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Synthesis and radical-scavenging activity of a dimethyl catechin analogue

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ABSTRACT

Catechin analogue **1** with methyl substituents *ortho* to the catechol hydroxyl groups was synthesized to improve the antioxidant ability of (+)-catechin. The synthetic scheme involved a solid acid catalyzed Friedel–Crafts coupling of a cinnamyl alcohol derivative to 3,5-dibenzyloxyphenol followed by hydroxylation and then cyclization through an intermediate orthoester. The antioxidative radical scavenging activity of **1** against galvinoxyl radical, an oxyl radical, was found to be 28-fold more potent than (+)-catechin.

Among natural bioactive compounds distributed in fruits, vegetables, and beverages of plant origin, phenolic antioxidants (ArOH), such as flavonoids, tocopherol, and resveratrol, are widely recognized for their biological and pharmacological effects that include anti-carcinogenic, anti-cardiovascular anti-neurodegenerative, and anti-inflammatory properties.[1–4] These properties are principally attributed to the capacity of these compounds to trap reactive oxygen species and to chelate metal ions, which through the Fenton reaction could generate radicals.[5] Therefore, this so-called antioxidative ability of phenolic compounds is frequently cited as the key to their success in the prevention and/or reduction of oxidative stress-related chronic diseases and age-related disorders. Consideration of antioxidative ability in drug development has led to interest in improving the radical scavenging activity of phenolic compounds based on the antioxidant mechanism.[6] Antioxidant activity via the direct quenching of free radicals is expressed in two mechanisms: a one-step hydrogen atom transfer from the phenolic OH group to the free radical and a single-electron transfer from ArOH to the free radical with concomitant formation of the radical cation $ArOH^{\cdot+}$.[7] The former process, characterized by vitamin E, is based on the capacity of a phenol to donate a hydrogen atom to a free radical.[8] Catechin and resveratrol (see Fig. 1 for the structures) scavenge free radicals by the latter mechanism.[9,10] In this case, the ionization potential (IP) of the phenol is important for the radical scavenging efficacy. Hence, introduction of electron-donating substituents at positions *ortho* and/or *para* to the phenolic hydroxyl group would decrease the IP of ArOH, thereby enhancing the radical scavenging ability. In this regard, planar catechin analogues, in which an isopropyl fragment as electron-donor was introduced into (+)-catechin by reaction with acetone, exhibited 5-fold more potent radical scavenging activity than (+)-catechin.[11] An epigallocatechin analogue with a similarly introduced isopropyl fragment also displayed increased radical scavenging activity.[12] In the case of *ortho*-alkyl substituents, the $ArOH^{\cdot+}$ formed in the reaction with a free radical is stabilized by compensation of the electron vacancy by either an inductive effect or through hyperconjugation, resulting in improved radical scavenging ability. Resveratrol analogues with one and two methyl groups at positions *ortho* to the phenol hydroxyl group showed 14- and 36-fold acceleration in the ability to scavenge free radicals, respectively.[13]

The aim of the present study was to synthesize the catechin analogue **1** in which both positions *ortho* to the catechol hydroxyl groups were substituted with methyl groups. Compared with (+)-catechin, the dimethyl analogue of catechin showed greatly enhanced radical scavenging ability against galvinoxyl radical as a model radical for reactive oxygen species. This result indicates

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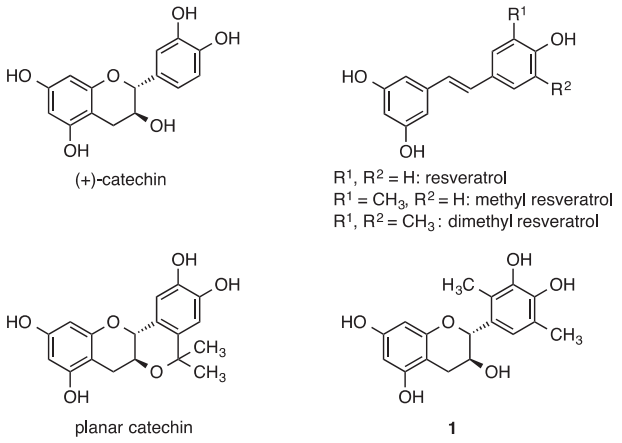


Figure 1. Structures of catechin, resveratrol, and their analogues.

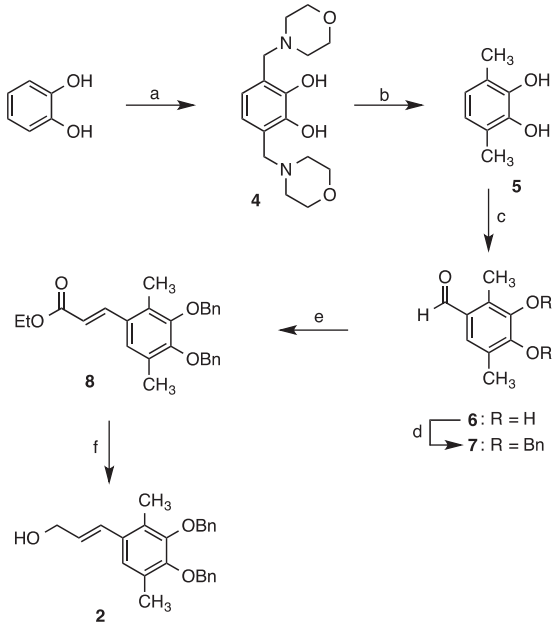
there is a role for the antioxidant chemistry of phenols via a radical scavenging mechanism that is accelerated by electron-donating substituents.

There are numerous methods for construction of the flavonoid skeleton using a biomimetic strategy.[14,15] The synthetic strategy employed here is an efficient and general approach to access the flavan framework of **1**. This process consisted of a solid acid catalyzed Friedel–Crafts coupling of cinnamyl alcohol derivative **2**, that included an embedded dimethylcatechol moiety, with 3,5-dibenzyloxyphenol **3**, followed by hydroxylation and cyclization to give the corresponding dimethyl catechin derivative.

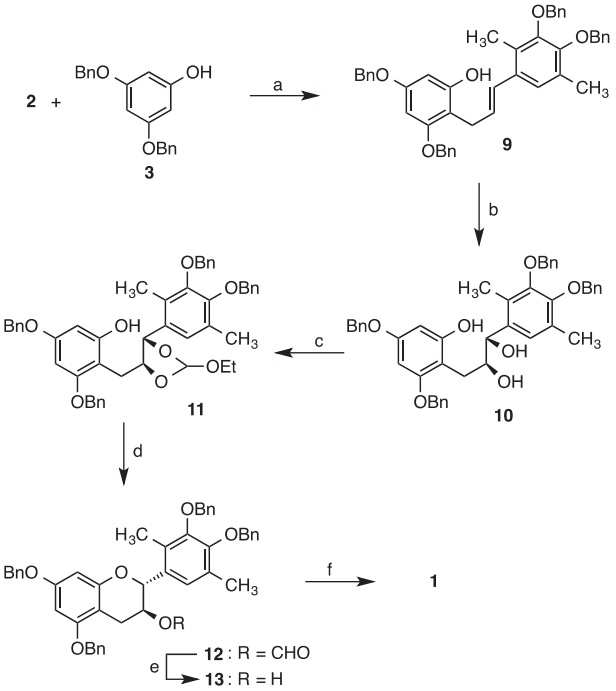
The synthesis of **2** is outlined in Scheme 1. Introduction of methyl groups at both *ortho* positions of the catechol could be readily accomplished by Mannich reaction of catechol using morpholine and formamide to afford **4**. Palladium catalyzed hydrogenation of **4** was carried out with heating at 70 °C under a high pressure of hydrogen gas to give dimethylcatechol **5**. Since **5** was highly sensitive, the subsequent reactions, formylation with $CH(OEt)_3$ followed by benzyl protection of the hydroxyl groups of catechol **6**, were performed using the crude reaction products. The resulting compound **7** was subjected to Knoevenagel condensation with monoethyl malonate in pyridine containing a catalytic amount of piperidine to produce α,β-unsaturated ester **8**. Reduction of **8** with LAH/$AlCl_3$ gave the corresponding cinnamyl alcohol **2**.

Phenol **3**, obtained by the method of Lehmann and Jahr,[16] was subjected to Friedel–Crafts alkylation with **2** using H_2SO_4/SiO_2 as catalyst in CS_2/CH_2Cl_2 to furnish the coupled product **9** as shown in Scheme 2. Oxidation of **9** with NMO in the presence of a catalytic amount of OsO_4 gave the *cis*-diol product **10**, which was converted into *ortho*-ester **11** with triethyl orthoformate and a catalytic amount of pyridinium *p*-toluenesulfonate (PPTS) at room temperature. The reaction was continued with heating at 60 °C to form the flavan framework as intermediate formate ester **12**. De-esterification of **12** with K_2CO_3 in 1,2-dimethoxyethane/methanol and debenzylation using $Pd(OH)_2$ and H_2 in THF/methanol afforded **1** (Fig. 1).

The radical scavenging activity of **1** was evaluated in a non-aqueous system using galvinoxyl radical (GO·) as an oxyl radical species. Because of its odd electron, GO· exhibits a strong absorption band at 428 nm, and a solution of GO· appears yellow in color. As the electron is paired, the absorption vanishes, and the resulting decolorization is stoichiometric with respect to the number of electrons taken up. Taking advantage of the color change of GO· in the presence of an antioxidant, the rate of radical scavenging of **1** toward galvinoxyl was measured. As shown in Figure 2, the decay rate of the absorbance at 428 nm followed pseudo first-order kinetics when the concentration of **1** was maintained at more than 10-fold excess to the GO· concentration. The pseudo first-order rate constant (k_{obs}) exhibited first-order dependence with respect to the concentration of **1** as shown in Figure 3. From the linear plot of k_{obs} versus [**1**], the second-order rate constant (k) for the radical scavenging of **1** toward GO· was determined to be 1.07×10^3 $mol^{-1}\,dm^3\,s^{-1}$. The k value for (+)-catechin was determined in the



Scheme 1. Reagents and conditions: (a) morpholine, formaldehyde, EtOH, rt, 61%; (b) H_2, 10% Pd/C, THF, 5 atm, 70 °C; (c) $CH(OEt)_3$, $AlCl_3$, toluene, rt; (d) BnBr, K_2CO_3, KI, acetone, rt, 3.2% (total yield from **4**); (e) monoethyl malonate, piperidine, pyridine, reflux, 87%; (f) LAH, $AlCl_3$, THF, rt, 48%.



Scheme 2. Reagents and conditions: (a) H_2SO_4/SiO_2, CH_2Cl_2, rt, (b) NMO, OsO_4, acetone, H_2O, rt, 37% (total yield from **2**); (c) $CH(OEt)_3$, PPTS, EDC, rt; (d) $CH(OEt)_3$, PPTS, EDC, 60 °C; (e) K_2CO_3, MeOH, DMF, rt, 89% (a total yield from **10**); (f) H_2, $Pd(OH)_2$, MeOH, THF, rt, 56%.

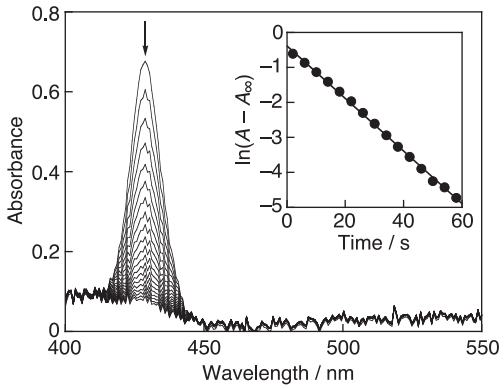


Figure 2. Spectral changes observed during the reaction between dimethyl catechin analogue **1** (6.8×10^{-5} mol dm^{-3}) and GO· (4.7×10^{-6} mol dm^{-3}) in deaerated MeCN at 298 K (interval: 2 s). Inset: first-order plot based on the decay of the absorption at 428 nm for GO·.

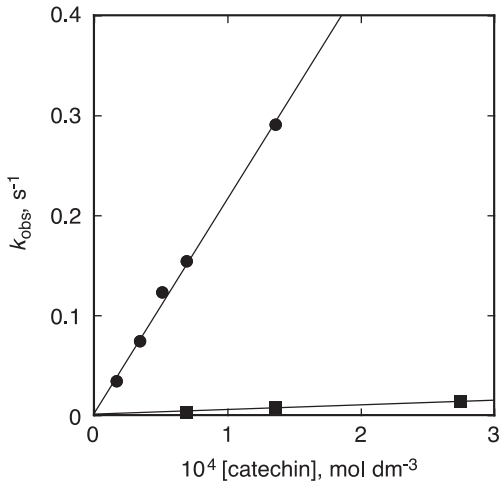


Figure 3. Plot of the pseudo first-order rate constants (k_{obs}) versus the concentrations of **1** and (+)-catechin for the radical scavenging reaction toward GO·.

same manner to be 3.87×10 mol^{-1} dm^3 s^{-1}. These results indicate that the introduction of methyl groups at both positions *ortho* to the catechol hydroxyl groups led to a 28-fold increase in radical scavenging activity.

In conclusion, the dimethyl analogue of catechin, **1**, was synthesized to demonstrate a strategy to enhance radical scavenging ability that may aid in the prevention and therapy of various oxidative stress related diseases. Indeed, the scavenging rate constant of GO· by **1** was greatly increased as compared to catechin. Since inductive and hyperconjugation effects of methyl groups can contribute to stabilization of the radical cation of catechol formed in the reaction with GO·, the enhanced radical scavenging activity of **1** supports the antioxidant chemistry of catechin occurring by the scavenging of oxyl free radicals through a single electron transfer mechanism. This simple derivatization by methyl substituents makes it possible to improve the radical scavenging activity of catechin without causing a fatal change in the overall structure that may be significantly associated with its inherent biological activities, and this strategy is likely to be of value in medicinal chemistry applications.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.03.029.

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