A Novel Quasi-Dimeric Oxidation Product of (+)-Catechin from Lipid Peroxidation

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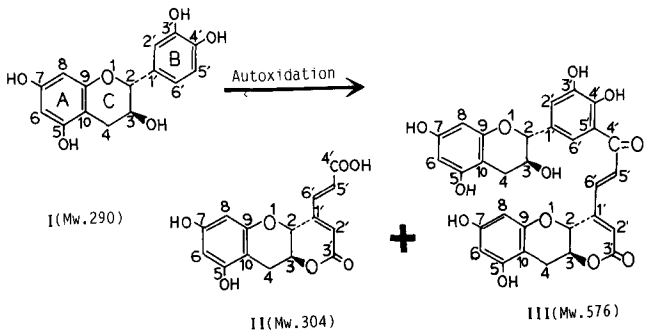
A novel quasi-dimeric oxidation product of (+)-catechin, formed during the radical-scavenging reaction that prevents lipid peroxidation, was isolated by chromatography, and its structure was elucidated by infrared, ultraviolet and ^1H and ^{13}C nuclear magnetic resonance spectra and mass spectrometry. It was 5′-[3-[3,4-(3′,5′-dihydroxy) benzo-8-oxo-2,7-dioxabicyclo[4.4.0]deca-3,9-dien-10-yl]acryloyl]-(+)-catechin. It is an unusual type of dimer of flavan-3-ol derivatives, which is different from the naturally formed dimer, procyanidin.

KEY WORDS: (+)-Catechin, oxidation product of (+)-catechin, lipid peroxidation.



SCHEME 1

Flavan-3-ol derivatives are important antioxidants, acting as radical scavengers in preventing the lipid peroxidation responsible for aging and some serious diseases such as arteriosclerosis and cancer (1,2). Mechanistic studies of their antioxidative reactions do not always address the structure of their oxidized state (3). (+)-Catechin(I) has a flavan-3-ol skeleton (see Scheme 1), and is one of the familiar antioxidants. Recently we reported that the structure of one of its major oxidation products (II) (see Scheme 1), formed where I acts as a radical scavenger in the antioxidative reaction of lipid, is an acrylic acid derivative (4). Here we wish to describe the isolation and the structure elucidation of another major oxidation product of I, a novel quasi-dimeric species (III) (see Scheme 1), formed under the same conditions.

MATERIALS AND METHODS

(+)-Catechin(I) was purchased from Sigma Chemicals Co., St. Louis, MO, and purified by silicic acid column chromatography. 2,2′-Azobis[2-methylpropanenitrile] (AIBN) was purchased from Kanto Chemicals Co., Tokyo, Japan, and used without further purification. All other chemicals were obtained commercially. All solvents used throughout the present work were distilled in an all-glass still before use.

The preparative radical-scavenging reaction of I was carried out in a model system mentioned earlier (4): an ethyl acetate solution of I (1.0 g) was irradiated with fluorescent lamps (15 W × 4) at 40°C for 20 days in the presence of AIBN (1.13 g). The reaction mixture was then concentrated by evaporation. The resulting residue was chromatographed on a Sephadex LH-20 column (35$^\phi$ × 500 mm) by successively eluting with ethanol-hexane (7:3), ethanol and methanol. The fraction containing III was purified by preparative high-performance liquid chromatography (HPLC) using an ODS column (20$^\phi$ × 250 mm) with water-acetonitrile-methanol-acetic acid (70:28:2:1, by vol) as the elution solvent.

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RESULTS AND DISCUSSION

The oxidation product (III) was obtained as yellowish brown powder (15.2 mg), m.p. 191.5–192.0°C (decomp.). Anal. Found: C, 57.66; H, 4.54%. Calcd. for $C_{30}H_{24}O_{12}\cdot 2\frac{1}{2}H_2O$; C, 57.97; H, 4.70%. $[\alpha]_D^{30}$ + 140.8° (*c* 0.22, acetone). Field desorption mass spectrometry (FDMS) *m/z* 576(M^+). Infrared (IR) ν_{max}^{KBr} 3390-3230, 1670, 1625 cm^{-1}. Ultraviolet (UV)-Vis λ_{max}^{MeOH} (log ε) 230(4.49sh), 280(4.17), 305(4.25), 400(3.54)nm. ^1H- and ^{13}C-nuclear magnetic resonance (NMR) data are shown in Tables 1 and 2, where peak assignments are based on 2D NMR (COSY and COLOC) spectra. The signals obtained from III could be separated into two sets, one originating from the segment derived from I and the other from II. Thus each signal obtained from III was assigned to segment I or II by comparing it with those obtained from pure I and II, and listed in two columns in the Tables.

The connecting positions between these two segments found in III were determined as follows. Two doublets at δ7.30(H-2′) and δ7.76(H-6′) corresponding to the catechin moiety showed the presence of substitution on the 5′-position in the B ring of I. The acryloyl moiety was indicated by a downfield shift of the 4′-carbon (δ194.5 compared with δ167.7 for II) suggesting the change from carboxyl to ketone. Moreover, long-range interactions ^3J between δ_C194.5 and δ_H7.76 were observed (COLOC) (5–7). These spectral data indicated that both segments connect on the B rings (see Scheme 1), one of which has been oxidatively cleaved between C-3′ and C-4′ of I to afford a conjugated carbonyl moiety. The data listed in Tables 1 and 2 and the above discussion led us to the structure of III, depicted in Scheme 1 along with those of I and II. III was identified as 5′-[3-[3,4-(3′,5′-dihydroxy)benzo-8-oxo-2,7-dioxabicyclo [4.4.0]deca-3,9-dien-10-yl]acryloyl]-(+)-catechin.

This novel oxidation production III is an unusual type of dimer of flavan-3-ol derivatives, which is different from the naturally found dimer, procyanidin (8); because III are formed via the reaction of the B-ring maintaining the A-ring skeleton of I. It should be noted that the

TABLE 1

^{13}C-NMR Spectral Data of I, II and III (75 MHz, δ ppm)

	I[a]	II[b]	III[a,c]	
2	82.0(d)	70.6(d)	82.5(d)	71.9(d)
3	68.1(d)	73.6(d)	68.5(d)	75.0(d)
4	28.2(t)	25.9(t)	29.0(t)	27.1(t)
5	156.6(s)	156.5(s)	157.7(s)[d]	157.8(s)[d]
6	96.1(d)	96.4(d)	96.5(d)	97.5(d)
7	157.2(s)	157.1(s)	157.2(s)[d]	158.4(s)
8	95.3(d)	94.1(d)	95.5(d)	96.0(d)
9	156.4(s)	154.1(s)	156.5(s)	155.4(s)
10	100.1(s)	97.9(s)	100.5(s)	99.6(s)
1′	131.6(s)	150.3(s)	131.4(s)	151.5(s)
2′	115.0(d)	120.7(d)	121.3(d)	123.2(d)
3′	145.3(s)	162.5(s)	147.4(s)	163.1(s)
4′	145.4(s)	167.7(s)	152.8(s)	194.5(s)
5′	115.7(d)	129.1(d)	120.4(s)	130.7(d)
6′	119.9(d)	136.3(d)	121.7(d)	137.8(d)

[a] Acetone-d_6.

[b] Dimethylsulfoxide (DMSO)-d_6.

[c] The first column corresponds to the (+)-catechin segment and the second to its oxidized segment (cf. Scheme 1).

[d] These assignments may be interchanged.

TABLE 2

^{1}H-NMR Spectral Data of I, II and III (300 MHz, δ ppm)*

	I[a]	II[b]	III[a,c]	
2	4.66	4.93	4.66	4.99
	(7.6)	(10.6;1.6)	(8.6)	(10.3;1.6)
3	4.11	4.64	4.13	4.71
	(8.4;7.6;5.5)	(10.8;10.6;5.9)	(9.3;8.6;5.8)	(10.9;10.3;6.2)
4	2.97	2.99	3.06	3.18
	(16.1;5.5)	(15.2;5.9)	(15.7;5.8)	(15.5;6.2)
	2.62	2.63	2.62	2.78
	(16.1;8.4)	(15.2;10.8)	(15.7;9.3)	(15.5;10.9)
6	6.09	6.02	6.06	6.13
	(2.3)	(2.0)	(2.2)	(2.2)
8	5.96	5.82	5.90	6.18
	(2.3)	(2.0)	(2.2)	(2.2)
2′	6.96	6.50	7.30	6.59
	(1.9)	(1.6)	(1.8)	(1.6)
5′	6.85	6.69		8.30
	(8.1)	(16.1)		(15.7)
6′	6.80	7.31	7.76	7.64
	(8.1;1.9)	(16.1)	(1.8)	(15.7)

*J-Values in parentheses are given in Hz. For superscripts a,b,c, see Table 1.

production of III has also been observed with HPLC in the radical-scavenging reactions that prevent lipid peroxidation. Investigations of the antioxidative activity of III and the mechanism of the radical-scavenging reaction of I in preventing lipid peroxidation are currently in progress.

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