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Planar Catechin Analogues with Alkyl Side Chains: A Potent Antioxidant and an α-Glucosidase Inhibitor

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As mitochondrial oxidative damage[1] or oxidative modification of low-density lipoprotein (LDL)[2] contribute significantly to a range of degenerative diseases and further production of reactive oxygen species (ROS), it might be advantageous to develop lipophilic antioxidants which would be able to suppress mitochondrial ROS production or LDL oxidation due to their affinity to lipid particles or membrane. Recently, we synthesized planar catechin analogue (**PC1**), in which the catechol and chroman structure in (+)-catechin are constrained to be planar, by the reaction of (+)-catechin with acetone in the presence of $BF_3{\cdot}Et_2O$.[3,4] The rate of hydrogen transfer from **PC1** to galvinoxyl radical (**G**•), a stable oxygen-centered radical, is about 5-fold faster than that of hydrogen transfer from the native (+)-catechin to **G**•. **PC1** also shows an enhanced protective effect against oxidative DNA damage induced by the Fenton reaction without the pro-oxidant effect, which is usually observed in the case of (+)-catechin. We also have found that **PC1**, as well as stilbene resveratrol[5] which is a typical cancer chemopreventive agent present in grapes, inhibits cell growth through induction of apoptosis in cancer cell lines (data not shown). Therefore, we envisioned that a conformationally constrained planar catechin might be valuable in the development of a new type of clinically useful antioxidant, if the hydrophobicity of **PC1** could be controlled so as to fine-tune its membrane binding and penetration into the phospholipid bilayer. Here, we describe a synthetic method for planar catechin analogues (**PCn**), the lipophilicity of which was controlled by changing the length of the alkyl chains. Also described are their remarkable antioxidative potencies and α-glucosidase inhibitory activities.

The synthesis of **PCn** was carried out by reacting catechin with various ketones having alkyl chains of different lengths. However, the previously reported method for the synthesis of **PC1**[3] is inapplicable to other **PCn** synthesis. Because the original reaction is carried out in a solution of acetone, the synthesis of **PCn** is limited to using the corresponding ketone as a solvent. Therefore, it was necessary to improve the synthetic method of **PC1** to be able to introduce various types of ketones into the catechin structure using a synthetic scheme applicable for any **PCn** production. We attempted to optimize the reaction using a combination of various acids and solvents, and finally, it was shown that the reaction using silyl Lewis acids such as TMSOTf, TESOTf, or TBSOTf gave the desired products in high yields. Typically, (+)-catechin and 1.2 equiv of ketone in THF was treated with 1.2 equiv of TMSOTf at −5 °C to form the desired **PCn**. This reaction was used to provide a series of **PC1** ≈ **PC6**, 44−76% yield (Scheme 1), with slightly different lipophilicity.

PCn were evaluated for their radical scavenging activities against DPPH (2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl) radical and AAPH (2,2′-azobis(2-amidinopropane) dihydrochloride)-derived peroxyl radical (Scheme 2). The hydrogen abstraction of **PCn** by DPPH radical in deaerated acetonitrile solution was monitored using the decrease of the visible absorption band at 543 nm due to DPPH radical that obeyed pseudo-first-order kinetics. The second-order rate constant (k_{HT}) for hydrogen abstraction of **PCn** by DPPH radical was then determined (Table 1). Similar to what was found with hydrogen abstraction by galvinoxyl radical,[3] the k_{HT} value (533 M^{-1} s^{-1}) of **PC1** is significantly larger than that of (+)-catechin (305 M^{-1} s^{-1}), indicating that the radical-scavenging activity of catechin using DPPH radical increased due to constraining the (+)-catechin in a planar configuration. In addition, it was found that the larger the number of carbon atoms there were in the alkyl chains, the greater the DPPH radical scavenging rates became, with the k_{HT} value of **PCn** plateauing at $n = 4$. The radical scavenging ability of **PCn** with longer side chains might be attributed to the −I effect of the side chain that stabilizes the cation radical formed after electron transfer from **PCn** to DPPH. The radical scavenging activities of **PCn** in aqueous solution were investigated using AAPH as a source of free radicals in phosphate buffer (Table 1). AAPH-derived peroxyl radicals react with luminol to generate prolonged luminescence,[6] and the antioxidative activities of **PCn** were determined using the concentration of **PCn** where the luminescence is reduced to 50%. As a result, the antioxidative activity of planar catechin in phosphate buffer was again stronger than that of catechin as well as its antioxidative activity in acetonitrile. The alkyl side chains also affect the antioxidative activity; an increase ($n = 1-3$) in the length of the alkyl chains tends to increase the antioxidative activity, with **PC3** showing the strongest antioxidative effect. However, further increase ($n = 4-6$) in the length of the side chain seems to weaken the antioxidative effects, which is consistent with the suggestion that longer alkyl side chains result in the formation of amphiphilic micelles in aqueous solvent.

For the evaluation of lipophilic **PCn** as antioxidants against biomolecular injury caused by ROS, the protecting effect of **PCn** on oxidative DNA damage induced by the Fenton reaction was

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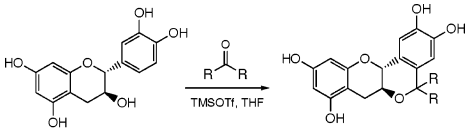
Scheme 1. Chemical Structure and Synthesis of PCn



PC1 R = CH_3, *76.3% yield*
PC2 R = CH_2CH_3, *73.5% yield*
PC3 R = $(CH_2)_2CH_3$, *66.7% yield*
PC4 R = $(CH_2)_3CH_3$, *59.1% yield*
PC5 R = $(CH_2)_4CH_3$, *52.8% yield*
PC6 R = $(CH_2)_5CH_3$, *44.6% yield*

Scheme 2. Radical Scavenging Reaction of PCn against DPPH• and AAPH

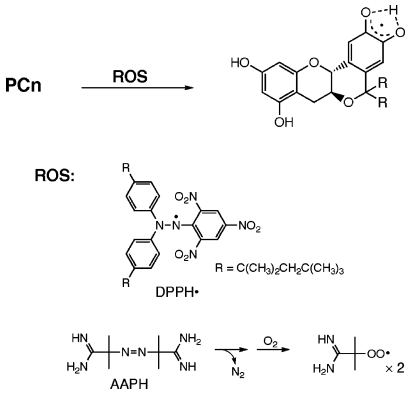


Table 1. Antioxidant Profile of Catechin and PCn Determined Using a DPPH and AAPH Scavenging Assay

compd	DPPH• k_{HT} (M^{-1} s^{-1})	AAPH IC_{50} (nM)
catechin	305	292
PC1	533	220
PC2	622	175
PC3	686	98
PC4	725	147
PC5	756	625
PC6	759	1700

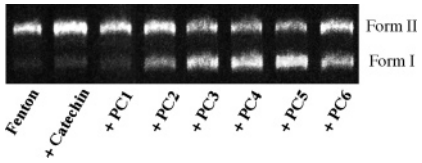


Figure 1. Effects of catechin and PCn on DNA breakage induced by the Fenton reaction (Fe^{3+}/H_2O_2). Assays were performed in 100 mM phosphate buffer, pH 7.0 containing 45 μM pBR322DNA, 10 mM H_2O_2, 100 μM $FeCl_3$, and 1 mM individual PCn for 1 h at 37 °C.

determined. Although **PC1** showed an excellent protecting effect against oxidative DNA scission compared with catechin,[3] the antioxidative activity of the series of **PCn** was evaluated under conditions in which the protecting effect of **PC1** appears to be weak. As shown in Figure 1, DNA cleaving activity induced by the Fenton reaction did not increase in the presence of **PCn**, and with an increase in the length of alkyl chains, the protecting effect of **PCn** on the oxidative DNA damage was greatly increased. The strong antioxidative activity might be attributed to a combination of radical scavenging activity and lipophilicity that tends to increase the binding between **PCn** and DNA. A small decrease in the protecting effect of **PC6** might be responsible for the diminishing radical scavenging ability under aqueous solution.

In addition to the antioxidative ability, (+)-catechin is known to be an inhibitor against α-glucosidase[7] that catalyzes the final step in the digestive process of carbohydrates. Therefore, the inhibitory effects of **PCn** on α-glucosidase from *Saccharomyces cerevisiae* and *Bacillus stearothermophilus* were evaluated (Table 2). Surprisingly, in contrast to the relative weak inhibitory effect of (+)-catechin with IC_{50} > 500 μM, **PCn** exhibited strong inhibitory effects with $IC_{50} = 0.7-47.5$ μM against both enzymes, with **PC1** (IC_{50} = 1.2 μM for *S. cerevisiae* and 0.7 μM for *B. stearothermophilus*) and **PC6** (IC_{50} = 0.9 μM for *S. cerevisiae* and 1.1 μM for *B. stearothermophilus*) showing especially high inhibition concentrations. The strong inhibitory effect of **PCn** on α-glucosidase suggested that these planar catechin analogues may be used as a lead compounds for the development of antidiabetic therapeutics, similar to acarbose and voglibose which are known to reduce postprandial hyperglycemia primarily by interfering with the carbohydrate digesting enzymes and delaying glucose absorption.

Table 2. Inhibitory Activities of Catechin and PCn against α-glucosidases

compd	*S. cerevisiae* IC_{50} (μM)	*B. stearothermophilus* IC_{50} (μM)
catechin	>500	>500
PC1	1.2	0.7
PC2	47.5	26.8
PC3	37.5	28.4
PC4	2.1	14.2
PC5	5.3	6.8
PC6	0.9	1.1

In summary, a practical method for the preparation of planar catechin analogues with various alkyl side chain lengths is described as well as the remarkable properties of these compounds as potent antioxidants and α-glucosidase inhibitors. In vivo studies to fully exploit these potential benefits of PCn are currently under way, and the results will be published in due time.

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Note Added after ASAP Publication. After this paper was published ASAP on May 3, 2006, Table 2 was corrected to show the *S. cerevisiae* IC_{50} value of 1.2 μM for **PC1.**

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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