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Enhanced Radical-Scavenging Activity of a Planar Catechin Analogue

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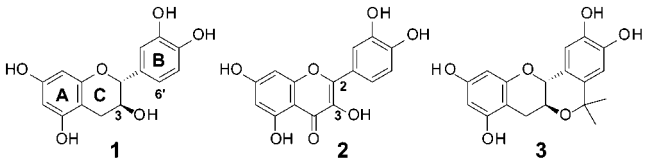
Oxidative stress is important in the pathogenesis of neuronal cell death in Alzheimer's[1] and Parkinson's[2] disease. The protective role of antioxidants against such pathogens has been widely studied, and this has promoted the development of antioxidants for the treatment of diseases associated with oxidative stress.[3−5] Vitamin E, which is an essential nutrient in humans, may be a clinically useful antioxidant. In fact, α-tocopherol reduces amyloid-induced cell death and suppresses the progression of Alzheimer's disease.[3] Flavonoids such as catechin (**1**) and quercetin (**2**) are plant phenolic pigment products that act as natural antioxidants. Quercetin, on one hand, has been shown to protect against oxidant injury and cell death[6] by scavenging free radicals,[7] protecting against lipid peroxidation,[8] and thereby terminating the chain-radical reaction.[9] On the other hand, there have been only a few reports on the use of catechin for the treatment of free radical-associated disease, whereas the mechanism to scavenge oxygen radical has been well-studied.[10] However, its ability to scavenge free radicals must be improved, and adequate lipophilicity is needed to penetrate the cell membrane before it is suitable for clinical use. The superior antioxidant ability of **2** results from the formation of a stable radical, due to the C2−C3 double bond and the resulting planar geometry which delocalizes the radical throughout the entire molecule.[11] Since the B ring in **1** is known to be perpendicular to the A ring,[12] the radical-scavenging ability of **1** might be improved by constraining the geometry of **1** to be planar. In this communication, we describe the first synthesis and characterization of the antioxidant properties of a planar catechin analogue (**3**) with respect to the chroman and catechol moieties of **1**, by taking advantage of the formation of a bridge between the 3-OH group on ring C and C6′ on ring B.



The planar catechin (**3**) was synthesized via an oxa-Pictet−Spengler reaction[13] using catechin and acetone with $BF_3{\cdot}Et_2O$ as the acid. The structure was characterized by ^1H and ^{13}C NMR and UV−visible spectroscopy. The ^1H signals of the four protons of the phenolic OH groups showed that the catechol moiety on ring B did not react with acetone. As shown in Figure 1, the planar geometry of **3** was substantiated by single-crystal X-ray crystallography of a tetra-*O*-silylated analogue (**4**), in which the four OH groups on the A and B rings are substituted by *t*-Bu(Me)$_2$SiO groups. X-ray analysis also confirmed that the stereochemistry of 3-H on ring C was maintained throughout the reaction without any acid-catalyzed racemization.

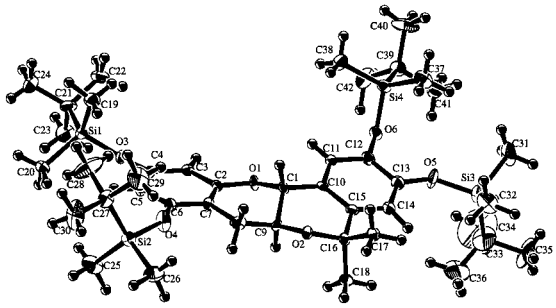


Figure 1. X-ray structure of tetra-*O*-silylated analogue (**4**) of **3**, showing ellipsoids at 50% probability.

The radical-scavenging activities of **1** and **3** as well as that of **2** were compared using galvinoxyl radical (G•) as an oxyl radical species.[14] Upon addition of **1** to a deaerated MeCN solution of G•, the absorption band at 428 nm due to G• disappeared immediately as shown in Figure 2. This indicates that hydrogen abstraction from one of the OH groups on the B ring of **1** by G• takes place to give catechin radical and hydrogenated G• (GH). The decay of the absorbance at 428 nm due to G• obeyed pseudo-first-order kinetics when the concentration of **1** was maintained at more than 10-fold excess of the G• concentration (inset of Figure 2). The dependence of the observed pseudo-first-order rate constant (k_{obs}) on the concentration of **1** is shown in Figure 3, which demonstrates a linear correlation between k_{obs} and the concentration of **1**. From the linear plot of k_{obs} vs the catechin concentration in Figure 3, we determined that the second-order rate constant (k) for hydrogen abstraction of **1** by G• was 2.34×10^2 M^{-1} s^{-1}. The k values for **2** and **3** were determined in the same manner to be 1.08×10^3 and 1.12×10^3 M^{-1} s^{-1}, respectively. Thus, the k value for planar catechin (**3**) is about 5-fold larger than that for catechin (**1**), approximately the same as that for quercetine (**2**).

Hydroxyl radical is the most reactive among oxygen-derived free radicals responsible for aging and free radical-mediated injury. Therefore, the effects of **1**, **2**, and **3** on hydroxyl radical-mediated

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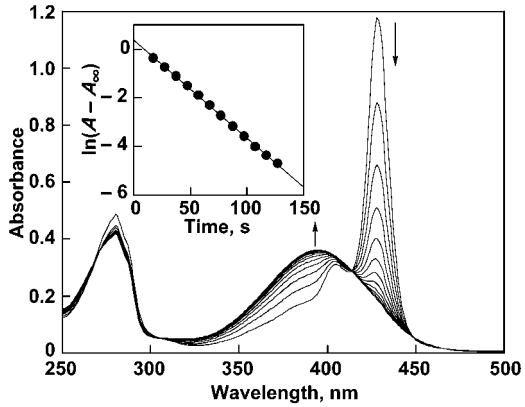

Figure 2. Spectral change in the reaction of **1** (1.5×10^{-4} M) with G• (2.4×10^{-6} M) in deaerated MeCN at 298 K (Interval: 10 s). (Inset) First-order plot based on the change in absorption at 428 nm.

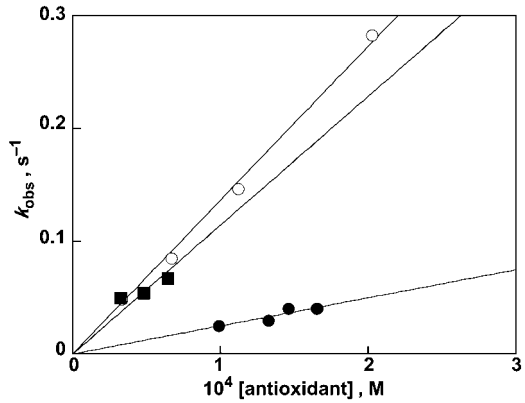

Figure 3. Plot of the pseudo-first-order rate constant (k_{obs}) vs the concentrations of **1** (●), **2** (■), and **3** (○) for hydrogen atom transfer from antioxidants to G• (2.4×10^{-6} M) in deaerated MeCN at 298 K.

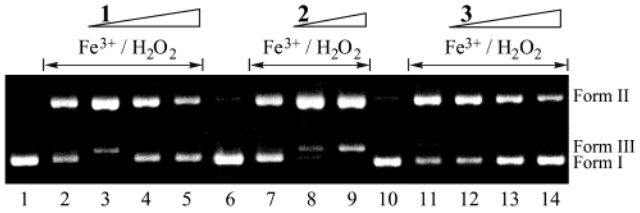

Figure 4. Effects of **1**, **2**, and **3** on DNA breakage induced by Fe^{3+}/H_2O_2. Assays were performed in 50 mM sodium cacodylate buffer, pH 7.2, containing 45 μM bp of pBR322DNA, for 1 h at 37 °C. Lanes 1, 6, and 10; DNA alone, lanes 2, 7, and 11; 10 mM H_2O_2 and 10 μM $FeCl_3$, lanes 3−5, 8, and 9, and 12−14; 10 mM H_2O_2 and 10 μM $FeCl_3$ in the presence of 0.25, 1.25, and 2.5 mM **1** (lanes 3−5), 0.25 and 1.25 mM **2**, and 0.25, 1.25 and 2.5 mM **3**.

DNA breakage were investigated. DNA-strand scission in supercoiled pBR322DNA was induced by a hydroxyl radical-generating system using hydrogen peroxide in the presence of Fe^{3+} (Fenton reaction). As shown in Figure 4, **1** at a high concentration (1.25 and 2.5 mM) suppressed DNA strand breakage, while at a low concentration (0.25 mM) it exhibited pro-oxidant properties, consistent with the enhanced DNA cleavage in comparison with cleavage without antioxidant. Quercetin (**2**) only showed pro-oxidant effects at 0.25 and 1.25 mM. In agreement with previously published results,[15] the measured pro-oxidant effects of **1** and **2** may be attributed to autoxidation of the antioxidant in the presence of transition metal, leading to the generation of primary radicals such as hydroxyl radical. In contrast to the pro-oxidant effects of **1** and **2**, the addition of **3** protected DNA from Fenton reaction-mediated damage at all of the concentrations tested, and **3** exhibited marked hydroxyl radical-scavenging ability, which exceeded that of catechin. Since **3** is very lipophilic compared to **1** (data not shown), the high radical-scavenging ability of **3** might be very useful for suppressing free-radical associated events, especially in the cell membrane.

In conclusion, we have described the first synthesis of planar catechin **3**, which was constrained by the formation of a bridge between the 3-OH group on ring C and C6′ on ring B. Preliminary experiments indicated that, despite the absence of a C2−C3 double bond, **3** showed enhanced radical-scavenging ability comparable to that of **2**. Efficient protection against DNA strand breakage induced by the Fenton reaction and the greater lipophilicity of **3** suggested that the construction of a planar catechin might be a new approach for the development of clinically useful antioxidants. The inducing planarity of **3** may pose the preferential stabilization of radicals through hyperconjugation between the π electrons on ring B and the σ electrons on C2 on ring C. In fact, a large amount of the spin density in the radical species generated via the antioxidative reaction of **3** is accumulated at the C2 position (data not shown). However, the effect of substitution to the para position from an OH ring on the B ring also should be considered as the essential factor for its enhanced reactivity. The detailed mechanism as well as the energetics of hydrogen abstraction from catechin analogues depending on the molecular structure is now under investigation.

Supporting Information Available: Experimental procedure for the preparation of **3**, kinetic measurements, the DNA-cleaving experiment, and crystallographic data for **4** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Katzman, R.; Kawas, C. In *Alzheimer Disease*; Terry, R. D, Katzman, R., Bick, K. L., Eds.; Raven Press: New York, 1994; pp103−119. (b) Behl, C.; Davis, J. B.; Lesley, R.; Schubert, D. *Cell* **1994**, *77*, 817. (c) Butterfield, D. A. *Chem. Res. Toxicol.* **1997**, *10*, 495. (d) Huang, X.; Atwood, C. S.; Hartshorn, M. A.; Multhaup, G.; Goldstein, L. E.; Scarpa, R. C.; Cuajungco, M. P.; Gray, D. N.; Lim, J.; Moir, R. D.; Tanzi, R. E.; Bush, A. I. *Biochemistry* **1999**, *38*, 7609.

(2) (a) Olanow, C. W. *Neurology* **1990**, *40*, 37. (b) Zang, L.-Y.; Misra, H. P. *J. Biol. Chem.* **1992**, *267*, 23601. (c) Pennathur, S.; Jackson-Lewis, V.; Przedborski, S.; Heinecke, J. W. *J. Biol. Chem.* **1999**, *274*, 34621.

(3) (a) Mayeux, R.; Sano, M. J. *N. Engl. J. Med.* **1999**, *341*, 1670. (b) Grundman, M. *Am. J. Clin. Nutr.* **2000**, *71*, 630S.

(4) (a) Skaper, S. D.; Fabris, M.; Ferrari, V.; Carbonare, M. D.; Leon, A. *Free Radical Biol. Med.* **1997**, *22*, 669. (b) Moosmann, B.; Behl, C. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 8867.

(5) The Parkinson Study Group *N. Engl. J. Med.* **1993**, *328*, 176.

(6) Greenspan, H. C.; Aruoma, O. I. *Immunol. Today* **1994**, *15*, 209.

(7) (a) Bors, W.; Michel, C.; Saran, M. *Methods Enzymol.* **1994**, *234*, 420. (b) Jovanovic, S. V.; Steenken, S.; Tosic, M.; Marjanovic, B.; Simic, M. G. *J. Am. Chem. Soc.* **1994**, *116*, 4846.

(8) Decharneux, T.; Dubois, F.; Beauloye, C.; Wattiaux-De Coninck, S.; Wattiaux, R. *Biochem. Pharmacol.* **1992**, *44*, 1243.

(9) Torel, J.; Cillard, J.; Cillard, P. *Phytochemistry* **1986**, *25*, 383.

(10) (a) Guo, Q.; Zhao, B.; Shen, S.; Hou, J.; Hu, J.; Bors, W.; Michel, C.; Stettmaier, K. *Arch. Biochem. Biophys.* **2000**, *374*, 347. (b) Dangles, O.; Fargeix, G.; Dufour, C. *J. Chem. Soc., Perkin Trans. 2* **2000**, 1653. (c) Valcic, S.; Burr, J. A.; Timmermann, B. N.; Liebler, D. *C. Chem. Res. Toxicol.* **2000**, *13*, 801.

(11) (a) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. *Free Radical Med.* **1996**, *20*, 933. (b) van Acker, S. A. B. E.; Bast, A.; Van Der Vijgh, W. J. F. *Antioxidants Health Dis.* **1998**, 221.

(12) Van Acker, S. A. B. E.; de Groot, M. J.; van den Berg, D.J.; Tromp, M. N. J. L.; Doone-Op den Kelder, G.; van der Vijgh, W. J. F.; Bast, A. *Chem. Res. Toxicol.* **1996**, *9*, 1305.

(13) Guiso, M.; Marra, C.; Cavarischia, C. *Tetrahedron Lett.* **2001**, *42*, 6531.

(14) Goto, N.; Shimizu, K.; Komuro, E.; Tsuchiya, J.; Noguchi, N.; Niki, E. *Biochim. Biophys. Acta* **1992**, *1128*, 147.

(15) (a) Hiramoto, K.; Ojima, N.; Sako, K.; Kikugawa, K. *Biol. Pharm. Bull.* **1996**, *19*, 558−563. (b) Yamashita, N.; Tanemura, H.; Kawanishi, S. *Mutat. Res.* **1999**, *425*, 107. (c) Nakanishi, I.; Fukuhara, K.; Ohkubo, K.; Shimada, T.; Kansui, H.; Kurihara, M.; Urano, S.; Fukuzumi, S.; Miyata, N. *Chem. Lett.* **2001**, 1152.

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