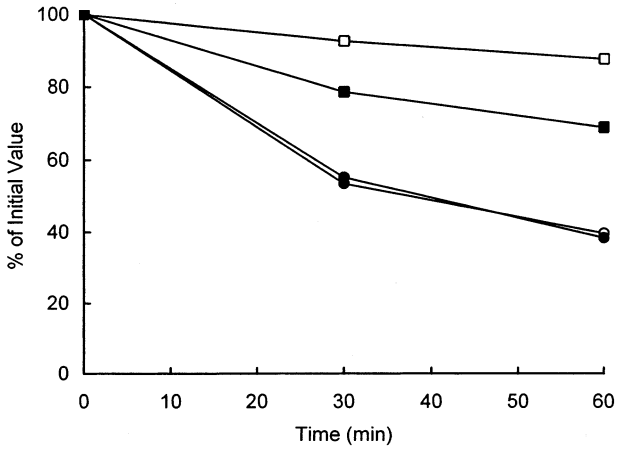

Figure 3. Stability of (–)-epigallocatechin-3-*O*-gallate and its O-methylated derivatives during heat treatment. Catechin solution (pH 6.0 buffer solution) was incubated at 90 °C for 60 min. (○) IV; (●) VI; (□) VII; (■) VIII.

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