Biosci. Biotechnol. Biochem., **74** (4), 875–877, 2010

Note

Epimerization of Tea Catechins under Weakly Acidic and Alkaline Conditions

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Received December 1, 2009; Accepted January 18, 2010; Online Publication, April 7, 2010
[doi:10.1271/bbb.90884]

Tea catechins in a buffer at pH 7 with N_2 replacing O_2 epimerized rapidly at 80 °C with less than 10% of oxidative side reactions and gave catechin epimers in a 50–63% yield. The epimerization of catechins with three hydroxyl groups was faster than with two groups, and of galloyl-free catechins was faster than catechins with a galloyl ester.

Key words: tea catechin; epimerization; catechin epimer; introduction N_2 gas

Green tea catechins like (−)-EC **1a**, (−)-EGC **2a**, (−)-ECg **3a**, and (−)-EGCg **4a**, as shown Fig. 1, possess a variety of biologically relevant properties which include anti-oxidative[1] and anti-viral[2] activities. Freudenberg *et al.*[3,4] found the epimerization of catechin in 1922 and obtained a catechin epimer in a low yield under alkaline conditions in 1924. Many studies about catechin epimerization have since been carried out. It has recently been suggested that green tea catechins in tea drinks were epimerized during brewing, sterilisation and long-term heating in tea shops and dispensers.[5,6] Recent studies have revealed that catechin epimers displayed more biologically relevant activities (inhibiting cholesterol absorption[7] and exhibiting antiallergic[8] and antioxidative[9] properties) than the original catechins. The changes in these biological activities only depend on changes in the configuration at C2. Investigating this epimerization would provide better insight into the mechanism governing these biological activities; it is therefore necessary to obtain catechin epimers in a good yield.

The epimerization mechanism shown in Fig. 1 for catechins under alkaline conditions was proposed by Metha and Whalley[10] in 1963. However, Seto *et al.*[11] have developed a useful epimerization method under weakly acidic conditions (pH 5) at 120 °C for 30 min and yielded catechin C2 epimers (−)-C **1b**, (−)-GC **2b**, (−)-Cg **3b**, and (−)-GCg **4b** from respective tea catechins **1a**–**4a** in a 19–36% yield. Although catechins are prone to oxidative degradation and polymerisation under high-pH conditions, their epimerization should be faster and more efficient under higher pH conditions if oxidative side reactions can be inhibited. In addition, tea infusions brewed with hard water (pH 7.2) have been found to contain more catechin epimers than with purified water (pH 5.9).[12] We therefore examined an efficient epimerization method for catechins that inhibited oxidative side reactions.

An epimerization experiment was conducted in which each tea catechin **1a**–**4a** with >98% respective purity was heated in a buffer, and the epimer of each in the reaction mixture was identified against an authentic sample by a reversed-phase HPLC analysis.

(−)-EC **1a** in a buffer at pH 8 was heated at 80 °C in a normal atmosphere to confirm how much catechin was degraded under alkaline conditions. During the reaction, the amounts of **1a** and **1b** were decreased rapidly, and the reaction mixture was turned brownish by catechin oxidants. N_2 gas was then introduced into the pure **1a** solution for 5 min to remove oxygen dissolved in the buffer. The **1a** buffer solution was then heated at 80 °C in the dark and was hardly discoloured by the reaction. ^{1}H-NMR data for the reaction mixture showed that it contained few oxidative by-products.

1a was then epimerized in buffers of various pH value. The relative epimerization rates of **1a** at 80 °C for 20 min in the various buffers, based on the rate in a pH 5 buffer solution, are shown in Fig. 2A. These rates show that the aqueous solutions of higher pH resulted in faster epimerization, the epimerization being about 50 times faster at pH 8 than at pH 5. Other tea catechins **2a**–**4a** were similarly epimerized, further validating the alkaline-promoted reaction mechanism.

Inhibiting most of the side reactions therefore enabled the rapid epimerization of catechins in the pH 8 buffer. Each tea catechin **1a**–**4a** was then favourably epimerized in the pH 8 buffer at 70 °C. This was conducted three times for catechins **1a**–**4a**, the average epimerization rate being shown in Fig. 2B. The three main factors relating the catechin structure to the epimerization reaction were therefore found. First, the catechins with three electron-donating hydroxyl groups in the B ring were epimerized faster than those with two groups (**2a** > **1a** and **4a** > **3a**). Second, the galloyl-free catechins were epimerized faster than the catechins with a galloyl ester at the 3 position that contained more acidic H_b at the 4″ position than that of H_a at the 4′ position in the B ring (Fig. 2C, **1a** > **3a** and **2a** > **4a**). Third, the epimer ratio at equilibrium of the catechins with a galloyl ester at the 3 position was 10% lower than that of the galloyl-free catechins (**1ab** and **2ab** > **3ab** and **4ab**).* These factors suggest the possibility of some sort of interaction between the B ring and galloyl group that reduced the stability difference between tea catechins **3a** and **4a** and their epimers **3b** and **4b**.

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* This trend was also observed in the data of Reference 11.

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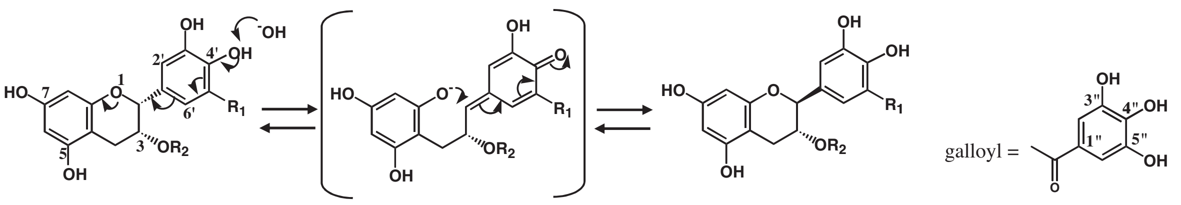
Abbreviations: (−)-EC, (−)-epicatechin; (−)-EGC, (−)-epigallocatechin; (−)-ECg, (−)-epicatechin gallate; (−)-EGCg, (−)-epigallocatechin gallate; (−)-C, (−)-catechin; (−)-GC, (−)-gallocatechin; (−)-Cg, (−)-catechin gallate; (−)-GCg, (−)-gallocatechin gallate



1a: R_1=H, R_2=H; (-)-Epicatechin [(-)-EC]
2a: R_1=OH, R_2=H; (-)-Epigallocatechin [(-)-EGC]
3a: R_1=H, R_2= galloyl; (-)-Epicatechin gallate [(-)-ECg]
4a: R_1=OH, R_2= galloyl; (-)-Epigallocatechin gallate [(-)-EGCg]

1b: R_1=H, R_2=H; (-)-Catechin [(-)-C]
2b: R_1=OH, R_2=H; (-)-Gallocatechin [(-)-GC]
3b: R_1=H, R_2= galloyl; (-)-Catechin gallate [(-)-Cg]
4b: R_1=OH, R_2= galloyl; (-)-Gallocatechin gallate [(-)-GCg]

Fig. 1. Epimerization of Tea Catechins **1a**–**4a** in an Aqueous Solution.

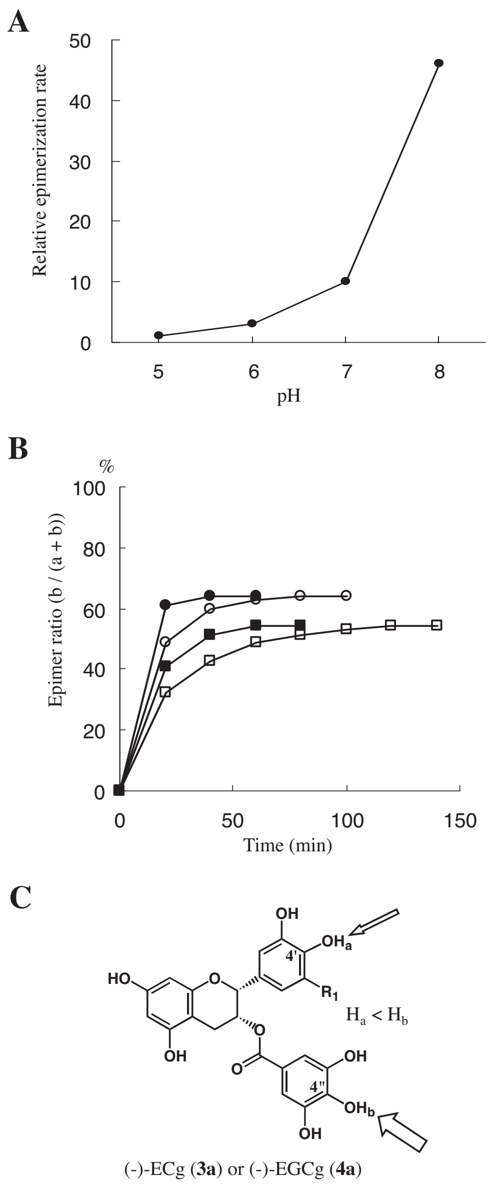


Fig. 2. Epimerization Rates of the Tea Catechins.

A, Relative epimerization rate of (−)-EC at 80 °C for 20 min in various pH buffers, based on the rate in the pH 5 buffer. B, Epimerization rate of (−)-EC **1a** (○), (−)-EGC **2a** (●), (−)-ECg **3a** (□), and (−)-EGCg **4a** (■) in the pH 8 buffer at 70 °C. The epimer ratio [b/(a + b)] of the reaction mixture was calculated from the peak area by an HPLC analysis. C, Relative acidity of the tea catechins.

Table 1. Epimerization Conditions and Yield of Catechins and Epimers

s.m.	pH	Temp. (°C)	Time (min)	Ratio b/(a + b) (%)	Yield* a + b (%)	Yield* b (%)
(−)-EC (**1a**)	7	80	90	64	92	59 (**1b**)
(−)-EGC (**2a**)	7	80	60	66	96	63 (**2b**)
(−)-ECg (**3a**)	7	100	30	55	93	51 (**3b**)
(−)-EGCg (**4a**)	7	100	20	54	93	50 (**4b**)

Each tea catechin (100 mg) in the buffer was heated. The epimer ratio [b/(a + b)] of the reaction mixture was calculated from the peak area by a HPLC analysis [NB-ODS 9 column (4.6 mmϕ × 250 mm); 280 nm detection; 1 ml/min eluents: 20% MeOH, 1% AcOH in H_2O (retention times of **2a** and **2b** were 9 and 4 min); 25% MeOH, 1% AcOH in H_2O (retention times of **1a**, **1b**, **4a** and **4b** were 17, 8, 10, and 16 min); 30% MeOH, 1% AcOH in H_2O (retention times of **3a** and **3b** were 12 and 16 min)].
*Isolated yield by HPLC in an ODS column.

The epimerization conditions for tea catechins **1a**–**4a** were then examined to more efficiently obtain the catechin epimers. Although epimerization of the catechins at pH 8 was rapid, the galloyl ester in **3a** and **4a** was partially hydrolysed. Epimerization was then carried out at pH 7 with less hydrolysis. The galloyl-free catechins (**1a** and **2a**) were heated at 80 °C, whereas the catechins with a galloyl ester (**3a** and **4a**) were heated at 100 °C because of their slow epimerization (Table 1).

These three factors affecting catechin epimerization were reproduced under those epimerization conditions. Inhibiting the oxidation of catechins during epimerization enabled recovery of the tea catechins in more than a 90% yield and of catechin epimers **1b**–**4b** in a 50–63% yield, this being nearly twice the previously reported[11] yield.

We consider that the results of this study help to elucidate the mechanism expressing the biological activities of catechins.

Acknowledgment

We thank Nagara Science Co., Ltd. (Gifu, Japan) for presenting authentic samples of the tea catechins and their epimers, and for the NB-ODS HPLC columns (4.6Φ × 250 mm and 10Φ × 250 mm).

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