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Structure of Polymeric Polyphenols of Cinnamon Bark Deduced from Condensation Products of Cinnamaldehyde with Catechin and Procyanidins

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Structures of two condensation products obtained by the reaction of cinnamaldehyde with (+)-catechin were determined by spectroscopic methods. One had two phenylpropanoid units at the C-6 and C-8 positions of the catechin skeleton. The other product had a dimeric structure with two catechin and two phenylpropanoid units. Matrix-assisted laser desorption time-of-flight mass spectrometric analysis of the reaction products of cinnamaldehyde with procyanidin B1 suggested that procyanidins were oligomerized in a manner similar to the reaction with catechin. Furthermore, ^{13}C NMR spectral comparison of the condensation products with the polymeric procyanidins obtained from commercial cinnamon bark strongly suggested that the procyanidins in the cinnamon bark also were polymerized by reaction with cinnamaldehyde.

KEYWORDS: Catechin; cinnamaldehyde; cinnamon bark; polyphenol; procyanidin

INTRODUCTION

Cinnamon bark [*Cinnamomum zeylanicum*, *Cinnamomum cassia* (Chinese cinnamon), *Cinnamomum burmannii* (Indonesian cinnamon), and *Cinnamomum sieboldii* (Japanese cinnamon)] is an important spice used worldwide as well as one of the most essential crude drugs in Oriental medicine. Cinnamaldehyde (**2**) is a major component of the essential oil that characterizes its flavor and aroma and shows various biological activities (*1*–*8*). In addition, cinnamon bark contains catechins and procyanidins, which have an astringent taste and antioxidative activities, and the structures of the procyanidins have been reported to have procyanidin B- and A-type linkages (*9*–*13*). The presence of polymeric polyphenols also was shown by normal-phase thin layer chromatography (TLC) and reversed-phase high-performance liquid chromatography (HPLC). The polymers remained at the TLC origin and were detected as a broad hump on the baseline by HPLC analysis. By analogy to the structures of procyanidin dimers to hexamers previously isolated from the same plant (*11, 12*), the structures of the polymer were deduced to be relatively simple procyanidin polymers with B- and A-type linkages. However, polyphenol fractions of cinnamon bark are dark reddish brown, which cannot be explained by the simple procyanidin structures. When fresh bark of Japanese cinnamon (*C. sieboldii*) was peeled from a branch, the color of the wood surface immediately changed from white to reddish brown. Since this dynamic change of color was not observed when the branch was heated in advance, the reaction was apparently catalyzed by enzymes. However, we found that a similar color change also was observed when a mixture of (+)-catechin (**1**) and cinnamaldehyde (**2**) was heated (100 °C) or irradiated with sun light. Even at room temperature, the color changed to red very slowly to give a complex mixture of products after 6 days. This study reports the chemical reaction of cinnamaldehyde with catechin and procyanidin B1. Furthermore, incorporation of cinnamaldehyde to polymeric procyanidins of commercial cinnamon bark was deduced by ^{13}C NMR spectroscopic comparison with the in vitro reaction products.

MATERIALS AND METHODS

Materials. (+)-Catechin was isolated from commercial gambir (extract of the leaves and twigs of *Uncaria gambir*) and recrystallized from H_2O (*14*). Procyanidin B1 was produced by degradation of polymeric procyanidin of *Areca catechu* with (+)-catechin according to the method previously reported (*15*). Cinnamaldehyde was purchased from Nacalai Tesque, Inc. Cinnamon bark (dried bark of *C. cassia*) was purchased from Uchida Wakanyaku Co., Ltd.

General Procedures. Infrared (IR) and ultraviolet (UV) spectra were obtained with Jasco FT/IR-410 and Jasco V-560 spectrophotometers (Jasco Co.), and optical rotations were measured with a Jasco DIP-370 digital polarimeter. ^1H and ^{13}C NMR, $^1H-^1H$ COSY, NOESY, HSQC, and HMBC spectra were recorded in acetone-d_6 at 27 °C with a Varian Unity plus 500 spectrometer (Varian Inc.) operating at 500 MHz for ^1H and 125 MHz for ^{13}C, respectively. Coupling constants are expressed in hertz, and chemical shifts are given on a δ (ppm) scale. HMQC, HMBC, and NOESY experiments were performed using standard Varian pulse sequences. The matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF MS) images were recorded on a Voyager-DE Pro spectrometer (Applied Biosystems), and 2, 5-dihydrohydroxybenzoic acid (10 mg/mL in 50% acetone containing 0.05% trifluoroacetic acid) was used as the matrix. Fast atom bombardment (FAB) MS was recorded on a JMS 700N spectrometer

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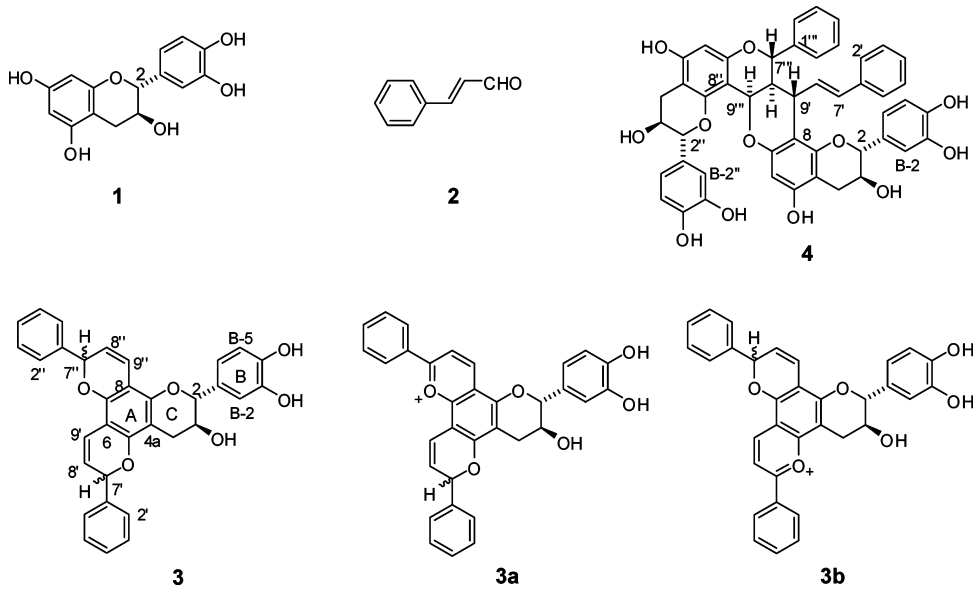

Figure 1. Structures of **1**−**4**, **3a**, and **3b**.

(JEOL Ltd.), and *m*-nitrobenzyl alcohol or glycerol was used as a matrix. Column chromatography was performed using 25−100 μm Sephadex LH-20 column (Amersham Pharmacia Biotech AB), Diaion HP20SS, MCI-gel CHP20 (Mitsubishi Chemical Co.), Chromatorex ODS (Fuji Silysia Chemical Ltd.), and silica gel 60 (Merck).

TLC was performed on 0.2 mm precoated Kieselgel 60 F_{254} plates (Merck) with toluene/ethyl formate/formic acid (2:7:1, v/v) and $CHCl_3$/MeOH/H_2O (90:10:1, v/v). Spots were detected by UV illumination and by spraying with 2% ethanolic $FeCl_3$ or 10% sulfuric acid reagent followed by heating. Detection of catechins and procyanidins was performed by spraying 1% ethanolic vanillin followed by concentrated HCl. Analytical HPLC was performed on a 250 mm × 4.6 mm i.d. Cosmosil 5C_{18}-AR II column (Nacalai Tesque, Inc.) with gradient elution from 4 to 30% (39 min) and 30 to 75% (15 min) of CH_3CN in 50 mM H_3PO_4 at a flow rate of 0.8 mL/min and detection with an MD-910 photodiode array detector (Jasco).

Reaction of (+)-Catechin and Cinnamaldehyde at Room Temperature. (+)-Catechin (**1**, 1.0 g) and cinnamaldehyde (**2**, 2.0 g) (**Figure 1**) were dissolved in acetone (30 mL), the organic solvent was evaporated under reduced pressure, and the resulting syrup was kept at room temperature for 6 days. The mixture was separated by silica gel column chromatography (35 cm × 3.0 cm i.d.) with $CHCl_3$/MeOH/H_2O (90:10:1, v/v). The first fraction positive to the $FeCl_3$ reagent was further purified by silica gel column chromatography (30 cm × 2.0 cm i.d.) with $CHCl_3$/MeOH/H_2O (90:10:1, v/v) to yield **3** (64.1 mg) as a precipitate powder from MeOH. Further elution of the first silica gel column with $CHCl_3$/MeOH/H_2O (40:10:1 and 14:6:1, v/v) gave a complex mixture of products.

Compound 3. Yellow powder (precipitated from MeOH), $[\alpha]^{15}_D$ 0° (*c* 0.25, acetone); MALDI-TOF MS *m/z* 519 $[M + H]^+$, 517 $[M - H]^+$ (base peak, corresponding to the M^+ of **3a** or **3b**); HR-FAB MS *m/z* 517.1658 ($[M - H]^+$ $C_{33}H_{25}O_6$ requires 517.1651); UV λ_{max} (nm) (log ϵ): 389 (3.42), 276 (4.29); IR ν_{max} (cm^{-1}): 3390, 2973, 1699, 1599, 1527, 1448, 1365, 1280, 1126; ^1H NMR (500 MHz, in acetone-d_6) (major isomer) δ: 7.47−7.29 (10H, m, benzene rings), 6.87 (1H, d, J = 2.0 Hz, B-ring H-2), 6.69−6.78 (4H, m, H-9′, 9″, B-ring H-5, 6), 5.89 (2H, br s, H-7′, 7″), 5.73 (2H, dd, J = 4, 10.0 Hz, H-8′, 8″), 4.63 (1H, d, J = 7.8 Hz, H-2), 3.93 (1H, ddd, J = 5.3, 7.8, 8.3 Hz, H-3), 2.79 (1H, dd, J = 5.3, 16.6 Hz, H-4), 2.50 (1H, dd, J = 8.3, 16.6 Hz, H-4). After measurement of the ^1H NMR spectrum, the concentration of isomers of **3** that were initially minor components was increased to give complex signals. ^{13}C NMR (125 MHz, in acetone-d_6) (mixture of four isomers) δ: 152.5 (C-5), 151.5 (C-8a), 148.5, 148.4 (C-7), 145.7, 145.6 (B-ring C-3, 4), 142.0, 141.9 (C-1′, 1″), 131.3, 131.2 (B-ring C-1), 129.3, 129.2 (C-3′, 5′, 3″, 5″), 128.9, 128.8 (C-4′, 4″), 127.51, 127.48, 127.40, 127.37 (C-2′, 6′, 2″, 6″), 121.14, 121.10, 121.05, 121.0, 120.9, 120.7 (C-8′, C-8″), 119.7, 119.6, 119.5, 119.4, 119.32, 119.28, 119.0, 118.8, 118.7 (C-9′, 9″, B-ring C-6), 115.7, 115.6 (B-ring C-5), 115.0, 114.9 (B-ring C-2), 104.3, 104.2, 104.14, 104.09 (C-6, 8), 102.7, 102.6 (C-4a), 83.0, 82.9, 82.8 (C-2), 77.4, 77.34, 77.27, 77.2, 77.04, 76.99, 76.97 (C-7′, 7″), 67.4, 67.3 (C-3), 28.5, 28.30, 28.27, 28.09 (C-4). The NMR signals were assigned with the aid of ^1H−^1H COSY, HSQC, and HMBC spectroscopic analysis. After measurement of NMR spectra, the color of the solution changed to red. The sample was analyzed by HPLC to show two large peaks at 51.9 and 52.2 min attributable to isomers of **3**, which were accompanied by a small peak at 31.8 min attributable to the pigment (**3a** or **3b**) produced from **3**. The UV spectrum of the peak showed absorption maxima at 467, 399, 307, 279, and 271 nm.

Reaction of (+)-Catechin and Cinnamaldehyde upon Heating. An acetone solution (50 mL) containing **1** (2.0 g) and **2** (5.0 g) was concentrated to dryness in vacuo, and the resulting syrup was heated at 100 °C for 50 min. The mixture was separated by Sephadex LH-20 column chromatography (30 cm × 3.0 cm i.d.) with EtOH to give four fractions. The first fraction contained **2**. The second fraction was further separated by MCI-gel CHP20 (30 cm × 3.0 cm i.d.) with H_2O that contained increasing proportions of MeOH (10% stepwise elution, each 200 mL), to give **1** (990 mg) and a crude crop of **4**. Purification by column chromatography on Sephadex LH-20 (20 cm × 2.5 cm i.d.) with H_2O-MeOH (40−100%, 10% stepwise elution, each 100 mL) and Chromatorex ODS (25 cm × 3.0 cm i.d.) with H_2O/MeOH (20−100%, 5% stepwise elution, each 100 mL) afforded **4** (16.9 mg).

Compound 4. Yellow amorphous powder, $[\alpha]^{20}_D$ −110.6° (*c* 0.12, MeOH); FAB MS *m/z* 809 $[M + H]^+$, 831 $[M + Na]^+$; HR-FAB MS *m/z* 809.2595 ($C_{48}H_{41}O_{12}$ requires 809.2598); UV λ_{max} (nm) (log ϵ): 283sh (3.99), 236sh (4.48); IR ν_{max} (cm^{-1}): 3340, 2915, 1619, 1520, 1447; ^1H and ^{13}C NMR data: see **Table 1**.

Reaction of Procyanidin B1 with Cinnamaldehyde. Procyanidin B1 (**5**) (20 mg) and **2** (20 mg) were dissolved in acetone (0.5 mL), and the organic solvent was evaporated under reduced pressure. The mixture was kept at room temperature for 8 days. The mixture was dissolved in a small amount of EtOH and subjected to Sephadex LH-20 column chromatography (15 cm × 1.0 cm i.d.) with EtOH. The nonpolar compounds including **2** were washed out with EtOH (30 mL). Subsequent elution of the column with 80% EtOH (20 mL) and then EtOH/H_2O/acetone (2:1:1, v/v) afforded a mixture of phenolic products (20.1 mg). MALDI-TOF MS of the products is shown in **Figure 5**. The products (2 mg) were dissolved in 60% EtOH solution (0.5 mL) that contained 4% mercaptoethanol and 0.1% HCl and heated at 70 °C for 7 h. HPLC analysis showed a small peak of epicatechin (t_R 22.3 min). In contrast, thiol degradation of **5** (2 mg) under similar conditions yielded epicatechin and epicatechin 4-hydroxyethylthioether (**5a**) (t_R 28.2 min) (*17*).

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for **3**[a]

position	^{1}H	^{13}C	HMBC (H to C)	position	^{1}H	^{13}C	HMBC (H to C)
2	4.55 (d, 7.6)	82.8	3, 4, 8a, B-1, B-2, B-6	2″	4.81 (d, 7.3)	82.6	3″, 4″, 8a″, B″-1, B″-2, B″-6
3	3.89 (m)	68.4		3″	4.02 (m)	68.0	
4	2.58 (dd, 16.1, 8.2)	28.7	2, 3, 4a, 5, 8a	4″	2.63 (dd, 16.1, 8.0)	28.2	2″, 3″, 4a″, 5″, 8a″
	2.89 (dd, 16.1, 5.3)				2.92 (dd, 16.1, 5.3)		
4a		101.4		4a″		101.5	
5		156.0		5″		157.7	
6	5.99 (s)	95.8	4a, 5, 7, 8	6″	6.03 (s)	95.6	4a″, 5″, 7″ 8″
7		152.9		7″		155.2	
8	100.4		8″		102.8		
8a	153.9		8a″		154.6		
B-1	132.1		B″-1		132.2		
B-2	6.78 (d, 1.9)	115.3	2, B-1, B-3, B-4, B-6	B″-2	6.88 (d, 1.8)	115.1	2″, B″-3, B″-4, B″-6
B-3		145.3	*b*	B″-3		145.5	*b*
B-4		145.4	*b*	B″-4		145.6	*b*
B-5	6.59 (d, 8.2)	115.6	B-1, B-3, B-4	B″-5	6.69 (d, 8.1)	115.8	B″-1, B″-3, B″-4, B″-6
B-6	6.64 (dd, 8.2, 1.9)	119.6	2, B-2, B-4	B″-6	6.73 (dd, 8.1, 1.8)	119.3	2″, B″-2, B″-4
1′		138.5		1‴		140.2	
2′, 6′	7.29 (2H, m)	127.0	4′, 7′	2‴, 6‴	7.31 (2H, m)	128.2	4‴, 7‴
3′, 5′	7.24 (2H, br t, 7.5)	129.2	1′, 2′, 4′, 6′	3‴, 5‴	7.37 (2H, br t, 7.3)	129.2	1‴, 2‴, 4‴, 6‴
4′	7.14 (dt, 7.5, 1.4)	127.6	2′, 6′	4‴	7.30 (m)	129.1	2‴, 6‴
7′	6.12 (dd, 15.9, 1.3)	131.1	1′, 2′, 6′, 8′, 9′	7‴	4.88 (d, 11.0)	77.4	1‴, 2‴, 6‴, 8‴, 9‴
8′	6.28 (dd, 15.9, 5.9)	133.8	8, 1′, 7′, 9′, 8‴	8‴	2.38 (ddd, 11.0, 1.5, 2.3)	41.4	8, 9′, 7‴
9′	3.27 (ddd, 5.9, 1.5, 1.3)	33.8	7, 8, 8a, 7′, 8′, 7‴, 8‴, 9‴	9‴	5.47 (d, 2.3)	62.7	7″, 8″, 8a″, 7‴
3-OH	3.97 (d, 4.6)		2, 3, 4	3″-OH	4.13 (d, 4.6)		2″, 3″, 4″
5-OH	8.38 (br s)		4a, 5	5″-OH	8.65 (br s)		4a″, 5″

[a] Measured in acetone-d_6, δ in ppm, J in Hz. [b] Assignments may be interchanged in each column.

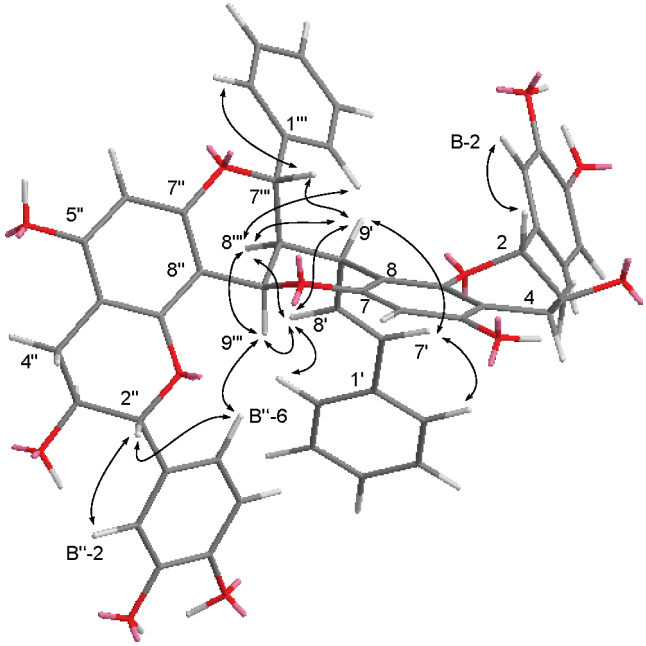

Figure 2. Important NOESY correlations (arrows) observed for **4**.

Extraction of Polyphenols from Cinnamon Bark. Commercial cinnamon bark (100 g) was extracted 3 times with 60% acetone (1 L) at 50 °C for 2 h. The extract was concentrated under reduced pressure until the organic solvent was removed, and precipitates formed in the resulting aqueous solution were dissolved by the addition of a small amount of MeOH. The solution was applied to Sephadex LH-20 column chromatography (32 cm × 4.0 cm i.d.) with H_2O that contained increasing proportions of MeOH (20% stepwise elution, each 300 mL) and finally with 50% aqueous acetone. The fractions that contained catechins and procyanidins, which were positive to $FeCl_3$ and vanillin/HCl reagents on TLC, were collected and concentrated until the organic solvent was completely removed. Contaminating nonpolar compounds were completely removed by partitioning with Et_2O 3 times, and the aqueous layer was lyophilized to give a polyphenol fraction (1.83 g). The lyophilized powder was subjected to gel permeation chromatography on Sephadex LH-20 (32 cm × 5.0 cm i.d.) with acetone/7 M urea (3:2 v/v, adjusted to pH 2 with concd HCl) (*16*). The first fraction, which contained polyphenols with large molecular weights, was collected and concentrated until the acetone was removed. The aqueous solution was directly applied to a Diaion HP20SS column (20 cm × 2.0 cm i.d.) with water to remove urea and HCl, and then the polyphenols were eluted with 80% MeOH to yield polymeric polyphenols (661.7 mg) as a brown amorphous powder. For ^{13}C NMR, see **Figure 7**.

Thiol Degradation of Polymeric Polyphenols. A solution of the polymeric polyphenols in 70% EtOH (25 mg/mL, 0.2 mL) was mixed with a 60% EtOH solution (0.8 mL) that contained 5% mercaptoethanol and 0.1% HCl and heated at 70 °C for 7 h. HPLC analysis showed two major peaks. The retention times and UV peak absorptions coincided with those of epicatechin (t_R 22.3 min) and epicatechin 4-hydroxyethylthioether (t_R 28.2 min), in that order of elution (*17*). The large broad hump on the baseline between t_R 40 and 50 min observed in the chromatogram of the original polymers decreased but did not completely disappear. Thiol degradation of the reaction products of **5** and **2** was performed in a manner similar to that described for cinnamon polymeric polyphenols.

RESULTS AND DISCUSSION

A mixture of catechin (**1**) and cinnamaldehyde (**2**) was kept without solvents at room temperature for 6 days. The reaction products were separated by silica gel column chromatography to give an unstable colorless product **3** as an insoluble precipitate from MeOH. Among the reaction products, **3** showed the highest R_f value on normal-phase TLC. The product was dark green with the $FeCl_3$ reagent, which suggested that **3** was derived from catechin. In the ^{1}H NMR spectrum, signals arising from catechin B- and C-rings were similar to those of **1**; however, the absence of signals due to the A-ring indicated that cinnamaldehyde was attached to the catechin C-6 and C-8 positions. With the aid of the $^{1}H-^{1}H$ COSY spectrum, the presence of two monosubstituted benzene rings, two oxygenated methines (H-7′ and H-7″), and two sets of mutually coupled (J = 10 Hz) *cis*-olefinic protons (H-8′, H-9′, H-8″, and H-9″) was revealed. The oxygenated methine protons H-7′ and H-7″ correlated with the H-8′ and H-8″, respectively, as well as the phenyl proton signals (H-7′ with H-2′ and H-6′ and H-7″ with H-2″ and H-6″). In

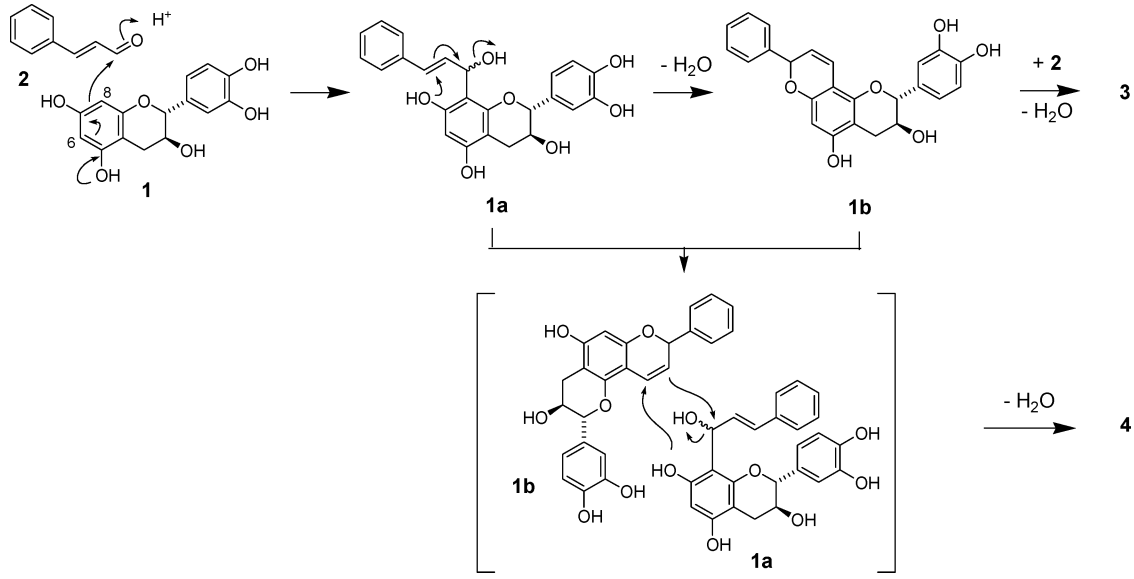

Figure 3. Production mechanisms of **3** and **4**.

addition, H-7′ and H-7″ showed $^{1}H-^{13}C$ long-range coupling with C-5 and C-7 of the catechin A-ring in the HMBC spectrum. This observation indicated that C-7′ and C-7″ were attached to catechin C-5 and C-7 through ether linkages. Furthermore, the signals attributable to H-9′ and H-9″ correlated with catechin A-ring carbon signals (C-5, C-6, C-7, C-8, and C-8a). These spectroscopic data indicated that **3** was a condensation product produced by coupling between A-ring C-6 and C-8 of **1** and two molecules of **2** (**Figure 1**). The product **3** was first isolated as a precipitate from methanol and exhibited reasonable ^{1}H NMR signals; however, this compound was unstable, and signals arising from several isomers gradually increased in the spectrum within several hours after dissolving NMR solvent (acetone-d_6). Chemical shifts of the newly observed signals were similar to that of **1**, which indicated that the structures of the isomers were closely related to **3** and suggested that the isomers differed in their configurations at C-7′ and C-7″ because these oxygen-bearing methines were positioned between benzene and the C-8═C-9 and C-8′═C-9′ double bonds, respectively, and were expected to be easily isomerized. The MALDI-TOF MS of **3** showed a small $[M + H]^+$ peak at *m/z* 519 along with a large $[M - H]^+$ peak. This strongly suggested that **3a** or **3b** oxidatively was generated from **3** upon irradiation of the laser beam during the MS measurement. Actually, **3** was unstable when it was exposed to air for a few days to give a small amount of red pigment, which was detected as a red spot on TLC. Although isolation failed, HPLC revealed that the pigment had UV−vis absorption maxima at 467, 399, 307, 279, and 271 nm. From the structure of **3**, it was deduced that there was a benzopyrylium ion moiety in the molecule. This also was supported by the production of related pigments by reactions between catechin and 4-hydroxy-3,5-dimethoxycinnamaldehyde (*18, 19*). It strongly was suggested that the dynamic color change observed when fresh bark of Japanese cinnamon was peeled off was explained by the formation of pigments related to **3a** or **3b** by enzymatic oxidation of **3** and related compounds. The yield of **3** was very low, and the composition of the remaining products was too complex to separate into individual components. The ^{13}C NMR and MALDI-TOF MS analyses of the mixture showed that the products were also condensation products of **1** and **2** with a larger molecular size (data not shown).

Similar condensation reactions of **1** and **2** occurred when the mixture was heated at 100 °C for 50 min. In this experiment, separation of the products by Sephadex LH-20 column chromatography yielded a new product **4** as a yellow amorphous powder. FAB MS showed the $[M + H]^+$ peak at *m/z* 809 and the $[M + Na]^+$ peak at *m/z* 831, which suggested that this compound was composed of two molecules of **1** and two of **2**. From the results of HR-FAB MS, the molecular formula was deduced to be $C_{48}H_{40}O_{12}$. The ^{1}H and ^{13}C NMR spectra also indicated the presence of two catechin skeletons and two phenylpropanoid units (**Table 1**). Substitution at the C-8 and C-8″ positions of the two catechin units was revealed by HMBC spectral analysis (**Table 1**), and assignment of the adjacent C-8a and C-8a″ signals was confirmed by correlations from H-2 and H-2″, respectively. These two carbons did not correlate with the A-ring singlet proton signals (H-6 and H-6″), which showed all other ^{2}J and ^{3}J correlations with the remaining A-ring carbons. Low-field shifts of the carbon signals attributable to C-8 and C-8″ (δ 100.4 and 102.8, respectively) also supported substitution at these positions. C-8, C-7, and C-8a showed cross-peaks with an aliphatic methine proton H-9′, which showed correlation peaks with a pair of trans-olefinic protons H-8′ and H-7′ ($J_{7',8'}$ = 15.9 Hz) in the $^{1}H-^{1}H$ COSY spectrum. Observation of HMBC correlations between the H-7′ and the benzene carbons indicated that this double bond was attached to a benzene ring (**Table 1**). The $^{1}H-^{1}H$ COSY spectrum also showed cross-peaks between H-9′ and H-8‴, and in turn, H-8‴ was coupled with two oxygen-bearing methine protons, H-7‴ and H-9‴. The connection of these four aliphatic methine carbons, C-9′, C-7‴, C-8‴, and C-9‴, also was confirmed by their mutual HMBC correlations (**Table 1**). Furthermore, H-9‴ exhibited HMBC correlations with C-8″, C-7″, and C-8a″, which indicated that C-9″ was attached to the C-8″ of one of the catechin units. The location of a benzene ring at C-7‴ was apparent from their mutual HMBC correlations, as shown in **Table 1**. On the basis of the molecular formula ($C_{48}H_{40}O_{12}$), the index of hydrogen deficiency was calculated to be 29. This indicated the presence of two additional rings in the molecule besides two catechin units, two benzene rings, and a double bond. Taking the structure of **3** into account, it was strongly suggested that two oxygen-bearing methine carbons C-7‴ and C-9‴ formed ether rings with the oxygen atoms attached at C-7

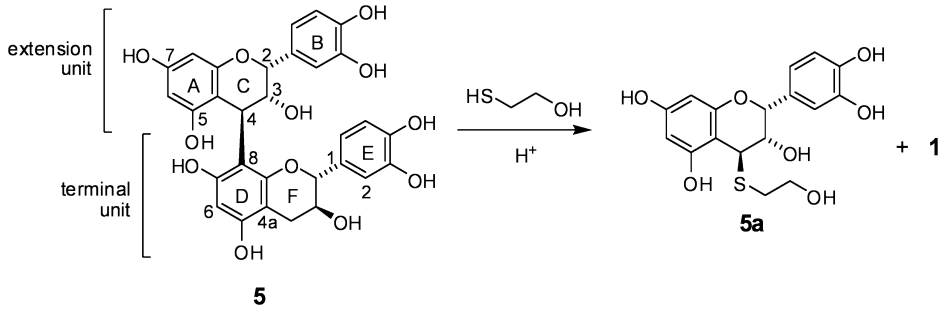

Figure 4. Thiol degradation of procyanidin B1 (**5**).

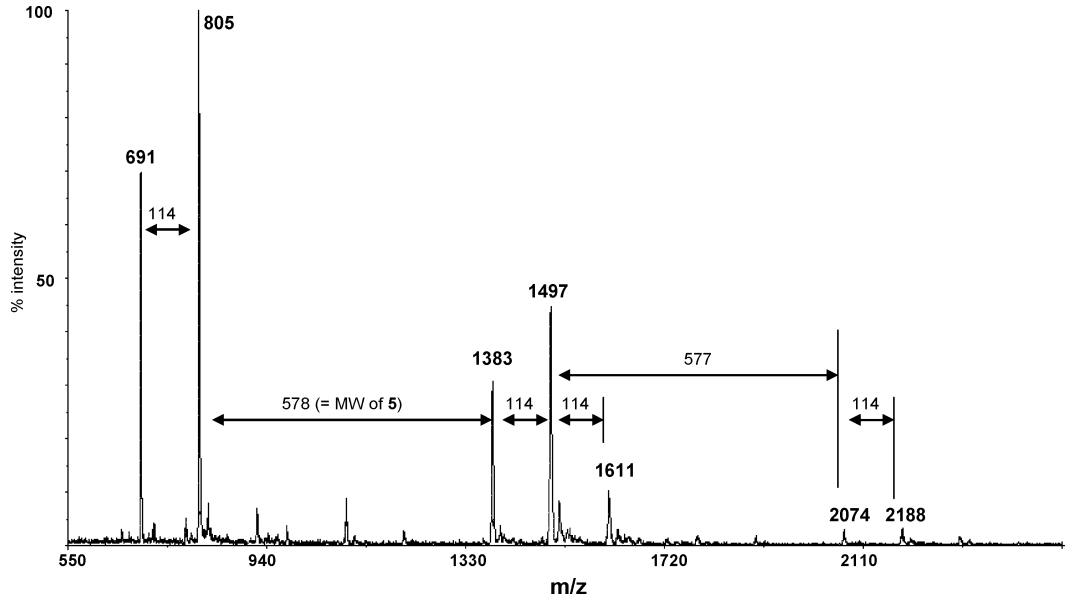

Figure 5. MALDI-TOF MS of reaction products of **2** and **5**.

and C-7″, respectively. Configuration of the methine carbons were determined by the mutual $^1H-^1H$ coupling constants and NOESY correlations (**Figure 2**). The large coupling constant between H-7‴ and H-8‴ (11.0 Hz) indicated that the dihedral angle between these protons was almost 180°. H-8‴ also coupled with H-9′, the coupling constant of which was 1.5 Hz. Since H-9′ showed NOESY correlations with both H-7‴ and H-8‴, H-9′ was spatially placed between H-7‴ and H-8‴. The coupling constant of H-8‴ with another methine proton H-9‴ was 2.3 Hz, and H-9‴ showed NOE to H-8‴, H-8′, and B″-ring protons (B″-2 and B″-6). The NOE between H-9‴ and B″-ring protons indicated that these two were oriented to the same side of the molecule. The absolute configuration at catechin C-2″ was *R* because (+)-catechin was used in this experiment. Therefore, these observations allowed us to construct the structure of **4**, including the absolute configuration shown in **Figure 1**.

The production mechanism of **3** and **4** is illustrated in **Figure 3**. It is known that the nucleophilicity of the C-8 position of the catechin A-ring is stronger than that of C-6 (*20*); therefore, C−C bond formation between **2** and **1** first occurs at the C-8 position of **1** to give **1a**. Then, simultaneous dehydration and benzopyran formation produces **1b**. Similar condensation at the C-6 position of **1b** yielded **3**, and reaction between **1b** and **1a** generated the dimer **4**.

To examine as to whether similar reactions occur with procyanidins, next procyanidin B1 (**5**) (**Figure 4**) and **2** were mixed and left to stand at room temperature for 8 days. After removal of the starting material by Sephadex LH-20 column chromatography, the mixture of the reaction products was examined by thiol degradation, MALDI-TOF MS (**Figure 5**), and ^{13}C NMR spectroscopy (**Figure 7**). The thiol degradation of the starting material **5** yielded epicatechin 4-hydroxyethyl thioether (**5a**) arising from the extension (upper) unit of **5** and **1** from the terminal (lower) unit (**Figure 4**). However, the reaction mixture produced by condensation between **5** and **2** did not yield **5a**. Only a small peak attributable to **1** was detected by HPLC analysis, which indicated that **2** also reacted with procyanidins. MALDI-TOF MS (**Figure 5**) showed some prominent peaks arising from the products, and their plausible structures, deduced from **3** and **4**, are shown in **Figure 6**. The results suggested that procyanidins were oligomerized by condensation with **2**. The ^{13}C NMR spectrum of the product mixture (**Figure 7B**) was closely related to that of **5** (**Figure 7C**), and the difference in the two spectra was the appearance of large peaks at δ 128 and small peaks in the range of δ 100−105. These peaks were related to those of the benzene moiety and the A-ring C-8 of **4**, respectively (**Table 1**). Furthermore, the spectrum of the reaction products resembled the ^{13}C NMR spectrum of the polymeric polyphenols separated from commercial cinnamon bark. The separation of the polymeric polyphenols was performed by a combination of absorption chromatography on Sephadex LH-20 with ethanol and gel permeation chromatography on the same Sephadex LH-20 with 7 M urea/acetone as the elution solvent (*16*). Thiol degradation of the polymeric polyphenols yielded **5a** and epicatechin as the major products; however, the ratio of thioether (representing extension units)/epicatechin (representing terminal units) was ca. 2:1, which indicated that the apparent average degree of polymerization was only 3. Although epicatechin trimers (procyanidin

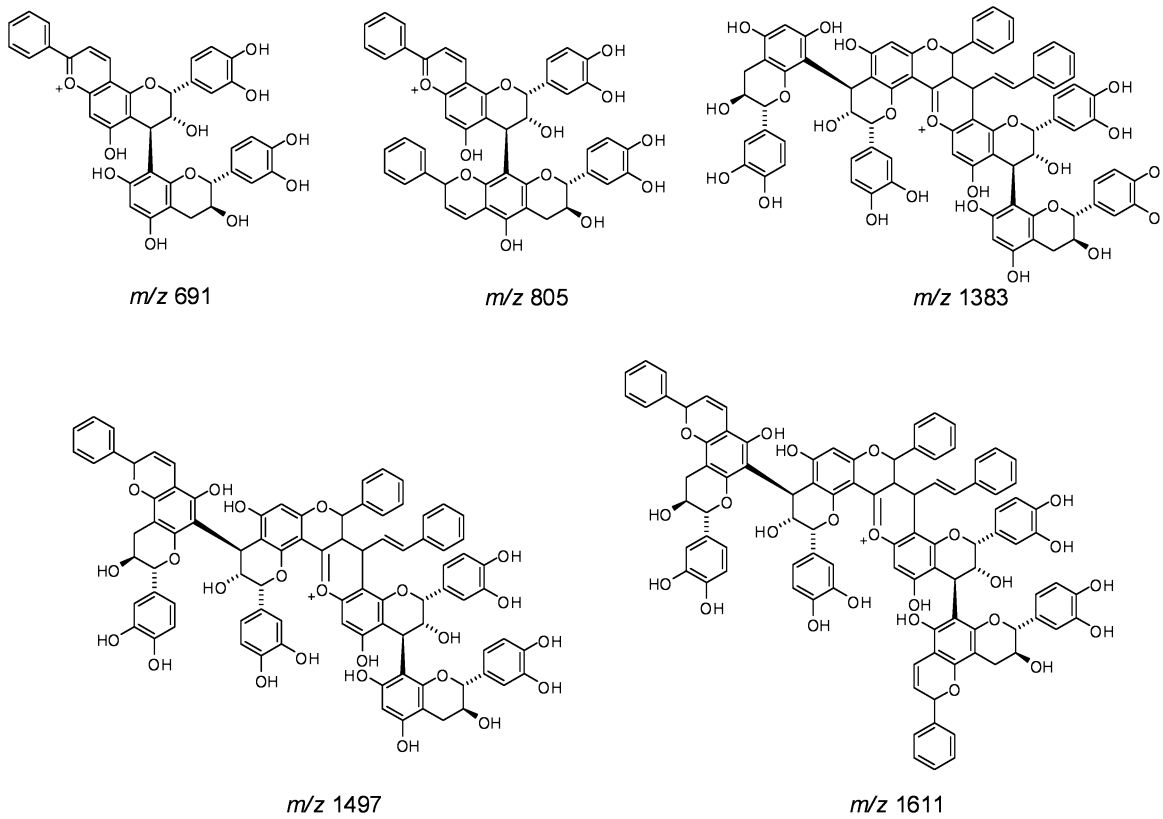

Figure 6. Possible structures of products detected by MALDI-TOF MS.

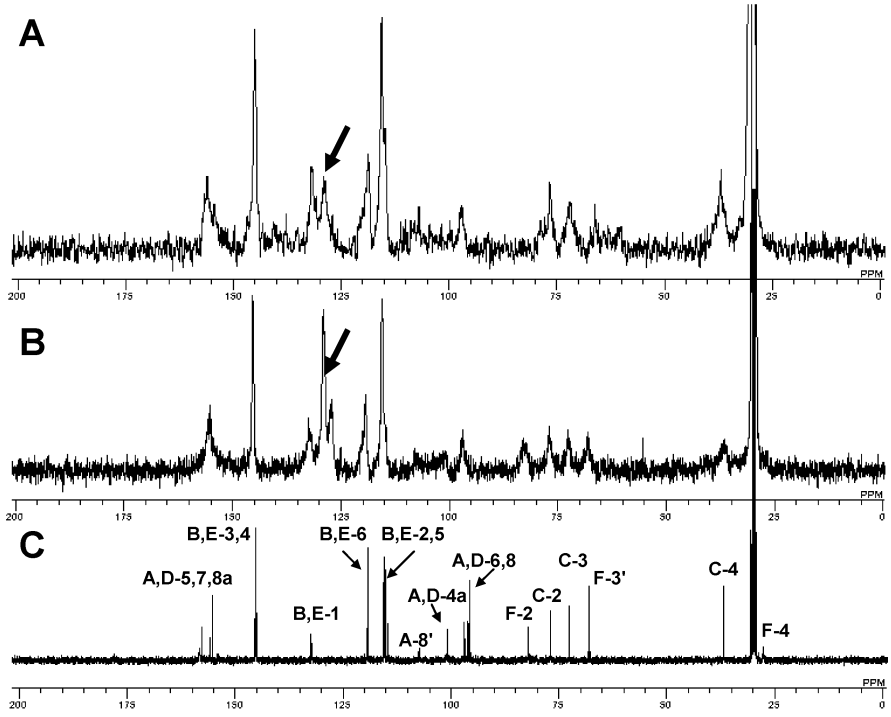

Figure 7. ^{13}C NMR spectra of polymeric polyphenols of cinnamon bark (**A**), mixture of reaction products of procyanidin B1 and cinnamaldehyde (**B**), and procyanidin B1 (**C**). Arrows indicates the peaks due to benzene rings of cinnamaldehyde moieties.

C-1, MW 866) can be detected as a clear spot on TLC and a sharp peak in HPLC, the polymeric polyphenols obtained from this experiment did not show the presence of such molecules. These observations strongly suggested that procyanidin oligomers and polymers of the commercial cinnamon bark were further polymerized by condensation with cinnamaldehyde.

In this study, we demonstrated that catechins and procyanidins reacted with cinnamaldehyde at room temperature to generate condensation products. Similar reactions probably occur during the drying process in production of commercial cinnamon bark. Actually, polymeric procyanidins obtained from commercial cinnamon bark showed ^{13}C NMR signals similar to those of the products generated by reaction of **5** and **2**. In addition, it was strongly suggested that oxidation of the products yielded benzopyrylium pigments, which explains the dynamic color change observed when the fresh bark of Japanese cinnamon

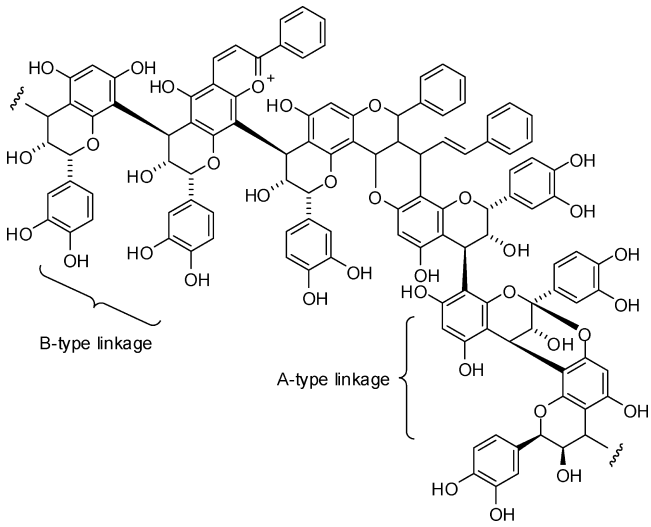

Figure 8. Possible structure of cinnamon polymeric polyphenols.

was peeled off. In that case, oxidation probably was catalyzed by plant enzymes. This may also explain the dark reddish brown color of the polyphenol fractions obtained from commercial cinnamon bark. On the basis of these results, a possible structure of cinnamon polymeric polyphenols is shown in **Figure 8**. A similar reaction of proanthocyanidins with aldehyde is known to occur in persimmon fruits; in that case, polymerization and insolubilization of the proanthocyanidins were caused by reaction with acetaldehyde generated by anaerobic metabolism (*17*). Similar insolubilization seemed to occur in cinnamon bark, and it was demonstrated by the following simple experiment: after repeated extraction of polyphenols from cinnamon bark powder, the remaining plant debris was directly subjected to thiol degradation. HPLC of the reaction mixture showed the production of epicatechin (from terminal units) and **5a** (from extension units), and the ratio (ca. 1:2 based on the peak area) was similar to that observed for degradation products from the extract. Production of polymeric polyphenols may have a significance in plant defense systems. When the polyphenols, such as **3**, were oxidized by the catalysis of enzymes, the oxygen molecules were simultaneously reduced to give reactive oxygen species such as superoxide anion and hydrogen peroxide, which act as antimicrobial agents. Thus, the results of this study are important from the viewpoint of not only food chemistry and pharmacognosy but also plant physiology.

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