

Food Chemistry (Research paper)

**A new catechin oxidation product and polymeric polyphenols of post-fermented tea**

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**Abstract:** A new epicatechin oxidation product with a 3,6-dihydro-6-oxo-2*H*-pyran-2-carboxylic acid moiety was isolated from a commercially available post-fermented tea that is produced by microbial fermentation of green tea. The structure of this product was determined by spectroscopic methods. A production mechanism that includes the oxygenative cleavage of the catechol B-ring of (-)-epicatechin is proposed. In addition, polymeric polyphenols were separated from the post-fermented tea and partially characterized by a <sup>13</sup>C-NMR spectroscopic and gel-permeation chromatography approach. The polymers appear to be primarily composed of epigalloacetechin-3-*O*-gallate and the molecular weight (Mn) of the acetylated form was estimated to be ~3500.

**Keywords:** Post-fermented tea; catechin; oxidation; tea; polyphenol

## 1. Introduction

Teas are produced from the leaves of *Camellia sinensis* (L.) O. Kuntze (Theaceae), and generally classified by the manufacturing process into four categories of unfermented (green), fermented (black), semi-fermented (oolong) and post-fermented (Pu-erh tea, dark tea and related products) (Ho, Lin, & Shahidi, 2008). The production of the post-fermented tea is limited to the areas of China and Japan, and the products of Yunnan, Hunan, Hubei, Guangxi, and Sichuan provinces have been well recognized in China. Consumption of post-fermented tea is much lower than the consumption of black and green teas. Owing to the much lower consumption of post-fermented tea, the chemical composition of this tea has not been characterized in detail. However, recent studies suggest some beneficial effects of post-fermented tea (Chiang, Weng, Lin-Shiau, Kuo, Tsai, & Lin, 2006; Duh, Yen, Yen, Wang, & Chang, 2004; Kuo, Weng, Chang, Tsai, Lin-Shiau, & Lin, 2005; Noguchi, Hamauzu, & Yasui, 2008; Oyaizu, Hotsumi, Takagi, Matsumoto, Fujita, Matsuzaki, & Yamashita, 2005; Oyaizu, Hotsumi, Takagi, Matsuzaki, Yamashita, Takenaga, & Itho, 2006). Black and oolong tea production includes a process called “tea fermentation”, in which constituents of fresh tea leaves are chemically altered by enzymes originally contained in the leaves (Roberts, 1962; Tanaka, Matsuo, & Kouno, 2010). In contrast, the post-fermented teas are produced by

aerobic or anaerobic microbial fermentation of heat-processed green tea leaves (Gong, Watanabe, Yagi, Etoh, Sakata, Ina, & Liu, 1993; Okada, Takahashi, Ohara, Uchimura, & Kozaki, 1996). During the fermentation process, the levels of tea catechins decrease because of decomposition by microbial enzymes and autoxidation; however, only a few catechin metabolites characteristic to post-fermented tea have been reported (Zhou, Zhang, Xu, & Yang, 2005). Thus we have examined the chemical constituents of a commercially available post-fermented tea product. Although it is known that the post-fermentation procedures differ between production areas, in this study we have examined the Hunan brick tea, which is produced by aerobic microbial fermentation of green tea in one of the major production regions of China.

## **2. Materials and methods**

### *2.1. Materials*

The post-fermented tea, Hunan brick tea, was purchased at a local market in Hunan, China, in September 2009. The Japanese green tea was produced by Nagasaki Agricultural and Forestry Technical Development Center, Higashisonogi tea laboratory, Nagasaki, Japan in 2009. Standard samples of tea catechins were separated from green tea and purified by crystallization from water. Standard samples of

4-(2-hydroxyethyl)thioethers of four tea catechins were prepared by thiolysis of persimmon tannins with 2-mercaptoethanol (Tanaka, Takahashi, Kouno, & Nonaka, 1994).

## 2.2. Analytical Procedures

UV spectra were obtained using a JASCO V-560 UV/VIS spectrophotometer (Jasco Co., Tokyo, Japan).  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded in acetone- $d_6$  +  $\text{D}_2\text{O}$  (9:1, v/v),  $\text{CD}_3\text{OD}$ , or  $\text{DMSO}-d_6$  at 27 °C with a JEOL JNM-AL400 spectrometer (JEOL Ltd., Tokyo, Japan) operating at a  $^1\text{H}$  frequency of 400 MHz.  $^1\text{H}$ - $^1\text{H}$  COSY, NOESY, HSQC and HMBC spectra were recorded using a mixture of acetone- $d_6$  +  $\text{D}_2\text{O}$  (9:1, v/v) and a Varian Unity plus 500 spectrometer (Varian Inc., Palo Alto, CA, USA) operating at a  $^1\text{H}$  frequency of 500 MHz. Coupling constants are expressed in Hz and chemical shifts are presented on a  $\delta$  (ppm) scale. HMQC, HMBC and NOESY experiments were performed using standard Varian pulse sequences. The matrix-assisted laser desorption time-of-flight mass spectra (MALDI TOF MS) were recorded on a Voyager-DE Pro spectrometer (Applied Biosystems, USA) and 2,5-dihydroxybenzoic acid (10 mg/ml in 50% acetone containing 0.05% trifluoroacetic acid) was used as the matrix. Fast atom bombardment (FAB) MS was recorded on a JMS 700N spectrometer

(JEOL Ltd., Japan) and *m*-nitrobenzyl alcohol or glycerol was used as a matrix. Elemental analysis was conducted with a PerkinElmer 2400 $\alpha$  analyzer (PerkinElmer Inc., Waltham).

Column chromatography was performed using Sephadex LH-20 (25–100  $\mu$ m, GE Healthcare Bio-Science AB, Uppsala), Diaion HP20SS (Mitsubishi Chemical, Japan), MCI gel CHP 20P (75–150  $\mu$ m; Mitsubishi Chemical, Tokyo, Japan) and Chromatorex ODS (100–200 mesh; Fuji Silysia Chemical, Kasugai, Japan) columns. Thin layer chromatography was performed on precoated Silica gel 60 F<sub>254</sub> plates (0.2 mm thick, Merck KGaA, Darmstadt, Germany) with toluene-ethyl formate-formic acid (1:7:1, v/v) and CHCl<sub>3</sub>-MeOH-water (14:6:1, v/v). Spots were detected using ultraviolet (UV) illumination and by spraying with 2% ethanolic FeCl<sub>3</sub> or a 10% sulfuric acid reagent followed by heating. Analytical HPLC was performed using a Cosmosil 5C<sub>18</sub>-AR II (Nacalai Tesque Inc., Kyoto, Japan) column (4.6 mm i.d.  $\times$  250 mm) with gradient elution from 4–30% (39 min) and 30–75% (15 min) of CH<sub>3</sub>CN in 50 mM H<sub>3</sub>PO<sub>4</sub>; the flow rate used was 0.8 ml/min, and detection was achieved using a Jasco photodiode array detector MD-910.

### 2.3. Extraction and separation

Post-fermented tea (300 g) was extracted with hot water (10 L, 100 °C for 5 min) and filtered. The filtrate was directly applied to a Sephadex LH-20 column (5.0 × 40 cm) with water containing increasing proportions of MeOH (10% stepwise gradient each 500 ml) to give five fractions. The first fraction, which was eluted with only water, was applied to a Diaion HP20SS (H<sub>2</sub>O-MeOH, 10% stepwise gradient from 0–100% MeOH). After removing the sugars via a washing process, elution with a 20–60% MeOH gradient afforded three fractions containing phenolic compounds: Fr. 1-1 (7.4 g), Fr. 1-2 (4.8 g) and Fr. 1-3 (2.8 g). Fr. 1-1 was separated using a Sephadex LH-20 column (H<sub>2</sub>O-MeOH containing 0.1% trifluoroacetic acid, 10% stepwise gradient from 0–100% MeOH) into 12 fractions. Fr. 1-1-5 (61 mg) was further purified by Chromatodex ODS (H<sub>2</sub>O-MeOH containing 0.1% trifluoroacetic acid, 5% stepwise gradient from 0–40% MeOH) to give compound **1** (8.1 mg). Fr. 4 (13.4 g) containing tea catechins and polymeric polyphenols was applied to a Sephadex LH-20 column (4 × 55 cm) with acetone-7 M urea (3:2, v/v, adjusted to pH 2 with conc. HCl) (Yanagida, Shoji, & Shibusawa, 2003). The fractions containing the polymers were collected and concentrated by rotary evaporator (below 45°C) until the acetone was completely removed. The aqueous solution was then applied to a Diaion HP20P column (3 × 30

cm) and the urea and HCl were removed by washing with water. Finally, elution of the polymeric polyphenols (540 mg) from the column was achieved using 80% MeOH. Following the removal of urea and HCl (see above) the fractions containing compounds with lower molecular weights were separated by successive column chromatography steps with Diaion HP20, Chromatorex ODS and Sephadex LH-20 to yield (+)-catechin (3.4 mg), (-)-gallocatechin (70.2 mg), strictinin (22.5 mg), 1,4,6-tri-*O*-galloyl- $\beta$ -D-glucose (34.8 mg) and myricitrin (15.7 mg). In this experiment, major catechins, that is epicatechin, epigallocatechin and their galloyl esters were not purified.

### *2.3. Isolation of polymeric polyphenols by precipitation method*

Post-fermented tea (50 g) was extracted with hot water (2 L, 100 °C) for 5 min and filtered. The filtrate was concentrated by rotary evaporator (below 45°C) to 300 ml, acidified to pH 3 by the addition of conc. HCl, and then partitioned with EtOAc (4 times). The aqueous layer was concentrated to remove residual EtOAc, and the resulting precipitates were collected by centrifugation (3000 rpm for 15 min). After washing with water (2 times), the precipitates were dissolved in a small amount of 80% MeOH and applied to a Sephadex LH-20 column (3.0 × 20 cm) using the same solvent. Caffeine

and other minor contaminants were eluted with 80 and 100% MeOH and polymeric polyphenols were eluted with 60% aq. acetone. The polymeric polyphenols (192 mg) were obtained as a dark brown powder. Elemental analysis: C 54.99; H, 4.92; N, 0.84. UV (MeOH)  $\lambda_{\max}$  nm: 275. IR  $\nu_{\max}$ (KBr)  $\text{cm}^{-1}$ : 3352, 2936, 1698, 1615, 1518, 1450, 1343 and 1233.

#### 2.4. Compound **1**

A tan amorphous powder.  $[\alpha]_{\text{D}} -40.8^{\circ}$  ( $c=0.1$ , MeOH). MALDI TOF MS  $m/z$ : 345  $[\text{M}+\text{Na}]^{+}$ , 361  $[\text{M}+\text{K}]^{+}$ , HR-FAB-MS  $m/z$ : 323.0776  $[\text{M}+\text{H}]^{+}$  (Calcd for  $\text{C}_{15}\text{H}_{15}\text{O}_8$ : 323.0767). IR  $\nu_{\max}$   $\text{cm}^{-1}$ : 3366, 2941, 1733, 1627 1519 and 1464. UV  $\lambda_{\max}$  (MeOH) nm (log  $\epsilon$ ): 278 (4.53). CD (MeOH,  $5.84 \times 10^{-5}$  mol/L)  $\Delta\epsilon$  (nm): 2.5 (282),  $-71.9$  (216).  $^1\text{H}$ -NMR (acetone- $d_6$  +  $\text{D}_2\text{O}$ )  $\delta$ : 6.02 (1H, d,  $J = 2.3$  Hz, H-6), 5.95 (1H, t,  $J = 1.8$  Hz, H-2'), 5.93 (1H, d,  $J = 2.3$  Hz, H-8), 5.62 (1H, ddd,  $J = 1.8, 3.4, 8.7$  Hz, H-5'), 5.05 (1H, br s, H-2), 4.37 (1H, ddd,  $J = 3.2, 4.6, 6.5$  Hz, H-3), 3.174 (1H, dd,  $J = 3.4, 16.5$  Hz, H-6'), 2.87 (1H, br dd,  $J = 4.6, 16.5$  Hz, H-4), 2.59 (1H, dd,  $J = 8.7, 16.5$  Hz, H-6) and 2.47 (1H, dd,  $J = 6.5, 16.5$  Hz, H-4).  $^{13}\text{C}$  NMR (acetone- $d_6$ )  $\delta$ : 172.2 (C-3'), 171.2 (C-4'), 170.4 (C-1'), 157.8 (C-7), 157.3 (C-5), 154.8 (C-8a), 118.4 (C-2'), 99.2 (C-4a), 96.5 (C-6), 95.1 (C-8), 79.6 (C-5'), 75.1 (C-2), 65.1 (C-3), 38.1 (C-6') and 27.3 (C-4).

### 2.5. Thiol degradation

A solution of polymeric polyphenols (10 mg) in 70% EtOH (0.5 ml) was mixed with 5% mercaptoethanol in 60% EtOH containing 0.1% HCl (1.5 ml) and heated at 70 °C for 7 h. The reaction mixture was analyzed by HPLC and the retention times and UV absorption of the peaks were compared with those of standard samples of (-)-epicatechin, (-)-epicatechin-3-*O*-gallate, (-)-epigallocatechin, (-)-epigallocatechin-3-*O*-gallate (**3**) and their 4-hydroxyethylthioethers.

### 2.6. Preparation of polymers of epigallocatechin-3-*O*-gallate

An aqueous solution of **3** (30 g in 3 L) was mixed with Japanese pear homogenate (2.4 kg) and vigorously stirred for 24 h (Tanaka, Watarumi, Matsuo, Kamei, & Kouno, 2003). Following the addition of EtOH (3 L), the mixture was filtered and the filtrate was applied to a Sephadex LH-20 column (10 × 45 cm) with water and the amount of MeOH in the solvent phase was increased. Fractions (23.5 g) mainly containing starting material and theasinensins A and D were eluted with 80–100% MeOH, and the fraction containing polymeric products (5.9 g) were eluted with 60% aq. acetone. The polymer fraction was subjected to Sephadex LH-20 column chromatography (4 × 55 cm) with acetone-7 M urea (3:2, v/v, adjusted to pH 2 with

conc. HCl). The fractions containing polymers were collected and concentrated by rotary evaporator (below 45°C) until the acetone was completely removed. The aqueous solution was then applied to a Diaion HP20P column (3 × 30 cm) and the urea was removed by washing the column with water. Finally, elution of the compounds from the column with 80% MeOH gave the polymers (2.07 g) as a black amorphous powder. Elemental analysis, C 54.57; H, 4.55; N, 0.41. UV (MeOH)  $\lambda_{\text{max}}$  nm: 278. IR  $\nu_{\text{max}}$ (KBr)  $\text{cm}^{-1}$ : 3369, 2959, 1698, 1614, 1519, 1450, 1338 and 1238.

### 2.7. GPC of polymeric polyphenols

The polymeric polyphenol (20 mg) was acetylated with  $\text{Ac}_2\text{O}$  (0.5 mL) and pyridine. (0.5 mL) at 60 °C for 5 h, and the mixture was poured into ice water. The product was collected as insoluble precipitates by centrifugation (3000 rpm, 5 min) and washed with water two times. High performance gel permeation chromatography was performed on a TSK gel  $\alpha$ -3000 column (7.8 × 300 mm) with *N,N*-dimethylformamide (DMF) containing 10 mM LiCl as the mobile phase at 40 °C (Fujihara, Nakagawa-Izumi, Ozawa, & Numata, 2007). Samples were dissolved in the elution solvent and 5  $\mu\text{l}$  were individually applied to the column and eluted using a flow rate of 0.5 ml/min; the eluate was monitored at 275 nm. Polystyrene standards (molecular

weights of 4,000, 25,000, 50,000 and 170,000), toluene (molecular weight 92) were used as the standards. The number average molecular weight (Mn) and weight average molecular weight (Mw) of Fr. 2217 were calculated from the SEC profile using an 807-IT integrator (Jasco).

### **3. Results and discussion**

High-performance liquid chromatography (HPLC) analysis of the 60% EtOH extracts indicated that the concentration of the four catechins, epicatechin (**2**), epigallocatechin and their galloyl esters, of post-fermented tea was much lower than that found in green tea (Fig. 1). For example, the peak areas of (-)-epigallocatechin and its galloyl ester **3** of the post-fermented tea was only 10.5 and 19.3%, respectively, of those of the green tea, indicating that large proportions of tea catechins are degraded by microorganisms; however, no apparent peak attributable to the metabolites derived from the catechins was observed in the chromatogram. This suggests that the catechins are decomposed to metabolites with only weak or no UV absorption. Furthermore, the appearance of a broad hump in the HPLC base-line and the detection of a FeCl<sub>3</sub>-positive substance at the origin on thin-layer chromatographic (TLC) analysis indicated the presence of polymeric polyphenols. It is also possible that the products were insoluble and could not

be extracted with water (Tanaka, et al., 1994).

Initially, we examined the minor metabolites detected as sharp peaks on HPLC analysis. The post-fermented tea was extracted with hot water, and fractionated by Sephadex LH-20. The fractions containing minor phenolic compounds were separated by a combination of column chromatography steps using Diaion HP20P, Chromatorex ODS and Sephadex LH-20 columns to yield a new compound **1** together with myricitrin, (+)-catechin, (-)-gallocatechin-3-*O*-gallate, theogallin (Hashimoto, Nonaka, & Nishioka, 1992), 1,4,6-tri-*O*-galloyl- $\beta$ -D-glucose (Nonaka, Sakai, & Nishioka, 1984) and strictinin (Okuda Yoshida Ashida & Yazaki, 1982). The isolated compounds, except for **1**, and four other major tea catechins are commonly found in green tea.

The compound **1** was obtained as a tan amorphous powder, and the molecular formula was indicated to be C<sub>15</sub>H<sub>14</sub>O<sub>8</sub> by HR-FAB-MS. The IR absorption at 1733 cm<sup>-1</sup> suggested the presence of carbonyl groups. The <sup>1</sup>H NMR spectrum is related to that of epicatechin (**2**) and exhibited the signals attributable to the A-ring aromatic protons ( $\delta$  6.02 and 5.93, each d, *J* = 2.3 Hz) and the C-ring H-2 ( $\delta$  5.05, br s), H-3 ( $\delta$  4.37, ddd, *J* = 3.2, 4.6, 6.5 Hz) and H-4 ( $\delta$  2.87, dd, *J* = 4.6, 16.5 Hz; 2.47, dd, *J* = 6.5, 16.5 Hz). A small coupling constant associated with the H-2 confirmed the 2,3-*cis* configuration. The remaining signals were assigned to one olefinic ( $\delta$  5.95, t, *J* = 1.8 Hz, H-2'), an

aliphatic methine ( $\delta$  5.62 ddd,  $J = 1.8, 3.4, 8.7$  Hz, H-5') and aliphatic methylene protons ( $\delta$  3.17, dd,  $J = 3.4, 16.5$  Hz; 2.59, dd,  $J = 8.7, 16.5$  Hz, H-6). The results indicated that the B-ring of **1** is non-aromatic and this was supported by very weak coloration with the FeCl<sub>3</sub> reagent. The <sup>13</sup>C NMR spectrum showed signals due to two carboxyl carbons [ $\delta$  172.2 (C-3') and 171.2 (C-4')], one double bond conjugated with a carbonyl group [ $\delta$  170.4 (C-1') and 118.4 (C-2')], one oxygen-bearing methine [ $\delta$  79.6 (C-5')] and an aliphatic methylene [ $\delta$  38.1 (C-6')]. The HMBC correlations of the C-ring H-2 to the olefinic C-1' and C-2' carbons confirmed the C-ring was attached to the double bond (Fig. 3). The location of the two carboxyl groups were determined to be as shown in Fig. 3 by the observation of correlations between C-3' and H-2', and between C-4' and H-5'/H-6'. Taking the degree of unsaturation (9) and chemical shift of the H-5' into account, the C-3 carboxyl group forms a  $\delta$ -lactone. The absolute configuration of the C-5' was deduced from the CD Cotton effect arising from the torsion of the lactone ring. The equatorial orientation of the C-4' carboxyl group was apparent from the large coupling constant of the H-5' ( $J_{5,6ax} = 8.7$  Hz). The CD spectrum showed a positive Cotton effect at 282 nm. On the assumption that the Cotton effect originated from the torsion of the  $-C=C-C=O$  moiety, the Cotton effect indicated the left-handed chirality of the conjugated carbonyl moiety (Fig. 4); thus, the configuration of the C-5' was deduced

to be *R* (Beecham, 1972; Snatzke, 1968). Based on these results, the structure of **1** is shown in Fig. 2. The production mechanism of **1** from **2** was proposed as shown in Fig. 5, in which oxygenative cleavage of the catechol B-ring via cyclic peroxide is the key reaction. Recently, it was reported that some microorganisms have the ability to convert (+)-catechin, an C-3 epimer of **2**, to metabolites with the same 3,6-dihydro-6-oxo-2*H*-pyran-2-carboxylic acid moiety in vitro (Das, Lamm, & Rosazza, 2010). Although the microorganisms involved in the production of post-fermented tea examined in this experiment are not known, it is apparent that the unknown microorganisms also have the ability to convert the catechol ring to the pyran carboxylic acid moiety.

During the post-fermentation process, the catechins are converted to polymeric substances, which were prepared from the fractions containing a polymeric substance by Sephadex LH-20 column chromatography in a gel-permeation chromatographic mode (Yanagida et al., 2003). In an alternative approach, the polymers were collected as precipitates of complexes with caffeine from a concentrated aqueous extract after the removal of non-polar compounds by partitioning with EtOAc, and purified by the usual Sephadex LH-20 column chromatography approach in an absorption chromatographic mode. The number-average molecular weight ( $M_n$ ) of the acetylated polymer was

estimated to be 3544 by gel permeation chromatography, which was 4.5-fold the mass of the octaacetyl derivative of **3**. The MALDI TOF MS did not show any informative peaks; however, the  $^{13}\text{C}$  NMR spectrum (Fig. 6A) showed resonances which were similar to those observed for a  $^{13}\text{C}$  NMR spectrum recorded on **3** (Fig. 6C), indicating that the polymer is composed of tea catechins with galloyl esters. The spectrum was also compared with that of the polymeric substance prepared by enzymatic oxidation of **3** (Fig. 6B). Elemental composition and infra-red absorptions of the oxidation products were similar to those observed for the polymers of post-fermented tea. In the  $^{13}\text{C}$  NMR spectrum of the polymer of **3**, the pyrogallol-type B-ring signals are significantly reduced compared with that of **3**, because oxidation and the subsequent coupling reactions are primarily taking place at the B-rings (Tanaka, et al., 2010). In contrast, the spectrum of the polymers of post-fermented tea exhibited the signals attributable to the B-ring carbons ( $\delta$  108, 128–135 and 145). This observation indicates that the polymers of post-fermented tea are not produced by oxidative coupling reactions. The appearance of a broad hump at the  $\delta$ 170–175 suggests the presence of carboxylic acids related to those of **1** in the molecule. It should be noted that the IR absorptions of the two polymers are almost identical, therefore indicating that the structures of the two compounds are similar and the UV spectra of both polymers showed absorptions at 278

nm mainly arising from galloyl moieties. Thiolysis, which cleaves proanthocyanidin C-4–C-8(6) linkages, was applied to the polymer (Tanaka et al., 1994); however, most of the polymers were not degraded (Fig. 7). Production of small amounts of catechins and their thioethers suggested the presence of procyanidins-like linkages in the polymer. The presence of the inter-flavan linkage is also suggested by the appearance of carbon signals in the range of  $\delta$  34–38 in the  $^{13}\text{C}$  NMR spectrum which can be attributed to the C-4 of the proanthocyanidin extension units (Fig. 6); however, the signals are much smaller than those of the C-4 methylene carbon signals at  $\delta$  25–28. To investigate the polymerization mechanism, a different strategy for spectral analysis or chemical degradation should be developed.

#### **4. Conclusions**

We are currently in the initial stages of the chemical analysis of post-fermented tea, including the examination of the metabolism of tea catechins by microorganisms. In this study, we isolated a new metabolite generated by the cleavage of the epicatechin B-ring. In addition, methods to separate polymeric polyphenols from post-fermented tea were developed and the polymers were partially characterized. Catechin degradation during microbial fermentation includes polymerization and oxidative cleavage of aromatic

rings and the phenomena suggests that the degradation of tea catechins is related to production of humic acids. The IR spectrum of polymers of post-fermented tea actually resembles that of humic acid (Grasset, & Amblès, 1998). The mechanism of catechin polymerization is unclear and we are currently characterizing the structure of the polymers.

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## Figure Captions

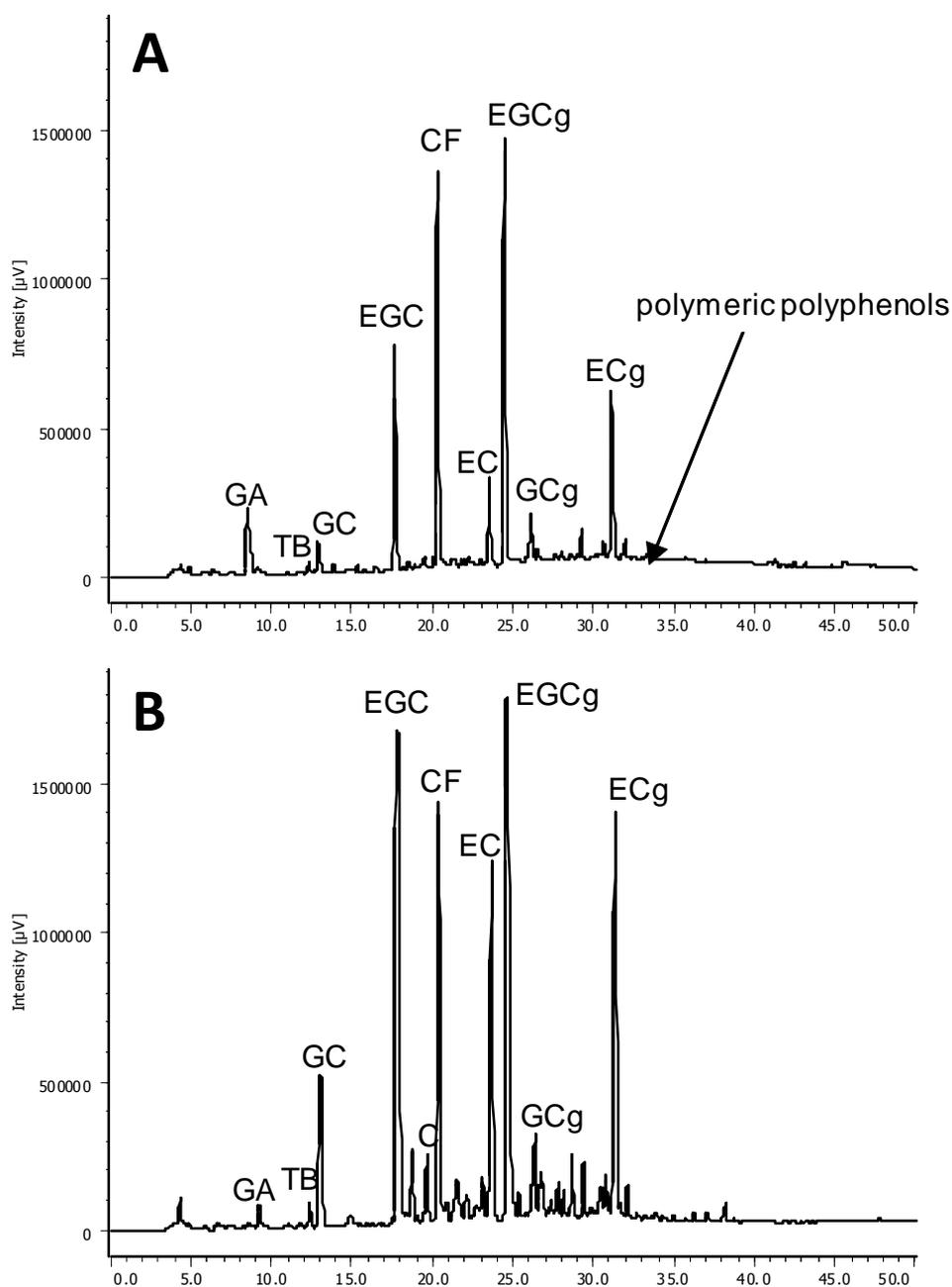


Fig. 1 HPLC profiles (max absorbance) of post-fermented tea (A) and green tea (B). CF: caffeine, EC: epicatechin (2), ECg: epicatechin-3-*O*-gallate, EGC: epigallocatechin, EGCg: epigallocatechin-3-*O*-gallate (3) (C), GA: gallic acid, GCg: gallocatechin-3-*O*-gallate, TB: theobromine.

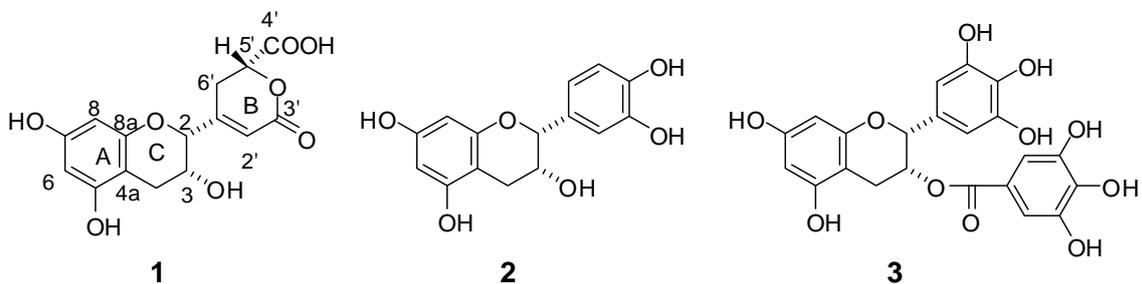


Fig. 2 Structures of 1 – 3.

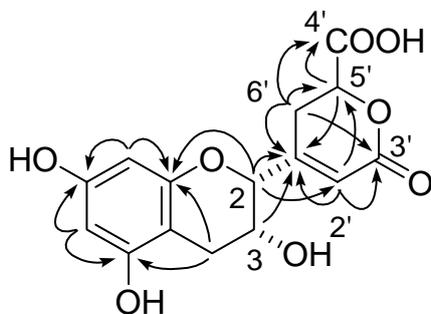


Fig. 3 Selected HMBC correlations of 1.

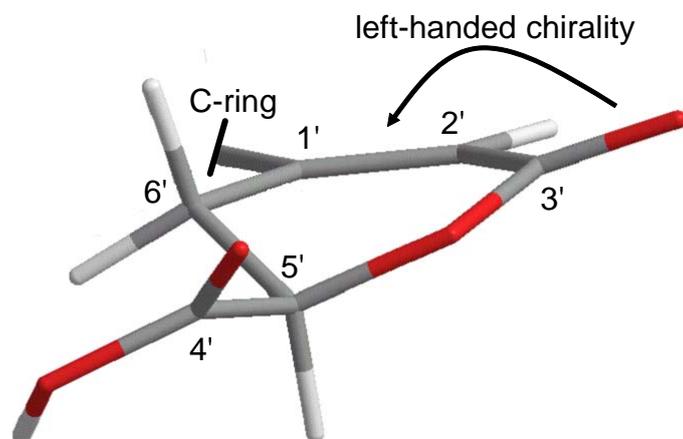


Fig. 4 Conformation of the B-ring of **1**.

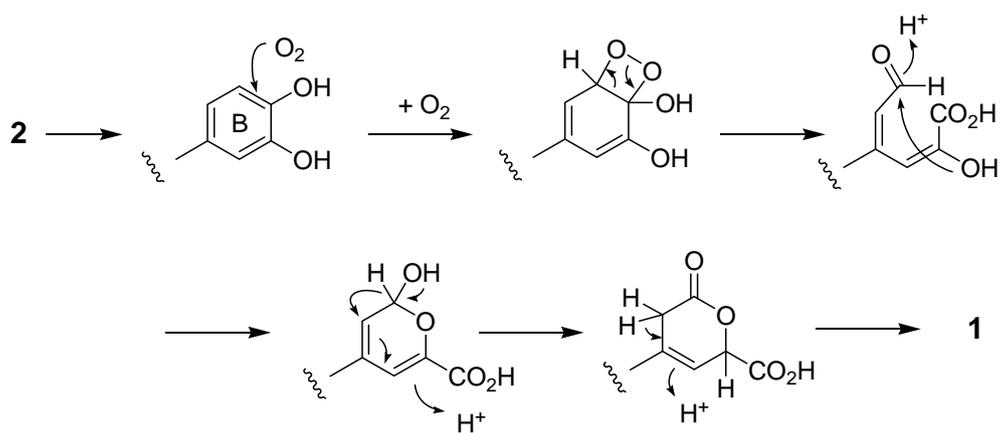


Fig. 5 The possible production mechanism of **1** from **2**

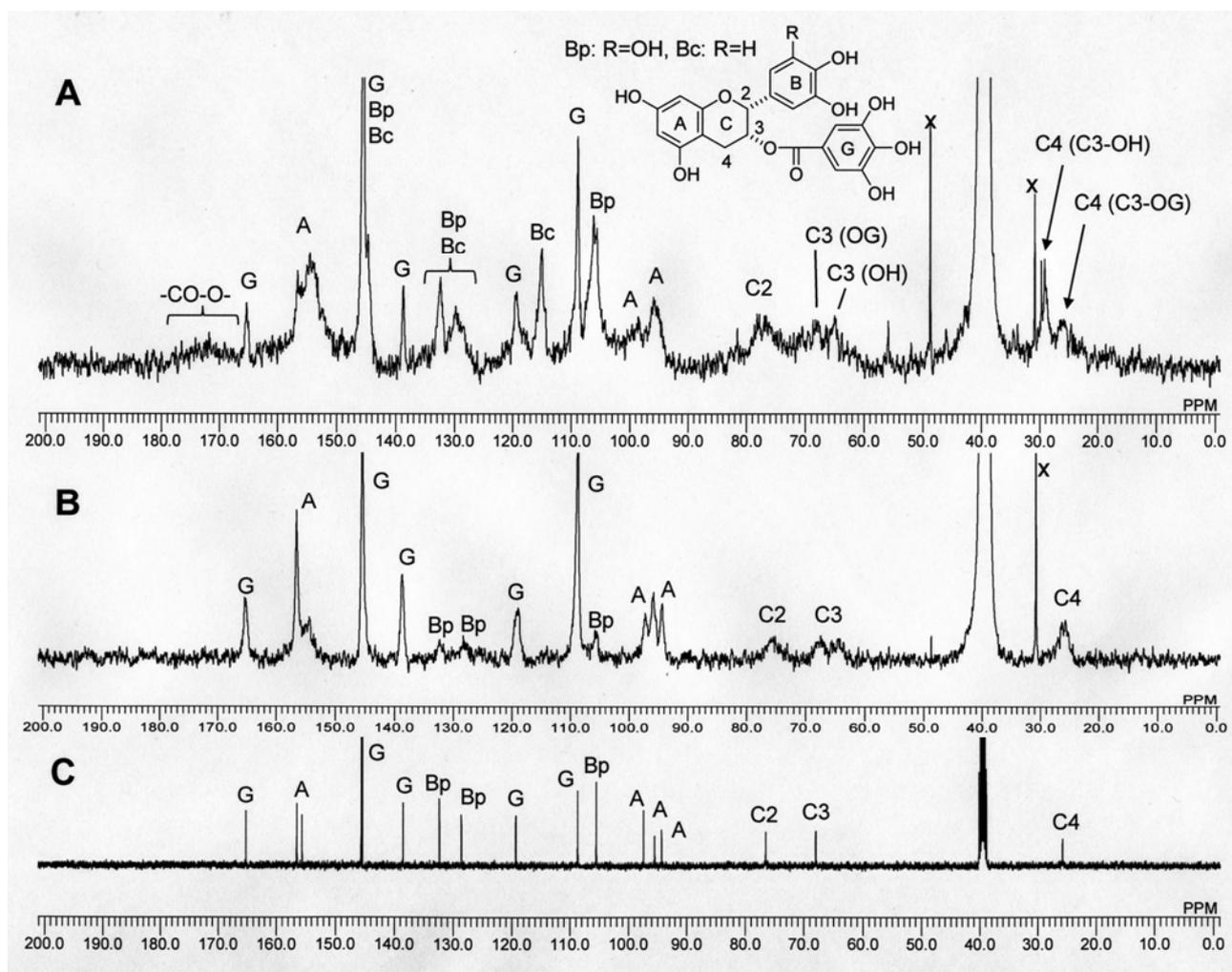


Fig. 6  $^{13}\text{C}$ -NMR spectra of polymeric polyphenols of post-fermented tea (A), polymers produced by enzymatic oxidation of epigallocatechin-3-O-gallate (B), and epigallocatechin-3-O-gallate (3). A: A-ring carbons, Bp: R = OH (pyrogallol type), Bc: R = H (catechol-type), C: C-ring carbons.

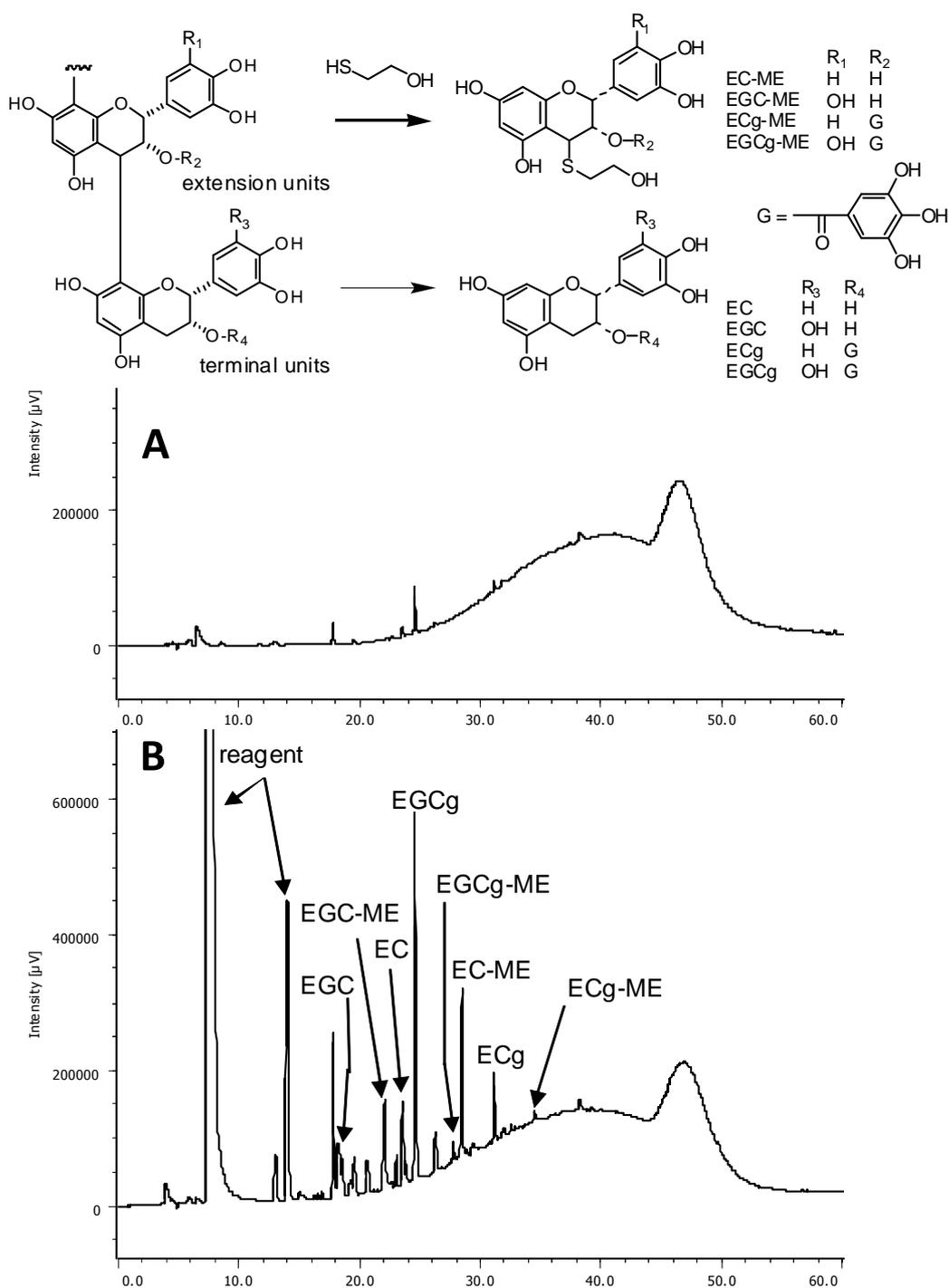


Fig. 7 Thiol degradation of polymeric polyphenols of post-fermented tea with 2-mercaptoethanol. A: before reaction, B: after thiol degradation. EC: epicatechin, EGC: epigallocatechin, ECg: epicatechin-3-*O*-gallate, EGCg: epigallocatechin-3-*O*-gallate (**3**), ME: hydroxyethylthio ethers.

## Graphical Abstract

### **A new catechin oxidation product and polymeric polyphenols of post-fermented tea**

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