Structural Characteristics for Superoxide Anion Radical Scavenging and Productive Activities of Green Tea Polyphenols Including Proanthocyanidin Dimers

Masashi SATO, Hajime TOYAZAKI, Yu YOSHIOKA, Nobutoshi YOKOI, and Toru YAMASAKI*

Department of Applied Biological Science, Kagawa University; 2393 Miki-Ikenobe, Kagawa 761–0795, Japan.
Received June 23, 2009; accepted October 25, 2009; published online October 30, 2009

The purpose of this paper is to report structural characteristics for superoxide anion radical (O_2^-) scavenging and productive activities of green tea polyphenols. (−)-Epicatechin 3-*O*-gallate (5), (−)-epigallocatechin (6), (−)-epigallocatechin 3-*O*-gallate (7), (+)-gallocatechin-(4α→8′)-epigallocatechin (8), and (−)-epigallocatechin-(2β→*O*→7′, 4β→8′)-epicatechin 3′-*O*-gallate (9) were isolated from the tea plant *Camellia sinensis* L. (+)-Epigallocatechin-(2β→*O*→7, 4β→8′)-epicatechin (10) was prepared by hydrolyzing 9. The polyphenols, as well as commercially available pyrogallol (1), methyl gallate (2), (+)-catechin (3), (−)-epicatechin (4), and the flavonol myricetin (11), produced O_2^- in descending order 1, 6≈11≈8, 7, 10, 2≈9, 5≈4. In the polyphenols with the pyrogallol-type B-ring and/or galloyl group, electron-withdrawing substituents (carbonyl and ketal carbons) and/or intramolecular hydrogen bonding constituted structural characteristics against the autoxidation reaction. The O_2^--productive activity partially counteracted O_2^--scavenging activity. However, such structural characteristics appeared to enhance the scavenging activity, accordingly the polyphenols in effect served as O_2^--scavengers in descending order 9≈7, 2, 11, 8, 10, 3≈4. On the other hand, 6, having no such structural characteristic, acted as a O_2^--generator, as well as 1. Further assessments covering tannins (*e.g.*, A-type proanthocyanidin dimer 9) are needed to identify which green tea polyphenols are the most desirable chemopreventive agents.

Key words *Camellia sinensis* L.; catechin; A-type proanthocyanidin dimer; superoxide anion radical; scavenging activity

A variety of polyphenols, such as catechins (flavan-3-ols),[1—3] B-type proanthocyanidin dimers,[4—6] and chalcan–flavan dimers,[6] occur in green tea leaf. A new gallate of A-type proanthocyanidin dimer has recently been isolated from the tea plant.[7] The green tea polyphenols, especially catechins, have been demonstrated to have health-protective effects, antilipoperoxidant,[8,9] anti-ischemic,[10] antiatherogenic,[11] antiallergic, and anti-inflammatory.[12] These protective effects are believed to come from the anti-oxidant activities of catechins, such as the superoxide anion radical (O_2^-)-,[13—16] hydroxyl radical-,[15,17] or lipid peroxy radical-[18,19] scavenging activities, or the singlet oxygen-quenching activity.[16]

On the other hand, O_2^- is shown to be involved in autoxidation reaction of green tea catechins,[20] and their adverse effects have been demonstrated, which are thought to be attributable to such pro-oxidant activities. A preparation of green tea catechins enhances tumor development of 1,2-dimethylhydrazine-initiated rat colon lesions.[21] Cell viability is reduced by treating rat hepatocytes with (−)-epigallocatechin 3-*O*-gallate and the cell death is associated with increased production of reactive oxygen species (ROS) besides depletion of reduced glutathione.[22]

This study focused on major green tea catechins and A- and B-types of proanthocyanidin dimers. It is the purpose of this paper to report the differences in O_2^--productive activity between the green tea polyphenols, structural characteristics causative of these differences, and the contribution of the characteristics to green tea polyphenols' O_2^--scavenging activity.

Experimental

General Remarks Optical rotations were measured with a Jasco P-1010. ^{1}H (400 MHz)- and ^{13}C (100 MHz)-NMR spectra were acquired with a Jeol JNM A-400 spectrometer. Rotating frame Overhauser enhancement spectroscopy (ROESY) spectra at 600.0 MHz (^{1}H) and 150.6 MHz (^{13}C) were taken with a Jeol ECA-600 spectrometer by a combination of the Ruben–States–Haberkorn procedure (States) and time proportional phase increment (TPPI).[23] UV absorbances were measured with a Shimadzu UV-1600 spectrophotometer.

Isolation and Enzymatic Hydrolysis of Green Tea Polyphenols From the water-soluble portion, that had been stocked at −20 °C, of a 70% aqueous acetone extract of fresh leaves of *Camellia sinensis* (L.) O. KUNTZE var. *sinensis* (cv., Yabukita), polyphenols **5**—**9** were isolated by essentially the same method as described previously.[7] An aliquot (160 ml) of the water-soluble portion, equivalent to 100 g dry material, was fractionated into fractions 1 (43.5 g), 2 (25.9 g), 3 (18.0 g), 4 (0.82 g), and 5 (10.2 g) by chromatography on a column (5×95 cm) of Sephadex LH 20 (25—100 μm, Pharmacia Biotech) using EtOH, aqueous EtOH (90% and 80%), EtOH–acetone–water (2 : 2 : 1), and 70% aqueous acetone, respectively, as mobile phases. Fraction 3 (100 mg) was chromatographed on a column (2×28 cm) of MCI GEL CHP 20P (75—150 μm, Mitsubishi Chemical) with water and aqueous EtOH (20% and 40%) as mobile phases. Five-milliliter eluent fractions were taken in a fraction collector to isolate (−)-epigallocatechin (**6**, 10.9 mg), (−)-epigallocatechin 3-*O*-gallate (**7**, 30.7 mg), and (−)-epicatechin 3-*O*-gallate (**5**, 4.2 mg). Fraction 4 (100 mg) was also chromatographed on CHP 20P (2×28 cm) with water and aqueous EtOH (20% and 40%) as mobile phases. Five-milliliter eluent fractions were collected and eluent fractions 175—186 and 416—418, respectively, gave the B-type proanthocyanidin dimer (+)-gallocatechin-(4α→8′)-epigallocatechin (**8**, 3.4 mg) and A-type proanthocyanidin dimer gallate (−)-epigallocatechin-(2β→*O*→7′, 4β→8′)-epicatechin 3′-*O*-gallate (**9**, 6.2 mg) that has been newly found.[7] The polyphenols were obtained in the following yields, *i.e.*, **5**, 0.12% of the oven-dried green tea leaf; **6**, 0.30%; **7**, 0.84%; **8**, 0.004%; and **9**, 0.008%. The core of **9**, (+)-epigallocatechin-(2β→*O*→7′, 4β→8′)-epicatechin (**10**),[24,25] was given by hydrolyzing **9** with tannase from *Aspergillus oryzae* (Wako Pure Chemical).[7]

Conformational Analysis by Molecular Mechanics (MM2) Calculations The generation and optimization of possible geometries and identification of the stablest conformers for **6**—**8** were performed by MM2 calculations based on submolecular properties.[26] The calculation program[27] was a Scigress Explorer CONFLEX 7.5 for Windows XP and 2000 (Fujitsu). A total of 5000 search steps was carried out and the conformations with energy differences of less than 25 kJ/mol from the global minimum were saved.

Other Chemicals The following chemicals were purchased from Wako; pyrogallol (**1**), methyl gallate (**2**), (+)-catechin (**3**), (−)-epicatechin (**4**) and the flavonol myricetin (**11**), nitroblue tetrazolium chloride (NBT), Triton

* To whom correspondence should be addressed. e-mail: toruyama@pe.kagawa-u.ac.jp

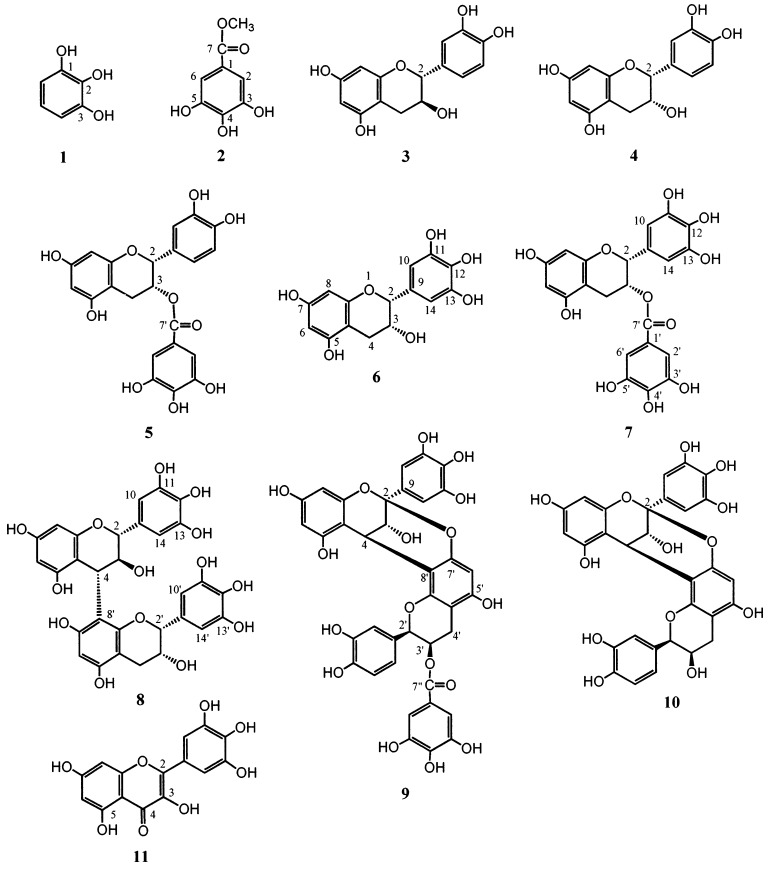

Fig. 1. Structures of Catechins **3**—**7**, Proanthocyanidin Dimers **8**—**10**, Flavonol **11**, and Related Compounds **1** and **2**

X-100, reduced nicotinamide adenine dinucleotide (NADH), phenazine methosulphate (PMS), tri(hydroxymethyl)aminomethane, sodium cacodylate trihydrate, and diethylenetriaminepenta-acetic acid (DTPA). Bovine erythrocyte Cu–Zn superoxide dismutase (SOD) was obtained from Sigma-Aldrich Chemical. The chemicals were of analytical grade. Water was purified through a Millipore Milli-Q-system and equilibrated with high purity O_2 at 20 °C, followed by preparation of aqueous solutions freshly before each experiment.

O_2^--Productive and Scavenging Activities Polyphenol assays with and without SOD for O_2^--productive activity were conducted at pH 8.2, 20 °C according to the procedure described by Minami and Yoshikawa,[28] *i.e.*, by detecting diformazan formed from NBT. For each polyphenol, the differences between O_2^--scavenging and productive activities were measured at pH 8.2, 20 °C by the procedure essentially identical with that described by Nishikimi *et al.*[29] or Ponti *et al.*,[30] *i.e.*, by bringing them into competition with NBT for O_2^- generated by the reoxidation of NADH-reduced PMS with O_2, except that Triton X-100 was used as a surfactant because diformazan is insoluble in water.[28] Absorbances were recorded at 540 nm after the first 5 min of reactions. Triplicate assays were conducted at each concentration.

Results and Discussion

O_2^--Dependent Autoxidation of Green Tea Polyphenols O_2^--productive activities of polyphenols are shown in Fig. 2. Polyphenol concentrations against increases in absorbance at 540 nm during a 5-min assay period, due to the formation of diformazan from NBT, were plotted into straight lines. When **1**, **7**, and **8** were assayed in the presence of SOD at a concentration of 1000 units/ml, the diformazan formation was inhibited to 96%, 98%, and 95%, respectively (Fig. 3), showing that these three polyphenols underwent a O_2^--dependent autoxidation reaction. The results were supported by the earlier data for **1**,[31] accordingly the other polyphenols also were thought to have autoxidized to produce O_2^-. As Fig. 2 shows,

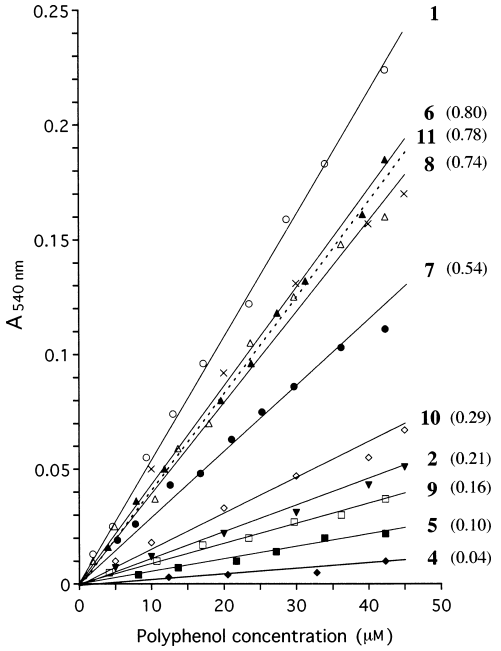

Fig. 2. Five-min Autoxidation Reaction of Polyphenols **1**, **2**, and **4**—**11**

Ratios of increases in absorbance at 540 nm for the polyphenols, including **6** (—▲—), **8** (—△—), and **11** (----×----), to that for **1** are shown in parentheses. Cuvettes contained 2—45 μM each polyphenol, 258 μM NBT, 1.3 mM DTPA, and 1.68% (v/v) Triton X-100 in a total volume of 950 μl buffered by 26.3 mM Tris–cacodylic buffer. The reaction was started by adding one polyphenol in deaerated water (2—100 μl) to each aerobic solution containing the other components. After the first 5 min of reactions, 3.0 ml of 2 M formic buffer at pH 3.5 containing 1.68% (v/v) Triton X-100 was added to halt the reactions.

certain of the polyphenols with the pyrogallol-type B-ring(s) remarkably surpassed those with the catechol-type B-ring in O_2^--productive activity, probably due to the greater oxidizability of the specific trihydroxy structure.[32] The significance of the pyrogallol-type B-ring in O_2^--dependent autoxidation reaction of flavonoids has been emphasized in the literature.[20,33] However, some polyphenols exhibited limited O_2^--productive activities, despite having the pyrogallol-type B-ring.

Electron-Withdrawing Substituents against Autoxidation Methyl gallate (**2**) exerted a low O_2^--productive activity. The carbonyl carbon atom (C-7) withdraws electrons from the aromatic ring through the single bond C-7–C-1, removing, in turn, electrons from the hydroxyl O atoms. Therefore, the bonding electrons between the O and H atoms are attracted more toward the O atoms, resulting in increased O–H bond polarization.[34] This will be responsible not only for increased acidity[34] but for retardation of the rate of one-electron transfer to O_2 (autoxidation reaction). Such retardation also will occur in the galloyl group of **5**, **7**, or **9**.

In spite of having the pyrogallol-type B-ring, A-type proanthocyanidin dimer **10** exerted a limited O_2^--productive activity. This compound is a ketal and so C-2, which has two electronegative O atoms, strongly withdraws electrons from the B-ring. This ultimately leads to increased O–H bond polarization, thus retarding the rate of autoxidation reaction. In the case of **9**, the low O_2^--productive activity is attributed to both ketal structure and galloyl group.

On the other hand, catechin **6**, B-type proanthocyanidin dimer **8**, and flavonol **11** were highly active. C-2 of these three compounds and C-2′ of **8** each have one O atom only, accordingly the electron-withdrawing effect of these carbons is smaller than that of the ketal carbon (C-2) of **9** or **10** and, therefore, appears to be insufficient to retard the rate of autoxidation reaction. In the case of **11**, the carbonyl carbon (C-4) of the C (γ-pyrone)-ring is located far from the hydroxyl groups on the B-ring and, moreover, the double bond C-2=C-3 is present, and so C-4 is of no use for retardation of the rate of autoxidation reaction. Flavonol **11**, a minor green tea polyphenol,[35] has recently been demonstrated to induce autoxidation-dependent genotoxicity in eukaryotic cells.[33] It is remarkable that **6** and **8** bore comparison with **11** in O_2^--productive activity (Fig. 2). Pyrogallol (**1**) exhibited the highest activity, due to the absence of any substituent.

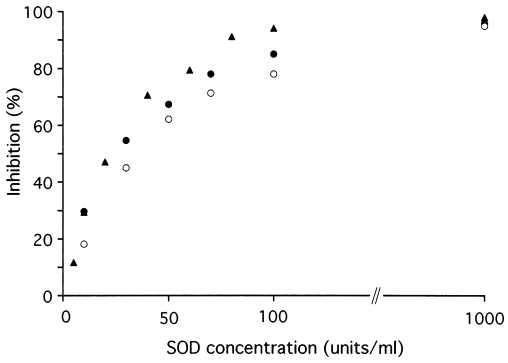

Fig. 3. Inhibition by SOD of Autoxidation Reaction of **1** (●), **7** (▲), and **8** (○) (10 μM)

In the presence of the enzyme at various concentrations, assays were initiated according to the procedure described in the legend for Fig. 2. One unit of the enzyme was the amount of the enzyme that inhibited the rate of cytochome c reduction by 50% in a coupled system with xanthine and xanthine oxidase at pH 7.8, 25 °C.

Intramolecular Hydrogen Bonding against Autoxidation Compared with the high O_2^--productive activity of **6**, that of gallate **7** was markedly low (Fig. 2). Upon identification of the stablest conformers for **7** and **6** by MM2 calculations (Fig. 4), the distributions were found to be 60% and 28.4% those of all possible geometries, respectively. In **7**, the B-ring and galloyl group were in close contact. For spatial distances in a straight line between the planes of the two aromatic rings, that between C-12 (the B-ring) and C-5′ (the galloyl group) was the shortest, 3.0 Å. The relatively wide distribution of 60% implies that the molecule of **7** is rather rigid and, furthermore, the short spatial distances suggest that the B-ring and galloyl group interact with each other. The ROESY spectrum of **7** exhibited a cross-peak between one singlet (δ 6.67) due to H-10 and H-14 and another singlet (δ 7.05) due to H-2′ and H-6′. The ROESY data supported the results of MM2 calculations. It has recently been suggested that the B-ring hydroxyl groups and galloyl group link to each other by intramolecular hydrogen bonding,[36] by which an excess of electrons on the relevant O atom can be reserved into the space between this atom and the counterpart H atom to form a new covalent bond.[37] Therefore, such hydrogen bonding appears to be responsible for retardation of the rate of autoxidation reaction. In the case of **6**, the B-ring stood alone (Fig. 4). The high O_2^--productive activity of **6** appears to be largely attributable both to the solitary configuration and to the small electron-withdrawing effect of C-2.

Difference between O_2^--Productive and Scavenging Activities Catechins **6** and **7** have been shown to react with O_2^- with efficient rate constants of $k=4.1\times10^5$ M^{-1} s^{-1} and $k=7.3\times10^5$ M^{-1} s^{-1}, respectively, at pH 7.0.[14] NBT's reactivity with O_2^- ($k=5.94\times10^4$ M^{-1} s^{-1} at pH 9.8)[38] is thought to

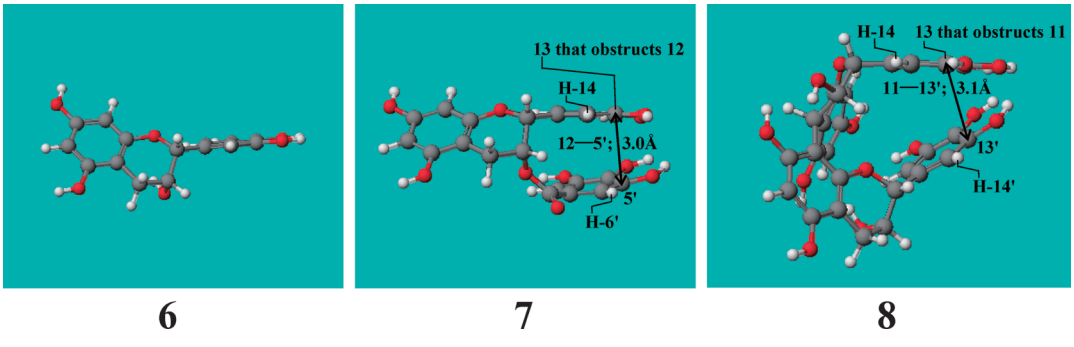

Fig. 4. Stablest Conformers for **6**—**8** Identified by MM2 Calculations

Lateral views. Grey, red, and white balls show C, O, and H atoms, respectively. The shortest intervals are indicated in the figures.

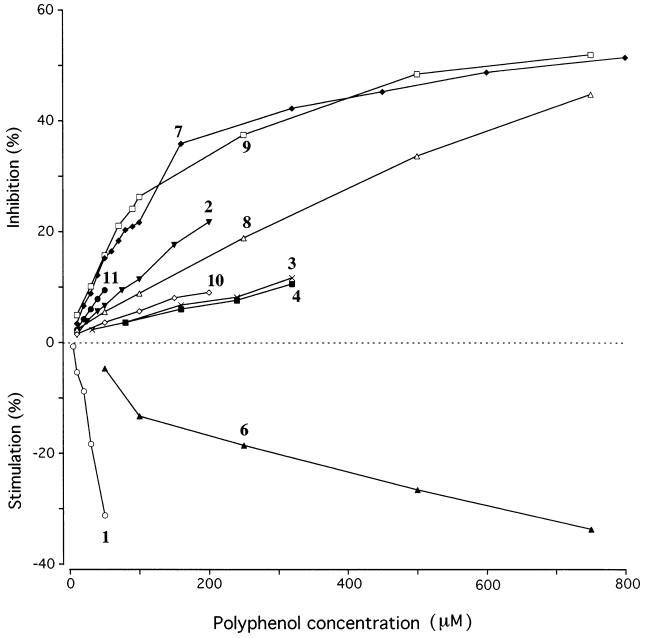

Fig. 5. Inhibition or Stimulation by Polyphenols of NBT Reduction

Cuvettes contained 5—800 μM each of polyphenols, including **11** slightly soluble in water, 80 μM NBT, 5.2 μM PMS, 73 μM NADH, 0.74 mM DTPA, and 1.55% (v/v) Triton X-100 in a total volume of 3.1 ml buffered by 16.3 mM Tris–cacodylic buffer. The reaction under aerobic conditions was initiated by adding NADH into the other components. The balance of O_2^--scavenging and productive activities was assessed by: inhibition (%)$=100(A_{NBT}-A_{(phenol+NBT)})/A_{NBT}$, in which $A_{(phenol+NBT)}<A_{NBT}$; or stimulation (%)$=100(A_{(phenol+NBT)}-A_{NBT})/A_{NBT}$, in which $A_{(phenol+NBT)}>A_{NBT}$.

be almost equal to that of **7**. Nevertheless, the IC_{50} value for **7**, *i.e.*, the concentration required to yield a 50% inhibition of NBT (80 μM) reduction by O_2^-, reached 690 μM; incidentally, that for **9** was 610 μM (Fig. 5). The results suggest that O_2^--scavenging activity is partially counteracted by O_2^--productive activity. In **3** and **4** with the catechol-type B-ring, favorable differences between O_2^--scavenging and productive activities were seen at negligible levels. For polyphenols with the pyrogallol-type B-ring or galloyl group, **9**, **2**, and **10**, in addition to **7**, in effect served as O_2^--scavengers. According to Lee-Ruff, O_2^--scavenging reaction by a number of catechols involves a sequence of one H atom (electron and proton) and proton abstractions to produce semiquinones and hydrogen peroxide.[39] Therefore, the retardation of the rate of one-electron transfer to O_2 by the electron-withdrawing or hydrogen bonding effect appeared to be favorable for the H atom abstraction, resulting in increased O_2^--scavenging activity.

In spite of showing the high O_2^--productive activities, **8** and **11** also served as O_2^--scavengers (Fig. 5). In the stablest conformer for **8** identified by MM2 calculations (the distribution, 95%), among spatial distances between the two B-rings, that between C-11 (the upper unit) and C-13′ (the lower unit) was the shortest, 3.1 Å (Fig. 4). This result was not supported by the ROESY experiment probably because the essential H-10 (or H-14) and H-10′ (or H-14′) are located far from each other (Fig. 4). However, it is possible that the hydroxyl groups on the two B-rings link to each other by intramolecular hydrogen bonding, accounting for the scavenging effect of **8**. In the case of **11**, the two hydroxyl groups on the A-ring are susceptible to the electron-withdrawing effect of the C-ring carbonyl C-4 and, furthermore, the carbonyl O atom is connected by intramolecular hydrogen bonding to either the hydroxyl group on the A-ring C-5 or that on the C-ring C-3.[40] Accordingly, the O_2^--scavenging activity of **11** appeared to be dependent on both electron-withdrawing and intramolecular hydrogen bonding effects.

In contrast to the polyphenols, pyrogallol (**1**), having no substituent, was a typical O_2^--generator, consistent with the results described in the literature.[33,41] It is most remarkable that catechin **6** also acted as a O_2^--generator, probably due to the structure insufficient to retard the rate of autoxidation reaction, *i.e.*, the essential lack of any structure to enhance O_2^--scavenging activity. There are many reports regarding health-protective effects of catechin gallate **7**.[12,42—44] However, **7** is probably hydrolyzed to **6** in the gastrointestinal tract,[45] accordingly **7** may be a double-edged sword. Further assessments of green tea polyphenols covering tannins, such as A-type proanthocyanidin dimer **9**, are concluded to be needed to identify the most desirable chemopreventive agents.

References

1) Vuataz L., Brandenberger H., Egli R. H., *J. Chromatogr.*, **2**, 173—187 (1959).
2) Coxon D. T., Holmes A., Ollis W. D., Vora V. C., Grant M. S., Tee J. L., *Tetrahedron*, **28**, 2819—2826 (1972).
3) Saijo R., *Agric. Biol. Chem.*, **46**, 1969—1970 (1982).
4) Nonaka G., Kawahara O., Nishioka I., *Chem. Pharm. Bull.*, **31**, 3906—3914 (1983).
5) Nonaka G., Sakai R., Nishioka I., *Phytochemistry*, **23**, 1753—1755 (1984).
6) Hashimoto F., Nonaka G., Nishioka I., *Chem. Pharm. Bull.*, **37**, 77—85 (1989).
7) Mukai D., Matsuda N., Yoshioka Y., Sato M., Yamasaki T., *J. Nat. Med.*, **62**, 155—159 (2008).
8) Terao J., Piskula M., Yao Q., *Arch. Biochem. Biophys.*, **308**, 278—284 (1994).
9) Salah N., Miller N. J., Paganga G., Tijburg L., Bolwell G. P., Rice-Evans C., *Arch. Biochem. Biophys.*, **322**, 339—346 (1995).
10) Suzuki M., Tabuchi M., Ikeda M., Umegaki K., Tomita T., *Med. Sci. Monit.*, **10**, BR166—BR174 (2004).
11) Miura Y., Chiba T., Tomita I., Koizumi H., Miura S., Umegaki K., Hara Y., Ikeda M., Tomita T., *J. Nutr.*, **131**, 27—32 (2001).
12) Middleton E., Kandaswami C., Theoharides T. C., *Pharmacol. Rev.*, **52**, 673—751 (2000).
13) Baumann J., Wurm G., von Bruchhausen F., *Arch. Pharm.*, **313**, 330—337 (1980).
14) Jovanovic S. V., Hara Y., Steenken S., Simic M. G., *J. Am. Chem. Soc.*, **117**, 9881—9888 (1995).
15) Kashima M., *Chem. Pharm. Bull.*, **47**, 279—283 (1999).
16) Guo Q., Zhao B., Shen S., Hou J., Hu J., Xin W., *Biochim. Biophys. Acta*, **1427**, 13—23 (1999).
17) Husain S. R., Cillard J., Cillard P., *Phytochemistry*, **26**, 2489—2491 (1987).
18) Torel J., Cillard J., Cillard P., *Phytochemistry*, **25**, 383—385 (1986).
19) Kondo K., Kurihara M., Miyata N., Suzuki T., Toyoda M., *Arch. Biochem. Biophys.*, **362**, 79—86 (1999).
20) Miura Y. H., Tomita I., Watanabe T., Hirayama T., Fukui S., *Biol. Pharm. Bull.*, **21**, 93—96 (1998).
21) Hirose M., Hoshiya T., Mizoguchi Y., Nakamura A., Akagi K., Shirai T., *Cancer Lett.*, **168**, 23—29 (2001).
22) Galati G., Lin A., Sultan A. M., O'Brien P. J., *Free Radic. Biol. Med.*, **40**, 570—580 (2006).
23) Berger S., Braun S., "200 and More NMR Experiments," Wiley-VCH, Weinheim, 2004, pp. 362—366.
24) Barreiros A. L. B. S., David J. P., de Queiroz L. P., David J. M., *Phytochemistry*, **55**, 805—808 (2000).
25) Ma C.-M., Nakamura N., Hattori M., Kakuda H., Qiao J.-C., Yu H.-I., *J. Nat. Prod.*, **63**, 238—242 (2000).
26) Ôsawa E., Musso H., *Angew. Chem. Int. Ed. Engl.*, **22**, 1—22 (1983).
27) Goto H., Ôsawa E., *J. Am. Chem. Soc.*, **111**, 8950—8951 (1989).
28) Minami M., Yoshikawa H., *Clin. Chim. Acta*, **92**, 337—342 (1979).

29) Nishikimi M., Rao N. A., Yagi K., *Biochem. Biophys. Res. Commun.*, **46**, 849—854 (1972).
30) Ponti V., Diazani M. U., Cheeseman K., Slater T. F., *Chem.-Biol. Interact.*, **23**, 281—291 (1978).
31) Marklund S., Marklund G., *Eur. J. Biochem.*, **47**, 469—474 (1974).
32) Pannala A. S., Chan T. S., O'Brien P. J., Rice-Evans C. A., *Biochem. Biophys. Res. Commun.*, **282**, 1161—1168 (2001).
33) Silva I. D., Gaspar J., Gomes da Costa G., Rodrigues A. S., Laires A., Rueff J., *Chem.-Biol. Interact.*, **124**, 29—51 (2000).
34) Conrow K., McDonald R. N., "Deductive Organic Chemistry," Addison-Wesley Publishing, Reading, MA, 1966, pp. 50—78.
35) Sonehara N., Izumi K., Matsumoto Y., *Jissen-Joshi-Dai. Ki.*, **6**, 12—15 (1965).
36) Takeuchi Y., Okuno K., Yoshioka H., Yoshioka H., *J. Radioanal. Neucl. Chem.*, **272**, 455—459 (2007).
37) Nishimoto, K., "Graduate Organic Chemistry," Vol. 1, ed. by Iwamura H., Noyori R., Nakai T., Kitagawa I., Kôdansha Scientific, Tokyo, 1989, pp. 47—67.
38) Bielski B. H. J., Richter H. W., *J. Am. Chem. Soc.*, **99**, 3019—3023 (1977).
39) Lee-Ruff E., *Chem. Soc. Rev.*, **6**, 195—214 (1977).
40) Cantrell J. S., "Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure–Activity Relationships," ed. by Cody V., Middleton E., Harborne J. B., Alan R. Liss, New York, 1986, pp. 391—394.
41) Yamada J., Yoshimura S., Yamakawa H., Sawada M., Nakagawa M., Hara S., Kaku Y., Iwama T., Naganawa T., Banno Y., Nakashima S., Sakai N., *Neurosci. Res.*, **45**, 1—8 (2003).
42) Mukhtar H., Ahmad N., *Am. J. Clin. Nutr.*, **71** (Suppl.), S1698—S1702 (2000).
43) Tachibana H., Koga K., Fujimura Y., Yamada K., *Nat. Struct. Mol. Biol.*, **11**, 380—381 (2004).
44) Nagle D. G., Ferreira D., Zhou Y.-D., *Phytochemistry*, **67**, 1849—1855 (2006).
45) Uyeta M., Taue S., Mazaki M., *Mutat. Res.*, **88**, 233—240 (1981).