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Chemical Studies of the Antioxidant Mechanism of Tea Catechins: Radical Reaction Products of Epicatechin with Peroxyl Radicals

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Abstract—Tea catechins, an important class of polyphenols, have been shown to have antioxidant activity and are thought to act as antioxidants in biological systems. However, the mechanisms of their antioxidant reactions remain unclear. The objective of this study was to characterize the reaction products of epicatechin with peroxyl radicals generated by thermolysis of the azo initiator azo-bisisobutyrylnitrile (AIBN). Structural elucidation of these products can provide insights into specific mechanisms of antioxidant reactions. Eight reaction products were isolated and identified using high-field 1D and 2D NMR spectral analysis. The observation of these compounds confirmed that the B-ring is the initial site for formation of reaction products in the peroxyl radical oxidant system.

Introduction

Tea is one of the most widely consumed beverages in the world; it has been used as a daily beverage and crude medicine in China and Japan for thousands of years. Catechins are a group of polyphenolic compounds found abundantly in green tea. The main polyphenolic components in green tea are (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin gallate (EGCG). These tea catechins have received considerable attention in recent years due to their diverse pharmacological potential, which includes antimutagenic activity and anticarcinogenic effects.[1–3] A commonly discussed mechanism for the biological and pharmacological activity of green tea involves the antioxidative activities of catechins.[4,5] A large number of researchers have reported that catechins effectively suppress lipid peroxidation in biological tissues and subcellular fractions such as microsomes and low-density lipoproteins (LDL).[6–10] The scavenging effects of tea catechins on free radicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH),[11] superoxide anions,[12] lipid free radicals,[13] and hydroxyl radicals,[14] have also been reported. It is believed that characteristic reaction products may be used as novel markers for antioxidant reactions of tea catechins in living systems. Therefore, detailed studies of the specific mechanisms of the antioxidation of catechins in different oxidation systems are of great scientific interest. Recently, numerous mechanistic studies have been conducted on EGC and EGCG with different oxidants such as the peroxyl radical system, the peroxidase/hydrogen peroxide oxidant system, the DPPH oxidant system, and the H_2O_2 oxidant system.[15–19] These reports indicate that the use of different oxidants can result in the formation of different oxidation products from catechins; moreover, the main site of catechin antioxidant action appears to depend not only on the oxidant used but also on the structures of the catechins. Thus, it is of interest to study the antioxidant mechanisms of the major tea catechins EC and ECG, which have an *o*-dihydroxy B ring rather than the trihydroxy B ring found in EGC and EGCG. We recently reported the structures of two major oxidation products of EC (**4** and **5**) with DPPH.[20]

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To extend our study of the antioxidant mechanisms of catechins, we reacted epicatechin with peroxyl radicals generated by thermolysis of the initiator 2,2′-azo-bis-isobutyronitrile (AIBN). The antioxidant progress of this reaction is thought to proceed in the following stages:

(1) Radical generation

$$RN{=}NR \xrightarrow{\Delta} 2R^{\bullet} + N_2$$

$$2R^{\bullet} + 2O_2 \rightarrow 2ROO^{\bullet}$$

(2) Radical trapping

$$ROO^{\bullet} + AH \rightleftharpoons ROOH + A^{\bullet}$$

(3) Radical termination

$$A^{\bullet} + X^{\bullet} \rightarrow \text{nonradical material}$$

AIBN decomposes thermally to yield alkyl radicals (R•), which then react rapidly with oxygen to generate peroxyl radicals (ROO•). AH is the phenolic antioxidant, A• is the antioxidant radical, and X• is another radical species or the same species as A•. Although the second stage is a reversible process, the third stage is irreversible and produces stable radical termination compounds. Structural information about these nonradical products can contribute importantly to the elucidation of antioxidant mechanisms. In the study presented here, we have succeeded in isolating and characterizing the reaction products of epicatechin (**1**) with alkylperoxyl radicals from AIBN in a homogeneous acetone system. Eight reaction products (**2**–**9**) were isolated and identified (Fig. 1). Their molecular structures were determined on the basis of detailed high field 1D and 2D NMR spectral analysis and mass spectrometry (MS).

Results

Eight major oxidation products, (**2**–**9**) from the reaction of epicatechin (**1**) with peroxyl radical were isolated and identified on the basis of their spectral data.

Compound **2**, a pale amorphous solid, was assigned the molecular formula $C_{30}H_{26}O_{12}$ based on negative-ion APCI-MS ($[M-H]^-$ at m/z 577), as well as from its ^{13}C NMR spectrum. The ^1H and ^{13}C resonances of **2** generally appeared as two sets of epicatechin carbon signals (Table 1), especially for those peaks associated with the heterocyclic C-ring. The major differences in the ^{13}C spectra of the two catechin moieties were (a) the presence of one additional quaternary carbon observed at δ 125.2 ppm in lieu of an unsubstituted aromatic carbon from the A-ring; and (b) the downfield shift of one of the hydroxylated carbons of the B ring (C-3′ or C-4′). In addition, the ^1H NMR spectrum of **2** showed only three signals for the A-ring at (δ_H 5.86 d, $J=2.4$ Hz; 5.91 d, $J=2.4$ Hz; 6.09 s) instead of the expected four signals, and the signals from H-2′ and H-6′ of one of the B-rings were downfield shifted (Table 1). All of these spectral features suggest a biphenyl type of linkage through a C–O bond between the two epicatechin moieties.

Since one of the A-ring signals in the ^1H NMR spectrum of **2** is absent, it was presumed that the biphenyl ether linkage was located between positions C-3′ or C-4′ of the B-ring for part A and positions C-6 or C-8 of the A-ring for part B. In the ^1H NMR spectrum of **2**, the chemical shifts of the B-ring signals for the part A structure resonate at lower field than those of analogous signals in part B, especially for positions H-2′ and H-6′ (Table 1). In addition, in the ^{13}C NMR spectrum of **2** the chemical shift (δ_C 148.0 ppm) of one of the oxygenated B-ring carbons of part A appears at a lower field than those for part B (δ_C 145.8 ppm) and the other oxygenated carbon (δ_C 146.8 ppm) for part A. In the

Table 1. NMR spectral data for compounds **2** and **3** (CD_3OD) (δ in ppm, J in Hz)

	2				**3**			
	Part A		Part B		Part A		Part B	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	4.82 s	80.0 d	4.77 s	79.9 d	3.85 s	73.2 d	5.05 s	81.0 d
3	4.16 m	67.6 d	4.11 m	67.2 d	4.49 m	65.3 d	4.31 m	66.7 d
4	2.83 dd 3.0, 16.8 2.91 dd 4.2, 16.8	28.6 t	2.61 dd 2.4, 16.8 2.77 dd 4.8, 16.8	28.2 t	2.82 brd 16.8 2.90 dd 4.2,16.8	29.7 t	2.67 dd 4.8,17.4 2.85 brd 17.4	25.2 t
5		158.0 s		154.3 s		157.7* s		169.0 s
5a		100.2 s		101.2 s		99.1 s		104.4 s
6	5.86 d 2.4	96.0 d	6.09 s	96.6 d	5.93 d 1.8	96.8 d	6.13 s	92.3 d
7		158.0 s		149.9 s		157.9* s		167.0 s
8	5.91 d 2.4	96.5 d		125.2 s	5.97 d 1.8	95.7 d		104.2 s
8a		157.2 s		149.5 s		155.9 s		156.5 s
1′		132.2 s		132.1 s		91.9 s		131.4 s
2′	6.88 d 1.8	116.7 d	6.65 d 1.8	115.5 d	2.20 d 10.8 2.95 d 10.8	41.1 t	7.05 d 1.8	115.3 d
3′		148.0 s		145.8 s		95.6 s		146.4 s
4′		146.8 s		145.8 s		193.5 s		146.3 s
5′	6.86 d 8.4	114.8 d	6.66 d 8.4	116.0 d	6.54 s	112.8 d	6.82 d 7.8	116.3 d
6′	7.06 dd 1.8, 8.4	121.8 d	6.59 dd 1.8, 8.4	119.5 d		165.0 s	6.87 dd 1.8, 7.8	119.4 d

* Assignments may be interchanged in each column.

HMBC spectrum of **2** (Fig. 2), H-2′ (δ_H 6.88 ppm), H-5′ (δ_H 6.86 ppm) and H-6′ (δ_H 7.06 ppm) for the part A structure showed cross peaks with δ_C 146.8 ppm, while only H-2′ and H-5′ showed cross peaks with δ_C 148.0 ppm. Thus, δ_C 148.0 and δ_C 146.8 ppm were assigned to C-3′ and C-4′, respectively. All of these observations indicated that position 3′ of the B-ring in the part A structure is the linkage point.

The remaining outstanding issue was whether the linkage point in the A-ring for the part B structure was at C-6 or C-8. To resolve this question, we carefully analyzed the cross peaks in the HMBC spectrum (Fig. 2). The HMBC spectrum showed correlations between δ_C 149.5 and H-2 (δ_H 4.82 ppm), δ_C 149.5 and H-4 (δ_H 2.61 and 2.77 ppm), and δ_C 154.3 ppm and H-4, thus establishing the signal at δ 149.5 ppm as that of C-8a and δ 154.3 ppm as that of C-5. This carbon nucleus resonating at 149.5 ppm showed no coupling to the only A-ring proton (δ 6.09 ppm), whereas the carbon at 154.3 ppm had a cross peak with the only A-ring proton appearing in the HMBC spectrum. For these reasons, a linkage through an ether bond was established between position C-3′ of the B ring of part A and position C-8 of the A ring of part B. Thus compound **2** was assigned as epicatechin [3′O, 8]-epicatechin. Full assignments of the ^1H and ^{13}C NMR signals of **2** were made using HMBC, HMQC, and COSY experiments (Table 1).

Compound **3** was isolated as a yellow amorphous solid. The negative-ion APCI-MS of **3** displayed a molecular ion peak at m/z $[M-H]^-$ 575, which along with its ^{13}C NMR spectrum supported a molecular formula of $C_{30}H_{24}O_{12}$. This mass is 2 units less than that of **2,** indicating that **3** had one more unsaturation than **2**. The ^1H NMR spectrum showed two sets of C-ring signals (Table 1); however, only one set of B-ring signals (δ_H 7.05 d, $J=1.8$ Hz, 6.87 dd, $J=1.8$, 7.8 Hz, and 6.82 d,

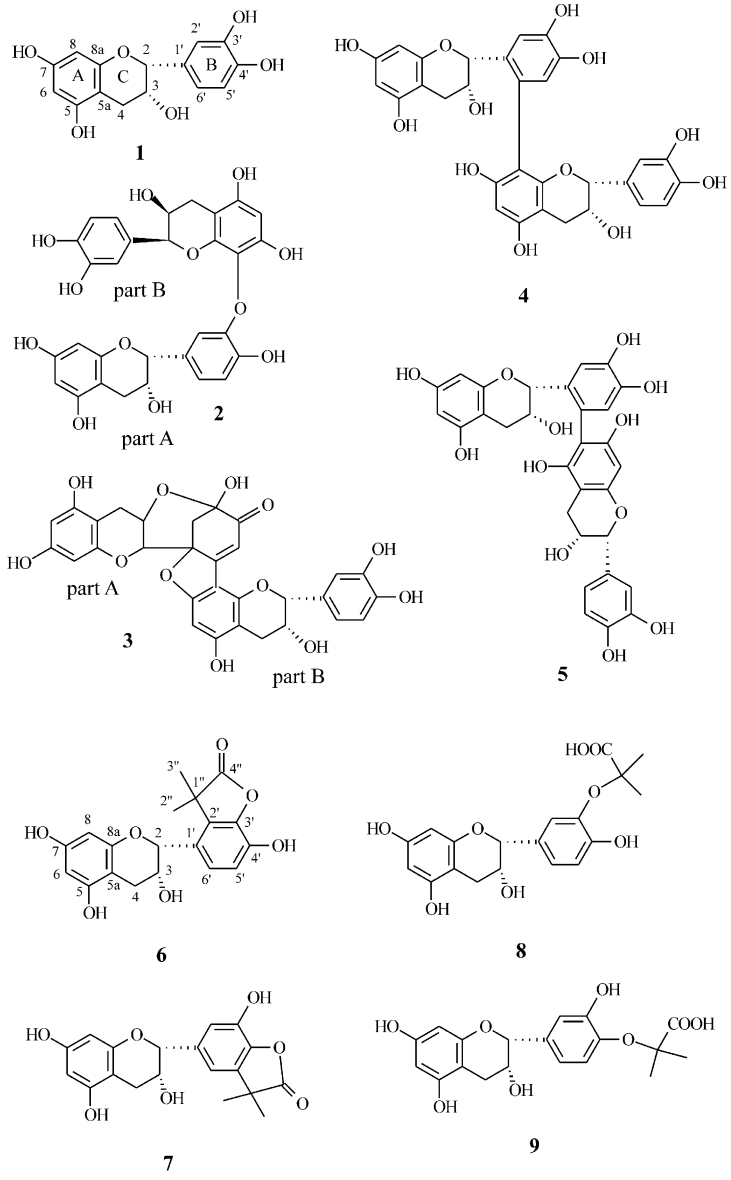

Figure 1. Structures of compounds **1**–**9**.

$J=7.8$ Hz) and one additional singlet signal (δ_H 6.54) were observed instead of the expected six from two B rings; and three signals were observed for the A ring at δ_H 6.93 d, $J=1.8$ Hz, 6.97 d, $J=1.8$ Hz, and 6.13 s instead of the expected four. In addition, two additional CH_2 signals appeared at δ_H 2.20 d, $J=10.8$ Hz and 2.98 d, $J=10.8$ Hz. In the COSY spectrum, these two peaks showed only a cross peak with each other, indicating that this CH_2 was connected to two quaternary carbons. The ^{13}C chemical shifts of **3** also appeared as a pair of epicatechin carbon signals (Table 1), especially in the high field region (δ_C 25 to 81 ppm) associated with the heterocyclic C ring, where the C-2, C-3 and C-4 carbon resonances showed up as twin peaks of comparable intensity. The major differences between the two epicatechins were found in the resonances for the B ring of one epicatechin unit and the A ring of the other epicatechin unit, for instance the presence of one carbonyl signal at δ_C 193.5 ppm, one methylene group at δ_C 41.1 ppm, two quaternary carbons at δ_C 91.9 and 95.6 ppm, and two additional quaternary carbons at δ_C 104.2 and 165.0 ppm from the A ring and B ring, respectively, in place of an unsubstituted aromatic carbon. The HMBC spectrum of **3** showed cross peaks between H-2 (δ_H 5.05) and δ_C 156.5, H-4 (δ_H 2.67 and 2.85 ppm) and δ_C 156.5, H-4 and 169.0 for the part B structure, thus establishing the signal at δ_C 156.5 ppm as that of C-8a, and δ_C 169.0 ppm as that of C-5. This 156.5 ppm carbon was not coupled to the only A-ring proton (δ_H 6.13 ppm), whereas the 169.0 ppm carbon had a cross peak with the only A-ring proton in the HMBC spectrum. Thus the linkage point in part B was deduced to occur at position C-8 of the A ring.

Spin correlations in the HMBC spectra were also observed between H-2 (δ_H 3.85) and δ_C 91.9, 41.1 and 165.0 ppm, H-3 (δ_H 4.49) and δ_C 95.6 ppm, δ_H 6.54 (δ_C 112.8 ppm) and δ_C 95.6 and 91.9 ppm, and δ_H 2.20 and 2.95 (two proton signals for δ_C 41.1 ppm) and δ_C 165.0, 95.6, 91.9 and 193.5 ppm for the part A structure. These features allowed assignment of δ_C 91.9 to C-1′, δ_C 41.1 to C-2′, δ_C 95.6 to C-3′, δ_C 193.5 to C-4′, δ_C 112.8 to C-5′ and δ_C 165.0 to C-6′. Taken together, the spectra indicated that in the part A structure, position C-3 is connected to position C-3′ through an ether linkage; between parts A and B structures, position C-8 for the part B structure is connected to C-6′ of the part A structure through a C-C bond, and position C-7 for the part B structure has a linkage with C-1′ of the part A structure through an ether bond. Therefore, **3** was identified as shown (Fig. 1). Full assignments of the ^1H and ^{13}C NMR spectra of **3** were achieved with HMBC, HMQC, and COSY experiments (Table 1). Similar compounds have been reported among the oxidation products of (+)-catechin obtained in the presence of peroxidase.[21]

Compound **4**, a yellow amorphous solid, was assigned the molecular formula $C_{30}H_{26}O_{12}$ based on negative-ion APCI-MS ($[M-H]^-$ at m/z 577) and its ^{13}C NMR data. The ^1H and ^{13}C NMR data of **4** were identical with those of epicatechin (8,6′) epicatechin that we reported from the reaction between epicatechin and the stable radical DPPH.[20] Thus, compound **4** was identified as epicatechin (8,6′) epicatechin (Fig. 1). This conclusion was further confirmed by comparing **4** with standard epicatechin (8,6′) epicatechin using TLC.

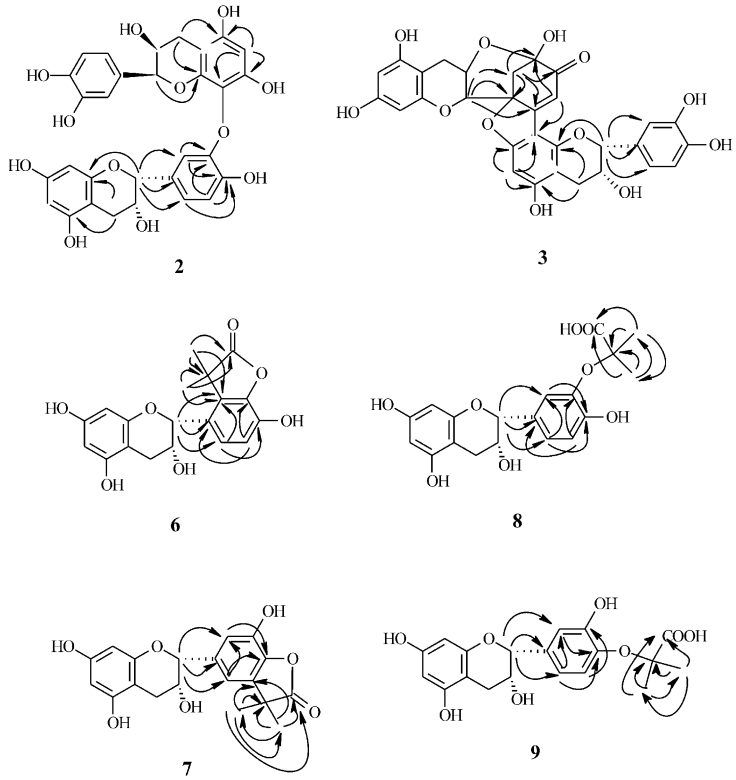

Figure 2. Significant HMBC (HC) correlations of compounds **2**, **3,** and **6**–**9**.

Compound **5** was isolated as a yellow amorphous solid. The negative-ion APCI-MS of **5** displayed a molecular ion peak at m/z $[M-H]^-$ 577, supporting a molecular formula of $C_{30}H_{26}O_{12}$, the same as that of **4**. The ^1H and ^{13}C NMR data of **5** were identical with those of epicatechin (6,6′) epicatechin that we reported from the reaction between epicatechin and the stable radical DPPH.[20] Thus, compound **5** was identified as epicatechin (6,6′) epicatechin (Fig. 1). As described above, this conclusion was checked by comparing the TLC of **5** with standard epicatechin (6,6′) epicatechin.

Compound **6** had the molecular formula $C_{19}H_{18}O_7$, established by positive-ion HRFAB-MS (m/z 359.1130, $[M+H]^+$; calcd for $C_{19}H_{19}O_7$: 359.1131) as well as ^{13}C NMR spectral data (Table 2). Comparing the NMR data of **6** and epicatechin, the major differences involved the signals of the B ring and the observation in **6** of signals from an AIBN-derived radical (δ_H 1.56 s, 1.58 s, each 3H; δ_C 25.6 q, 26.2 q, 45.9 s and 182.6 s). The ^1H NMR spectrum of **6** showed signals consistent with a 1,2,3,4-tetrasubstituted B-ring (δ_H 7.43 d, $J=9.0$ Hz; 6.86 d, $J=9.0$ Hz), instead of the 1,3,4-trisubstituted B-ring of epicatechin. Together these observations indicated that **6** is an adduct of epicatechin with an AIBN-derived radical located at position 2′ of the B ring. Because the molecular weight of **6** is 18 mass units less than the sum of epicatechin plus an AIBN-derived radical, it is reasonable to propose that **6** is a lactone between the –COOH group of the AIBN-derived radical and the –OH group at position 3′ of the B-ring. All of these structural features were confirmed by the HMBC spectrum (Fig. 2), which exhibited the following correlations: δ_H 5.06 ppm (H-2) with δ_C 126.7 (C-6′), 128.2 (C-1′), and 133.0 (C-2′) ppm; δ_H 7.43 (H-6′) with δ_C 76.1 (C-2), 133.0 (C-2′) and 142.7 (C-4′) ppm; δ_H 6.86 (H-5′) with δ_C 128.2 (C-1′), 140.6 (C-3′) and 142.7 (C-4′) ppm; δ_H 1.56 or 1.58 (H-2″ or H-3″) with δ_C 25.6 (C-3″ or 2″), 26.2 (C-2″ or C-3″), 45.9 (C-1″), 133.0 (C-2′), and 182.6 (C-4″) ppm. Thus, the structure of **6** was determined as shown (Fig. 1). Full assignments of the ^1H and ^{13}C NMR signals of **6** were made using HMBC and HMQC experiments (Table 2).

Compound **7** was isolated as a pale amorphous solid. The positive-ion APCI-MS of **7** displayed a molecular ion peak at m/z $[M-H]^+$ 359, supporting a molecular formula of $C_{19}H_{18}O_7$, identical to that of **6**. The NMR spectra of **7** also displayed signal patterns similar to that of **6**. The most significant differences in the spectra of compounds **6** and **7** involved the signals for the B ring. The ^1H NMR spectrum of **7** showed signals for a 1,3,4,5-tetrasubstituted B ring (δ_H 7.00 d, $J=1.2$ Hz; 6.96 d, $J=1.2$ Hz) instead of the 1,2,3,4-tetrasubstituted B-ring found for **6**. This observation was confirmed by the HMBC spectrum (Fig. 2), which showed correlations of H-2 (δ_H 4.84 ppm) with δ_C 113.3 (C-6′), 116.3 (C-2′), and 136.1 (C-1′) ppm; δ_H 7.00 (H-2′) with δ_C 79.9 (C-2), 113.3 (C-6′), 140.1 (C-4′) and 142.3 (C-3′) ppm; δ_H 6.96 (H-6′) with δ_C 44.9 (C-1″), 79.9 (C-2), 116.3 (C-2′), 138.4 (C-5′) and 140.1 (C-4′) ppm; δ_H 1.48 (H-2″ or H-3″) with δ_C 25.8 (C-3″ or 2″), 44.9 (C-1″), 138.4 (C-5′), and 182.9 (C-4″) ppm. Therefore, **7** was identified as shown (Fig. 1). Full assignments of the ^1H and ^{13}C NMR resonances of **7** were made from HMBC and HMQC experiments (Table 2).

Compound **8** was assigned the molecular formula $C_{19}H_{20}O_8$, established by positive-ion HRFAB-MS (m/z 377.1234, $[M+H]^+$; calcd for $C_{19}H_{21}O_8$: 377.1236) as well as the ^{13}C NMR spectral data (Table 2). Comparing the NMR data of **8** and epicatechin, the major differences were the observation of signals for an AIBN derived radical (δ_H 1.48 s, 6H; δ_C 27.0 q, 84.6 s and 183.9 s) and the downfield shift of one of the oxygenated carbons (δ_C 153.2) of the B ring. The HMBC spectrum

Table 2. NMR spectral data for compounds **6–9** (CD_3OD) (δ in ppm, J in Hz)

	6		**7**		**8**		**9**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	5.06 brs	76.1 d	4.84 brs	79.9 d	4.86 brs	79.9 d	4.86 brs	79.9 d
3	4.18 m	66.9 d	4.21 m	67.6 d	4.22 m	67.5 d	4.18 m	67.7 d
4	2.85 brd 16.8 2.93 dd 4.2, 16.8	30.2 t	2.76 brd 16.8 2.90 dd 4.8, 16.8	29.5 t	2.75 dd 3.0, 16.8 2.88 dd 4.8, 16.8	29.5 t	2.72 dd 3.0, 16.8 2.87 dd 4.2, 16.8	29.4 t
5		158.4* s		157.9 s		157.8* s		158.2* s
5a		100.0 s		100.1 s		100.2 s		100.3 s
6	5.90 d 2.4	96.1 d	5.95 d 2.4	96.6 d	5.94 d 1.8	96.1 d	5.93 d 1.8	96.0 d
7		157.9* s		158.2 s		158.2* s		158.3* s
8	5.98 d 2.4	96.8 d	5.97 d 2.4	96.8 d	5.95 d 1.8	96.6 d	5.95 d 1.8	96.7 d
8a		157.6 s		157.3 s		157.4 s		157.4 s
1′		128.2 s		138.4 s		137.5 s		131.2 s
2′		133.0 s	7.00 d 1.2	116.3 d	6.99 d 1.8	116.2 d	7.10 d 1.8	124.6 d
3′		140.6 s		142.3 s		153.2 s		144.1 s
4′		142.7 s		140.1 s		143.9 s		153.0 s
5′	6.86 d 9.0	117.3 d		136.1 s	6.92 d 9.0	125.7 d	6.79 d 8.4	125.2 d
6′	7.43 d 9.0	126.7 d	6.96 d 1.2	113.3 d	6.84 dd 1.8, 9.0	118.2 d	7.07 dd 1.8, 8.4	116.2 d
1″		45.9 s		44.9 s		84.6 s		84.7 s
2″	1.56* s	26.2* q	1.48 s	25.8 q	1.48 s	27.0 q	1.48* s	27.2 q
3″	1.58* s	25.6* q	1.48 s	25.8 q	1.48 s	27.0 q	1.46* s	26.9 q
4″		182.6 s		182.9 s		183.9 s		184.0 s

*Assignments may be interchanged in each column.

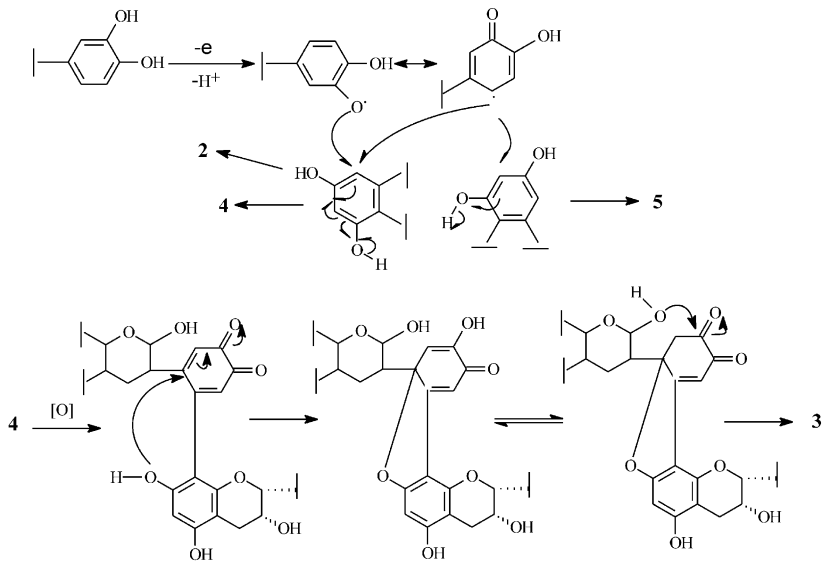

Figure 3. Proposed mechanisms for the formation of oxidation products **2**–**5**.

showed correlations of H-2′ (δ_H 6.99 ppm) with δ_C 79.9, 118.2, 143.9, and 153.2 ppm; H-5′ (δ_H 6.92 ppm) with δ_C 137.5, 143.9, and 153.2 ppm; H-6′ (δ_H 6.84 ppm) with δ_C 79.9, 116.2, and 143.9 ppm; δ_H 1.48 (H-2″ or H-3″) with δ_C 27.0, 84.6, and 183.9 ppm. Thus, δ_C 153.2 was assigned to C-3′, 143.9 to C-4′, and 84.6 to C-1″. The AIBN-derived radical group is connected at position 3′ through an ether linkage, so that **8** was determined as shown (Fig. 1). Assignments of all ^{1}H and ^{13}C NMR signals of **8** were made with HMBC and HMQC experiments (Table 2).

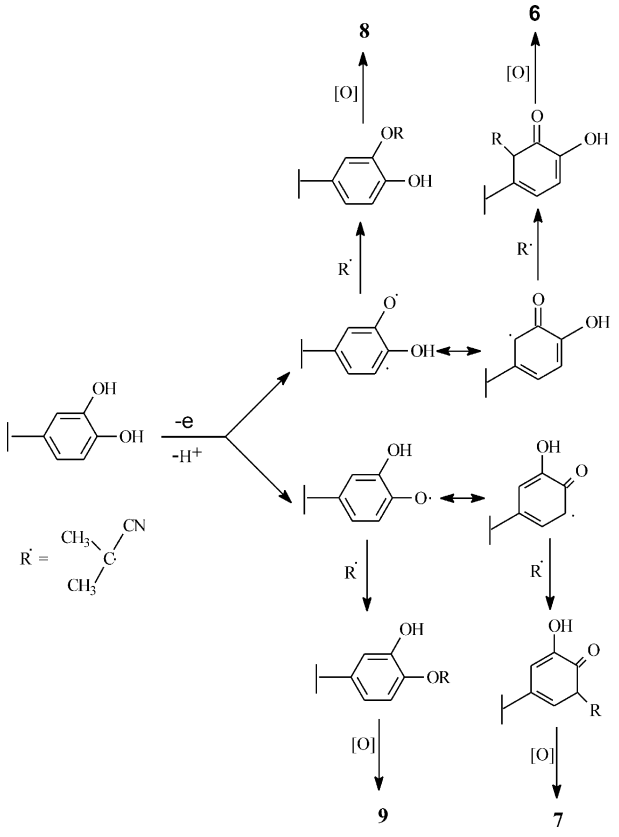

Figure 4. Proposed mechanisms for the formation of oxidation products **6**–**9**.

Compound **9** was isolated as a pale amorphous solid. The positive-ion APCI-MS of **9** displayed a molecular ion peak at m/z $[M+H]^+$ 377, consistent with a molecular formula of $C_{19}H_{20}O_8$, identical to that of **8**. The NMR spectrum of **9** displayed a pattern of signals similar to that of **8**. It also exhibited signals for an AIBN-derived radical (δ_H 1.46 s, 1.48 s, each 3H; δ_C 26.9 q, 27.2 q, 84.7 s and 184.0 s) and a downfield shift of one of the oxygenated carbons (δ_C 153.0) of the B ring. The HMBC spectrum showed correlations of δ_H 7.10 ppm (H-2′) δ with δ_C 79.9, 116.2, 144.1, and 153.0 ppm; δ_H 6.79 ppm (H-5′) δ with δ_C 131.2, 144.1, and 153.0 ppm; δ_H 7.07 ppm (H-6′) δ with δ_C 79.9, 124.6, and 153.0 ppm; δ_H 1.46 or 1.48 (H-2″ or H-3″) with δ_C 26.9, 27.2, 84.7, and 184.0 ppm. Thus, δ_C 153.0 was assigned to C-4′, 144.1 to C-3′, and 84.7 to C-1″, respectively. The AIBN-derived radical group was then connected at position 4′ through an ether linkage and **9** was determined as shown (Fig. 1). Full assignments of the ^{1}H and ^{13}C NMR signals of **9** were made using HMBC and HMQC experiments (Table 2).

Discussion

The purpose of this investigation was to isolate and characterize the reaction products of epicatechin with alkylperoxyl radicals derived from AIBN in a homogeneous solution. The chemical structures of these eight oxidant products suggest the antioxidant mechanisms for epicatechin illustrated in Figures 3 and 4. Epicatechin is proposed to react with peroxyl radicals by a single electron transfer followed by deprotonation from the hydroxyl group of the B ring to form a resonance pair. If the reaction is initiated at the hydroxyl group of C-3′, **2, 4, 6**

and **8** should be formed; conversely,**5, 7** and **9** should be formed if the reaction is initiated at the hydroxyl group of C-4′. **3** could be formed by further oxidation of **4**.

The formation of stable AIBN-derived radical adducts of epicatechin is particularly notable. These types of coupling require formation of either 3′- or 4′-phenoxy radical intermediates; such phenoxyl radicals can be tautomerized to the corresponding *o*-quinones and alkyl radicals may be attached at the C-2′, C-5′, 3′-phenoxy and 4′-phenoxy positions on the B ring. Further oxidation of the AIBN moiety then leads to formation of compounds **6–9**. The observation of these products reinforces the conclusion that the B ring is the initial site for formation of all reaction products in the peroxyl radical oxidant system. On the basis of this and prior work,[15–20] it can be concluded that the choice of oxidant alters the catechin products and that the main site of antioxidant action depends on both the oxidant used and the structures of the catechins. The identification of oxidant-specific products may provide analytical approaches for evaluating the antioxidant actions of catechins in biological systems. Thus, this strategy may be a valuable tool for the evaluation of possible catechins anticancer activity in living systems.

Experimental

General experimental procedures

^{1}H (600 MHz), ^{13}C (150 MHz) and all 2D NMR spectra were run on a Varian Unity INOVA NMR spectrometer with TMS as internal standard. FT-IR was performed on a Magna 550 spectrometer. The APCI MS experiments were performed on a Fisons/VG Platform II mass spectrometer. HRFAB MS was run on JEOL AX-505 double focusing mass spectrometer. Thin-layer chromatography was performed using Sigma-Aldrich TLC plates (250 μm thickness, 2–25 μm particle size), visualizing compounds by spraying with 5% (v/v) H_2SO_4 in ethanol solution. AIBN was purchased from Sigma Chemical Co.: ^{1}H NMR ($CDCl_3$, 600 MHz): δ_H 1.66 (s, CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz): δ_C 25.3 (q, CH_3), 68.3 (s, C), 119.3 (s, C≡N). (−)-epicatechin was purchased from Sigma Chemical Co. CD_3OD was purchased from Aldrich Chemical Co.

Oxidation of epicatechin (1) and isolation of reaction products 2–9. 1.0 g epicatechin was allowed to react with AIBN (3.0 g) in 25 mL acetone incubated at 50 °C for 16 h. After evaporation of the solvent in vacuo, the residue was first applied to a silica gel column and eluted with chloroform to remove the AIBN, and then with methanol to obtain a mixture of eight reaction products and unreacted epicatechin. The mixture was subjected to Sephadex LH-20 chromatography to generate five subfractions. Subfraction 3 was pure unreacted epicatechin (260 mg). Subfractions 4 and 5 were applied to a RP-C18 silica gel column eluted by a 70% methanol/water solvent mixture to give 17 mg of **4** and 24 mg of **5**, respectively. Subfraction 2 was applied to a silica gel column eluted with hexane/ethyl acetate (1/1) to give fraction **1**, then eluted with ethyl acetate to give fraction 2, and finally eluted with ethyl acetate/methanol/water (5/1.2/0.8) to give fraction 3. Fraction 1 was subjected to Sephadex LH-20 chromatography to give 20 mg of **6** and 27 mg of **7**. Fraction 2 was run on a RP-C18 silica gel column eluted by 15% methanol/water first to give 13 mg of **8**, and then eluted with 60% methanol/water to give 18 mg of **9**. Fraction 3 was applied to a RP-C18 silica gel column eluted by 20% methanol/water first to give 6 mg of **2**, and then eluted with 50% methanol/water to give 7 mg of **3**.

Compound **2** was isolated as a pale amorphous substance: ^{1}H NMR (CD_3OD, 600 MHz): see Table 1; ^{13}C NMR (CD_3OD, 150 MHz): see Table 1; negative APCI-MS *m*/*z* 577 $[M-H]^-$.

Compound **3** was isolated as a yellow amorphous substance: ^{1}H NMR (CD_3OD, 600 MHz): see Table 1; ^{13}C NMR (CD_3OD, 150 MHz): see Table 1; negative APCI-MS *m*/*z* 575 $[M-H]^-$.

Compound **4** was isolated as a yellow amorphous substance: ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz): the same as epicatechin-(6′,8)-epicatechin;[20] negative APCI-MS *m*/*z* 577 $[M-H]^-$.

Compound **5** was isolated as a yellow amorphous substance: ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz): the same as epicatechin-(6′,6)-epicatechin;[20] negative APCI-MS *m*/*z* 577 $[M-H]^-$.

Compound **6** was isolated as a pale amorphous substance: ^{1}H NMR (CD_3OD, 600 MHz): see Table 2; ^{13}C NMR (CD_3OD, 150 MHz): see Table 2; positive HRFAB-MS *m*/*z* 359.1130 $[M+H]^+$; calcd for $C_{19}H_{19}O_7$: 359.1131.

Compound **7** was isolated as a pale amorphous substance: ^{1}H NMR (CD_3OD, 600 MHz): see Table 2; ^{13}C NMR (CD_3OD, 150 MHz): see Table 2; negative APCI-MS *m*/*z* 357 $[M-H]^-$.

Compound **8** was isolated as a pale amorphous substance: ^{1}H NMR (CD_3OD, 600 MHz): see Table 2; ^{13}C NMR (CD_3OD, 150 MHz): see Table 2; negative HRFAB-MS *m*/*z* 377.1234, $[M+H]^+$; calcd for $C_{19}H_{21}O_8$: 377.1236.

Compound **9** was isolated as a pale amorphous substance: ^{1}H NMR (CD_3OD, 600 MHz): see Table 2; ^{13}C NMR (CD_3OD, 150 MHz): see Table 2; negative APCI-MS *m*/*z* 375 $[M-H]^-$.

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