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Synthesis and Enhanced Radical Scavenging Activity of a Conformationally Constrained Epigallocatechin Analogue

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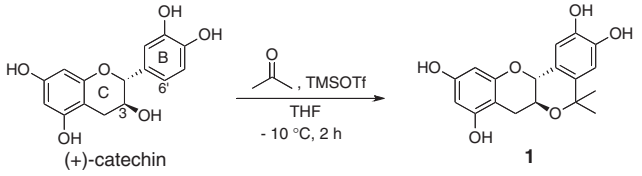
The freely rotating single bond between the pyrogallol and chroman substructures in epigallocatechin (EGC) was constrained by reaction of EGC with acetone in the presence of trimethylsilyl trifluoromethanesulfonate to prepare the rigidified analog in good yield. The synthesized analog was examined for free radical scavenging activity toward the galvinoxyl radical and was found to be 27-fold more potent than EGC.

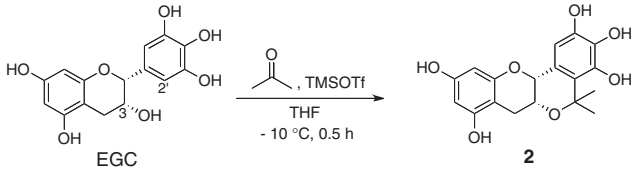
As oxidative stress is considered to be a crucial factor in cell damage, the protective role of antioxidants is receiving increased attention as an approach to suppressing oxidative stress by scavenging free radicals that are produced in the process of energy production and utilization. In fact, the antiaging benefit of antioxidants is due to their anti-inflammatory effect that delays or prevents the occurrence of cancer, diabetes, and brain disorders.[1–5] However, for clinical usage and/or chemoprevention of oxidative stress-associated diseases, the development of novel antioxidants with promising free radical scavenging activity that also have the appropriate pharmacokinetic and clinical pharmacology is required. Our goal was to develop an effective and practical chemopreventive agent based on a natural antioxidant lead, which was designed to target free radical generation in the progression of oxidative stress-related diseases such as cardiovascular disease, diabetes, and cancer. We have recently reported the synthesis of the (+)-catechin analog **1** in which the catechol and chroman moieties in (+)-catechin were constrained to the same plane by formation of a bridge between the 3-OH group on ring C and C6′ on ring B.[6,7] As shown in Scheme 1, the synthesis was accomplished by taking advantage of a Pictet–Spengler reaction between (+)-catechin and acetone to give the derivative **1**, which had 5-fold more potent radical scavenging activity compared with (+)-catechin. Interestingly, **1** was very lipophilic compared to (+)-catechin, and the high radical-scavenging ability of **1** was expected to be very effective for suppressing free-radical associated events, especially in cell membranes. The one-step chemical modification of (+)-catechin to generate a derivative with improved radical-scavenging activity could also be used as a way of modulating the pharmacokinetics of (+)-catechin by substituting lipophilic or hydrophilic ketones for acetone. Therefore, this method should be effective in developing synthetic versions of natural catechin that target oxidative stress generation in individual diseases. Among naturally occurring catechins, epigallocatechin (EGC) and its gallate derivative (EGCG) are the principal phenolic antioxidants found in a variety of plant products including green and black tea. EGCG has been shown to have cancer-preventive activity in a number of animal models, and numerous mechanisms to account for this activity have been proposed based on studies with human cell lines. In this study, the freely rotating single bond between the pyrogallol and chroman substructures in EGC was constrained by taking advantage of the same method used for the synthesis of **1**. Compared with EGC, the conformationally constrained EGC **2** showed greatly enhanced radical-scavenging activity against the galvinoxyl radical. This is the first report of a chemical modification of EGC that produced a derivative with improved radical scavenging activity.

As shown in Scheme 2, **2** was synthesized by the Pictet–Spengler reaction of EGC, 1.2 equivalents of acetone, and 1.2 equivalents of trimethylsilyl trifluoromethanesulfonate in dry THF at −10 °C under argon for 0.5 h. The reaction mixture was purified by column chromatography to obtain **2** as a pale yellow powder in 78.4% yield.[8] Analysis of the ^{1}H NMR of **2** in DMSO-d_6 exhibited a spectrum in which the 2′-H and 3-OH signals had disappeared while the other EGC protons remained along with two new methyl group signals, indicating the formation of an isopropyl bridge between rings B and C.

To evaluate the antioxidant activity of **2**, a radical-scavenging assay using the galvinoxyl radical (GO•) as an oxyl radical species was performed. When **2** was added to a deaerated



Scheme 1. Synthesis of **1** from (+)-catechin.



Scheme 2. Synthesis of **2** from EGC.

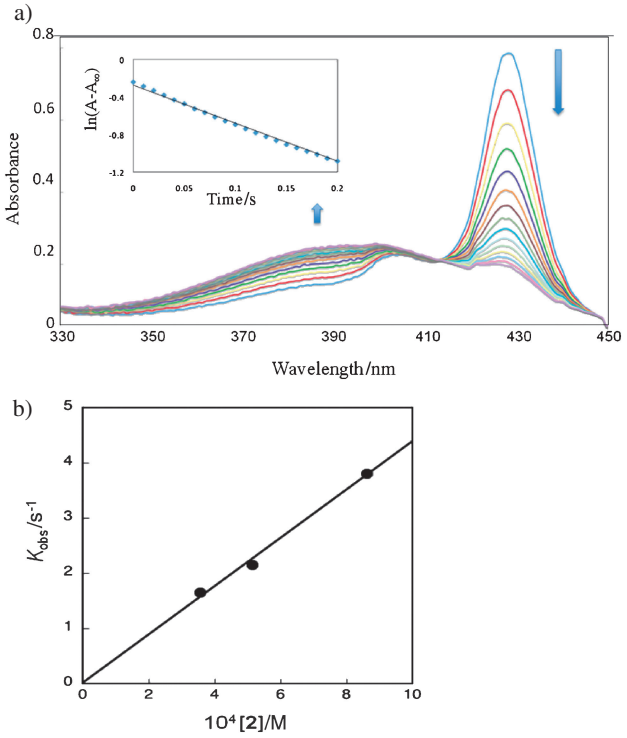


Figure 1. (a) Spectral changes in the reaction of **2** with GO$^{\bullet}$ in deaerated acetonitrile at 298 K (Interval: 10^{-2} s). (Inset) First-order plot based on the change in absorption at 428 nm. (b) Plot of the pseudo-first-order rate constants (k_{obs}) vs. [**2**] for the reaction of **2** with GO$^{\bullet}$.

acetonitrile solution of GO$^{\bullet}$, the visible absorption band at 428 nm due to GO$^{\bullet}$ immediately disappeared, indicating that GO$^{\bullet}$ scavenging by **2** took place to give the hydrogen-transfer product GO-H. As this spectral change was very fast, the rate of the radical-scavenging reaction of **2** was measured by monitoring the decrease in absorbance at 428 nm using a stopped-flow technique. As shown in Figure 1a, the decay of the absorbance at 428 nm obeyed pseudo-first-order kinetics when the concentration of **2** was maintained at more than 10-fold excess to the GO$^{\bullet}$ concentration. The pseudo-first-order rate constant (k_{obs}) increased linearly with an increase in the concentration of **2**, as shown in Figure 1b. From the slope of the linear plot of k_{obs} vs. [**2**], the second-order rate constant (k) for the radical-scavenging reaction was determined to be $4.34 \times 10^3\,M^{-1}\,s^{-1}$. The k value for EGC determined in the same manner was $1.62 \times 10^2\,M^{-1}\,s^{-1}$, showing that the radical-scavenging activity of **2** was about 27-fold greater than that of EGC.

Previously, it was found that hydrogen transfer from both (+)-catechin and **1** in the GO$^{\bullet}$ scavenging reaction proceeds by a one-electron transfer followed by proton-transfer mechanism.[9] In analogy with the catechins, the GO$^{\bullet-}$ scavenging reaction of **2** is thought to proceed via a one-electron transfer from **2** to GO$^{\bullet}$. Therefore, the strong radical-scavenging activity of **2** compared with EGC might be attributable to the electron-donating effect of the isopropyl group in **2**, i.e., the isopropyl group is capable of stabilizing the radical cation intermediate **2**$^{\bullet+}$ generated in the electron-transfer oxidation of **2** by GO$^{\bullet}$, which would account for the enhanced radical-scavenging activity.

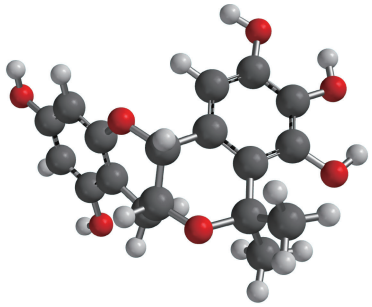


Figure 2. DFT optimized structure of **2** calculated using the B3LYP/6-31G* basis set.

In the case of **1**, the planar structure has an ability to delocalize the radical cation throughout the entire molecule, resulting in enhanced radical-scavenging activity. However, as shown in Figure 2, no such enhanced stabilizing delocalization of the radical cation is possible in the bent form of **2**. Because the electron-transfer mechanism for the radical-scavenging reaction of **2** depends on the electrochemical ease of one-electron oxidation, one-electron oxidation potentials (E_{ox}^0) of EGC and **2** were measured by electrochemical methods. Cyclic voltammetry (CV) was initially attempted, but the radical cation of EGC generated in the time scale of the CV experiment was too unstable to determine an accurate E_{ox}^0. Therefore, second harmonic alternating current voltammetry (SHACV) was performed, because this method is superior for directly evaluating the one-electron oxidation potential in cases of subsequent chemical reactions.[10] The one-electron oxidation potential (E_{ox}^0) of EGC and **2** in deaerated MeCN containing 0.1 M TBAP at 298 K was thus determined to be 1.18 and 0.86 V vs. SCE, respectively,[11] indicating that the electrochemical oxidation was easier for **2** than EGC. This experiment supports **2** undergoing one-electron oxidation by GO$^{\bullet}$ more easily than EGC and provides a further measure of the excellent radical-scavenging ability of **2**.

In summary, a conformationally constrained analog of EGC, in which C2′ on ring B and the 3-OH group of ring C were connected by an isopropyl group, was synthesized by the same method previously used to synthesize a planar catechin derivative from (+)-catechin. Much higher radical-scavenging activity for **2** than for EGC was observed in the reaction with GO$^{\bullet}$. Similar to the planar catechin analog, **2** was more lipophilic than EGC (data not shown), and it should be possible to optimize the phamacokinetics and pharmacodynamics of EGC analogs by varying the ketone reactant used in the synthesis. Therefore, the enhanced radical-scavenging activity of **2** strongly supports the development of conformationally constrained EGC analogs as potential chemopreventive drugs for oxidative stress related diseases.

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8 To a solution of EGC (306 mg, 1 mmol) and acetone (232 mg, 4 mmol), in dry THF (30 mL) at −10 °C, trimethylsilyl trifluoromethanesulfonate (TMSOTf, 267 mg, 4 mmol) was slowly added. After stirring for 0.5 h, the mixture was poured into water, extracted with diethyl ether (3 × 50 mL). The organic layer was washed with brine, dried over Na_2SO_4, filtered and the solvent was evaporated. The resultant solid was purified by column chromatography on silica gel (7:3:1 toluene–acetone–methanol) to give 271 mg (78.4%) of **2** a white solid; ^{1}H NMR (DMSO-d_6): δ 1.45 (s, 3H), 1.50 (s, 3H), 2.54 (d, $J = 16.6$ Hz, 1H), 2.73 (dd, $J = 5.2$ and 16.6 Hz, 1H), 4.12 (d, $J = 5.2$ Hz, 1H), 4.37 (s, 1H), 5.59 (d, $J = 2.4$ Hz, 1H), 5.87 (d, $J = 2.4$ Hz, 1H), 6.31 (s, 1H), 8.26 (s, 1H), 8.52 (s, 1H), 8.85 (s, 1H), 9.11 (s, 1H), 9.17 (s, 1H); ^{13}C NMR (acetone-d_6): δ 25.6, 27.3, 31.3, 63.6, 70.5, 75.1, 94.9, 95.2, 98.6, 112.1, 116.6, 124.0, 135.4, 143.6, 146.2, 156.0, 156.6; MS m/z: 346 $[M + H]^+$.
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