



Pergamon

Tetrahedron Letters 41 (2000) 485–488

TETRAHEDRON  
LETTERS

## Conversion of procyanidin B-type (catechin dimer) to A-type: evidence for abstraction of C-2 hydrogen in catechin during radical oxidation

Kazunari Kondo,<sup>a,\*</sup> Masaaki Kurihara,<sup>b</sup> Kiyoshi Fukuhara,<sup>b</sup> Takashi Tanaka,<sup>c</sup> Takashi Suzuki,<sup>a</sup> Naoki Miyata<sup>b</sup> and Masatake Toyoda<sup>a</sup>

<sup>a</sup>Division of Foods, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

<sup>b</sup>Division of Organic Chemistry, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

<sup>c</sup>School of Pharmaceutical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan

Received 1 October 1999; revised 29 October 1999; accepted 2 November 1999

### Abstract

Procyanidin B-1 and B-2 were converted into A-1 and A-2 by radical oxidation using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals under neutral conditions, respectively. Transformation of procyanidin B-type into A-type certainly shows abstraction of the hydrogen atom at the C-2 position during radical oxidation. © 2000 Elsevier Science Ltd. All rights reserved.

**Keywords:** polyphenols; antioxidant; radicals; bond dissociation enthalpy.

Catechins and their oligomers are an important class of polyphenols ubiquitously found in plants (Fig. 1). They have versatile biological effects such as anticancer,<sup>1</sup> antiallergy,<sup>2</sup> and antioxidant activity.<sup>3</sup> In general, polyphenols like catechins can scavenge free radicals by donating electrons located on the phenolic OH and are changed into the corresponding quinone compounds. Therefore, it has been considered that (–)-epicatechin (EC) with the catechol moiety can trap two radical molecules and be transformed into the corresponding *o*-quinone. However, this cannot explain the fact that the antioxidant effect of EC is greater than that of other flavonoids with the catechol moiety.

Recently, we have clarified the antioxidant effects of monomeric catechins and proposed their antioxidant mechanisms which would be closely related to the potential of catechins in biological fluids.<sup>4,5</sup> In our studies, it has been shown that EC is very effective in suppressing the production of lipid peroxidation and that EC can trap three molecules of peroxy radicals in the liposomal system.<sup>4</sup> The results from LC/MS/MS analyses of the reaction products and calculation of bond dissociation enthalpies (BDEs) for catechins showed that the third reaction site to trap peroxy radicals was the benzylic C-2 position in catechins as shown in Fig. 2, suggesting the importance of the presence of the C-2 hydrogen in catechins.<sup>6</sup>

\* Corresponding author. Fax: +81-3-3707-6950; e-mail: kondo@nihs.go.jp (K. Kondo)

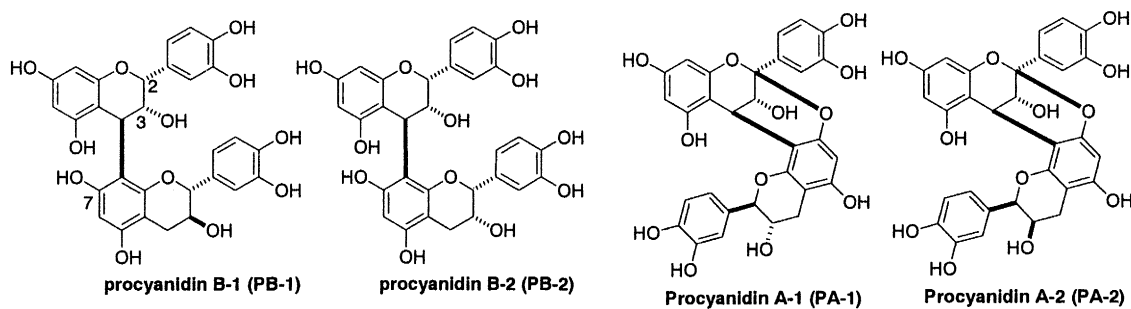


Fig. 1. Structures of procyanidin A- and B-types

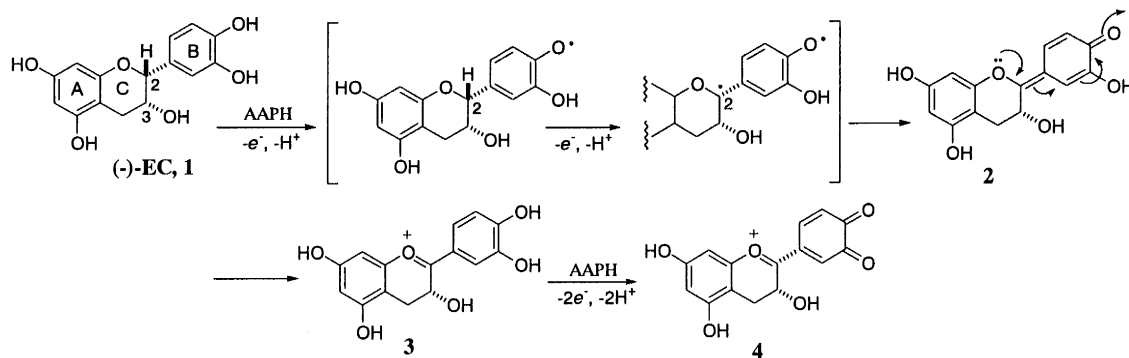


Fig. 2. Proposed antioxidant mechanisms of EC in radical oxidation

That is, EC donates an electron to peroxy radicals followed by the abstraction of the C-2 hydrogen. This result supports the fact that the antioxidant activity of EC is the same or greater than quercetin, which has the 2,3-double bond in conjunction with a 4-oxo function in the C-ring that is responsible for electron delocalization and lower oxidation potential.<sup>7</sup> However, direct evidence for the abstraction of the C-2 hydrogen could not be obtained. Attempts to elucidate the abstraction, isolation of adducts at the C-2 position of catechins during radical reaction, were unsuccessful. Transformation of procyanidin B-type to A-type in the alkaline/H<sub>2</sub>O<sub>2</sub> system has been reported previously.<sup>8</sup>

We were very interested in the generation of A-type procyanidins because the transformation is an oxidative intramolecular reaction. In the present study, an effort to clarify the abstraction of the C-2 hydrogen was done by analyzing the reaction products formed from procyanidin B-1 or B-2 (catechin dimers) with DPPH radicals under neutral conditions<sup>9</sup> and by BDEs for PB-1 and PB-2.<sup>10</sup> As shown in Fig. 3,<sup>11</sup> by the reaction of PB-1 with DPPH radicals under neutral conditions, PA-1 was immediately produced ( $t_R=11.87$  min) together with two other peaks, and the peak (PA-1) and its spectrum were identified by comparison with those of an authentic sample (PA-1).<sup>12</sup> PA-1 was not produced by DPPH radicals in ethanol, suggesting the reaction of product **2** into **3** as seen in Fig. 2. The BDE at the C-2 position of the upper catechin unit was low (68.7 kcal/mol), enough to be abstracted by radicals. Furthermore, electrochemical analysis was carried out to elucidate the mechanisms described in Fig. 2 (Fig. 4), indicating that two electrons are easily abstracted from EC. The result from the molecular mechanics calculation (MM2\*) suggests that the distance between the C-2 carbon of the upper catechin unit and the C-7 oxygen of the lower unit was close (4.6 Å) in the global minimum energy conformation for PB-1 quinone intermediate (data not shown).

These results show that the C-2 hydrogen of the upper catechin unit in PB-1 (catechin dimer) is certainly abstracted during the radical reaction and that PB-1 is probably converted to PA-1 by a quinone

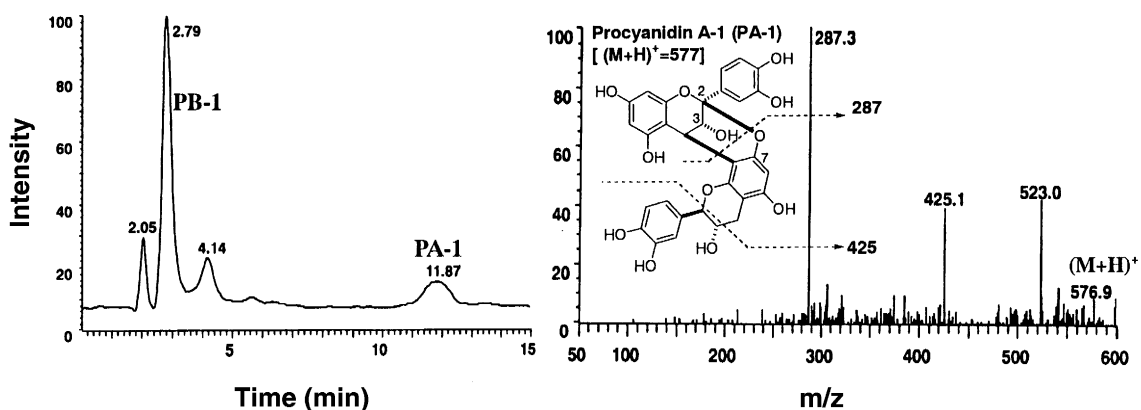


Fig. 3. Total ion chromatogram of reaction products formed from PB-1 and DPPH radicals (left) and mass spectrum of PA-1 formed from PB-1 (right)

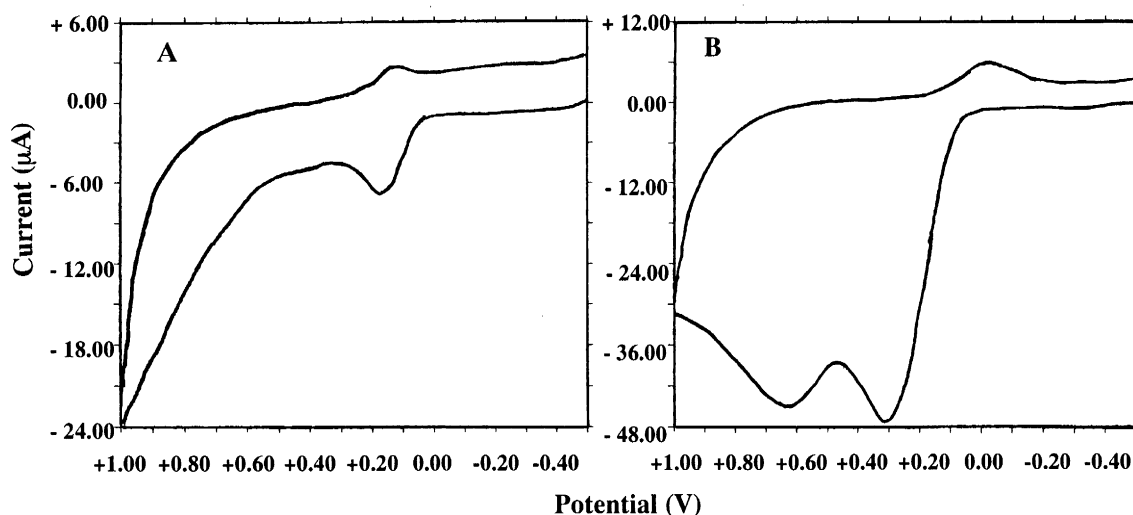


Fig. 4. Cyclic voltammograms for the oxidation of quercetin (A) and EC (B). Quercetin and EC (2 mM) were analyzed using glassy carbon electrode in 50 mM sodium cacodylate buffer (pH 7.2),  $\nu=200$  mV/s

intermediate mechanism. As a result, PB-1 as an antioxidant was converted into procyanidin A-1 (PA-1) during the radical oxidation under neutral conditions as shown in Fig. 5. PA-1 can also act as an antioxidant because of its catechol structure, indicating that procyanidins exert a strong scavenging activity. It is reported that the antioxidant effect of PA-2 is less than that of PB-2.<sup>13</sup> This also supports the importance of the C-2 hydrogen in catechin unit. Similarly, PA-2 was immediately produced by the reaction of PB-2 and DPPH radicals (data not shown). Procyanidin A-type naturally found in plants might be produced from procyanidin B-type via a radical process.

In conclusion, the strong effect of catechins and their oligomers on radical scavenging is based upon the presence of the C-2 hydrogen in addition to the *o*-dihydroxyl structure.

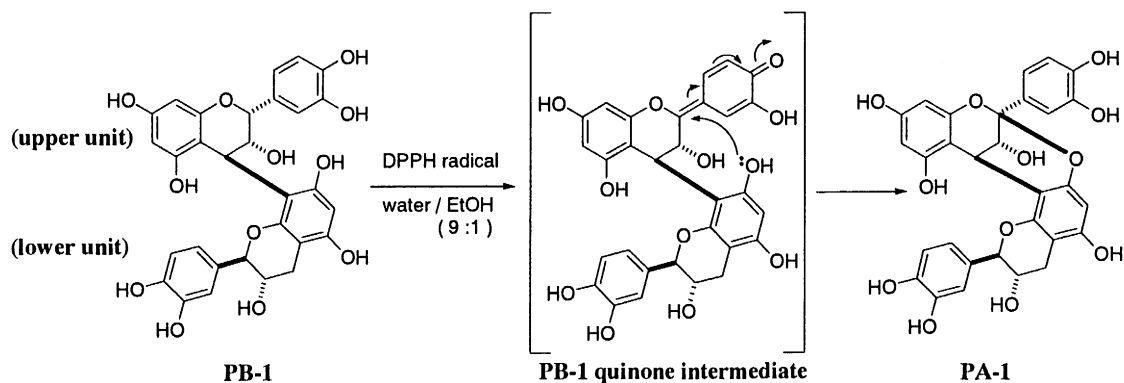


Fig. 5.

## Acknowledgements

This study was supported in part by a grant from the Health Science Foundation and by a grant from the Ministry of Health and Welfare.

## References

1. Brown, J. P. A. A. *Mutat. Res.* **1980**, 75, 243.
2. Sano, M.; Suzuki, M.; Miyase, T.; Yoshino, K.; Maeda-Yamamoto, M. *J. Agric. Food Chem.* **1999**, 47, 1906.
3. Ariga, T.; Hamamoto, M. *Fragrance J.* **1994**, 22, 52.
4. Kondo, K.; Kurihara, M.; Miyata, N.; Suzuki, T.; Toyoda, M. *Arch. Biochem. Biophys.* **1999**, 362, 79.
5. Kondo, K.; Kurihara, M.; Miyata, N.; Suzuki, T.; Toyoda, M. *Free Radical Biol. Med.* **1999**, 27, 855.
6. Although the BDE for the C-2 hydrogen in EC (69.1 kcal/mol) was lower than that for the phenolic OH (ca. 75 kcal/mol), the first abstraction (an electron and then hydrogen) from EC probably occurred at the phenolic OH.
7. Rice-Evans, C. A.; Miller, N. J.; Paganga, G. *Free Radical Biol. Med.* **1996**, 20, 933.
8. Satoshi, M.; Nanaka, G.; Nishioka, I. *Chem. Pharm. Bull.* **1987**, 35, 4717.
9. To 200  $\mu$ M of PB-1, DPPH (2 mM) was added and the mixture stood at 40°C. In LC/MS analysis, ODS column was used and MeOH/1 was used as an eluent. The flow rate was 0.2 ml/min.
10. The program SPARTAN (version 5.0, Wavefunction Inc., Irvine, CA) was used for molecular orbital (MO) calculations. First, the structures of procyanidins and their radicals were optimized by a conformational search (MacroModel<sup>®</sup> version 6.5, Schrödinger Inc. Jersey City, NJ; MonteCarlo method, MM2\* force field) and then the bond dissociation enthalpies (BDEs) were calculated using the semiempirical AM1 method. The BDEs at the phenolic OH were ca. 75 kcal/mol.
11. The ion peak ( $m/z$  425) by fragmentation and the ion peak ( $m/z$  287) by retro-Diels–Alder fission were observed as reported by Karchesy et al. in: *Anal. Chem.* **1986**, 58, 2563.
12. Procyanidin A-types (PA-1 and PA-2) are natural products found in plants such as peanut skins.
13. Saint-Cricq de Gaulejac, N.; Provost, C.; Vivas, N. *J. Agric. Food Chem.* **1999**, 47, 425.