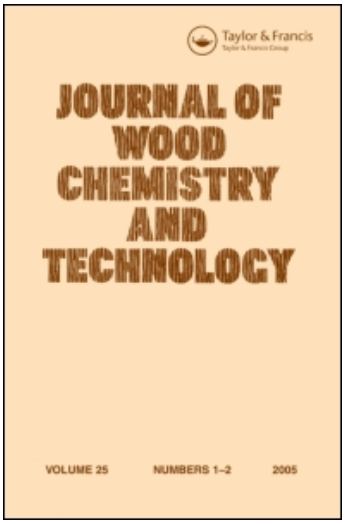

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Base-Catalyzed Reactions of Procyanidin B3: Formation of a Novel Catechinic Acid-Catechin Dimer

Koh Hashida[a]; Seiji Ohara[a]; Rei Makino[a]
[a] Wood Extractives Laboratory, Department of Forest Chemistry, Forestry and Forest Products Research Institute, Ibaraki, Japan

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Base-Catalyzed Reactions of Procyanidin B3: Formation of a Novel Catechinic Acid-Catechin Dimer

Koh Hashida, Seiji Ohara, and Rei Makino
Wood Extractives Laboratory, Department of Forest Chemistry,
Forestry and Forest Products Research Institute, Tsukuba Norin Kenkyu
Danchi-Nai, Ibaraki, Japan

Abstract: Reaction of procyanidin B3 (catechin-(4α-8)-catechin) at pH 12 and 40°C gave a novel doubly-linked catechinic acid-catechin dimer, catechinic acid-(8β-8, 9-*O*-7)-catechin, together with catechinic acid and a catechinic acid stereoisomer. The novel dimer had an unexpected β-interunit linkage, which is contrary to the α-interflavanoid bond of procyanidin B3, indicating that this compound was formed through the cleavage and recombination of the interflavanoid bond. The nature of the reaction products suggests that cleavage of both the interflavanoid bond and the pyran ring occurred and that the interflavanoid bond cleaved prior to the pyran ring.

Keywords: Base-catalyzed reaction, procyanidin B3, pyran ring, interflavanoid bond, catechinic acid, catechinic acid stereoisomer, catechinic acid-catechin dimer

INTRODUCTION

It is well known that condensed tannins have protein-adsorbing capacity and related various biological activities. The protein-adsorbing capacity of condensed tannins is probably improved by opening their pyran rings.[1,2] We have continued a series of studies on base-catalyzed reactions of condensed tannins because these reactions involve opening of the pyran rings.

Address correspondence to Koh Hashida, Wood Extractives Laboratory, Department of Forest Chemistry, Forestry and Forest Products Research Institute, P.O. Box 16, Tsukuba Norin Kenkyu Danchi-Nai, Ibaraki 305-8687, Japan. E-mail: koh@ffpri.affrc.go.jp

We have been studying base-catalyzed reactions of (+)-catechin (**1**, Figure 1) and (−)-epicatechin (**2**, Figure 1), abundant monomer units of condensed tannins. The base-catalyzed reactions of (+)-catechin gives *ent*-epicatechin,[3] catechinic acid (CA),[2,4] catechinic acid stereoisomer (CAS),[2] and diarylpropanol-catechinic acid dimer (DCAD).[5] In the reactions of (−)-epicatechin, the enantiomers of those compounds are formed.[6] These reactions probably proceed through opening of the pyran ring to give the quinone methide intermediates (**3** from **1**, **4** from **2**, Figure 1), followed by intramolecular or intermolecular nucleophilic attack on the quinine methide intermediate. These reactions of (+)-catechin and (−)-epicatechin indicate that the configuration of the hydroxyl group at C-3 should influence the stereoselectivity of the subsequent rearrangement and dimerization reactions.

Procyanidins, which are polymers of (+)-catechin and (−)-epicatechin, are widely distributed in plants such as cacaos,[7] grapes,[8] and conifers (bark).[9,10] There are several reports on base-catalyzed reactions of procyanidins. Reactions of polymeric procyanidins with toluene-α-thiol at pH 12 give benzylthio derivatives of diarylpropanoids, which are the pyran ring opened products of monomer units.[11] Reactions of polymeric procyanidins with phloroglucinol at pH 12 give epicatechin-(4β)-phloroglucinol, CA, and the

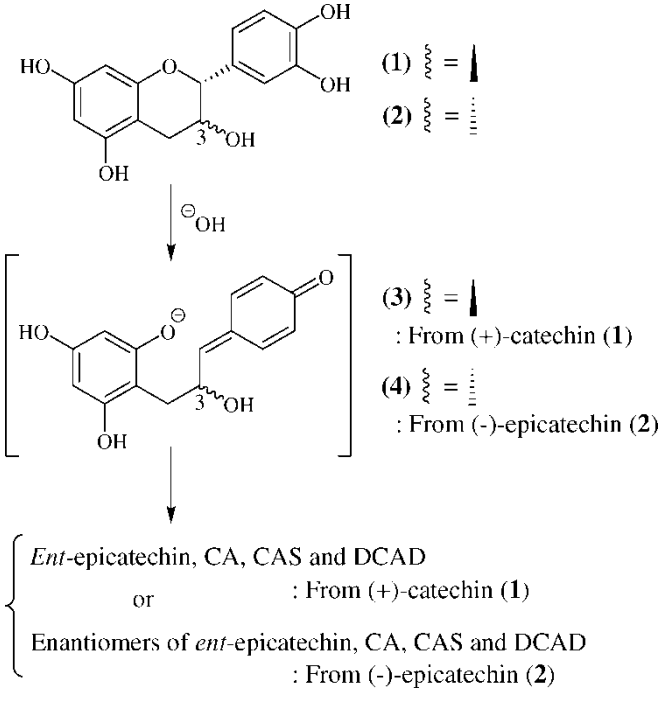

Figure 1. Base-catalyzed reactions of (+)-catechin (**1**) and (−)-epicatechin (**2**).

catechinic acid-type rearrangement products bound to phloroglucinol.[12] These studies indicate that the interflavanoid bond of procyanidins is more labile to cleavage than the pyran ring under alkaline conditions. Mild base-catalyzed reactions (pH 10) of procyanidin B2 and B3 have also been demonstrated to give tetrahydropyrano[2,3-*h*]chromenes and 4-aryl-2-flavanylbenzopyrans in addition to flavanol monomers, (+)-catechin, or (−)-epicatechin.[13,14] Under the mildly basic conditions, procyanidin B2 and B3 are presumably transformed to the B-ring quinone methide intermediate, followed by the migration (transfer reaction) of (+)-catechin, (−)-epicatechin, or phloroglucinol and the subsequent pyran recyclization. However, base-catalyzed reactions of procyanidins at high pH (>10) without any nucleophilic reagent such as toluene-α-thiol and phloroglucinol have not been demonstrated except for the formation of the high-molecular weight phenolics formed from base-catalyzed reactions of pine bark tannins.[15] In this study, the base-catalyzed reactions of procyanidin B3 (PC-B3, catechin-(4α-8)-catechin) at pH 12 and 40°C were investigated to clarify the reaction behavior of procyanidins, especially of the pyran ring and the interflavanoid bond.

RESULTS AND DISCUSSION

Base-catalyzed reaction of PC-B3 (**5,** Figure 2) at pH 12 and 40°C yielded several compounds. Separation of the products by Sephadex LH-20 column chromatography led to three compounds. Two compounds were identical with CA (**6**) and CAS (**7**), respectively, by comparison of FAB-MS and ^{1}H- and ^{13}C-NMR spectra with those of authentic compounds.[2] These compounds should come from the terminal unit of PC-B3. The third compound (**8**) was a novel compound which was not formed from the base-catalyzed reactions of (+)-catechin. The FAB-MS spectrum of **8** showed $[M + H]^+ = 579$, indicating the same molecular weight as the starting material. The ^{1}H- and ^{13}C-NMR spectra of **8** in pyridine-d_5 (shown in Table 1 and 2) resembled those of both CA and (+)-catechin. In addition, a variety of 2D NMR experiments suggested that **8** might consist of an upper CA unit and a terminal catechin unit. However, the carbon signals due to the enolizable β diketone moiety of the upper CA unit (C-2A and C-4A of **8**) could not be detected, and the C-9A signal was shifted far up field compared to the corresponding carbonyl carbon signal of CA (C-9 of **6**). Similar phenomena were observed for the C-2, C-4, and C-9 signals of CAS (**7**),[2] therefore, this compound was expected to have a partial structure similar to CAS.

Because the complete identification of **8** could not be performed in the free phenolic form, structural analyses were done using a methyl ether derivative. Methylation of **8** gave two main products similar to those of CA and CAS. The FAB-MS spectra of both compounds showed the same $[M + H]^+ = 677$, indicating that these were heptamethyl ether derivatives of **8**. From the results of the ^{1}H- and ^{13}C-NMR (Table 1 and 2) and 2D

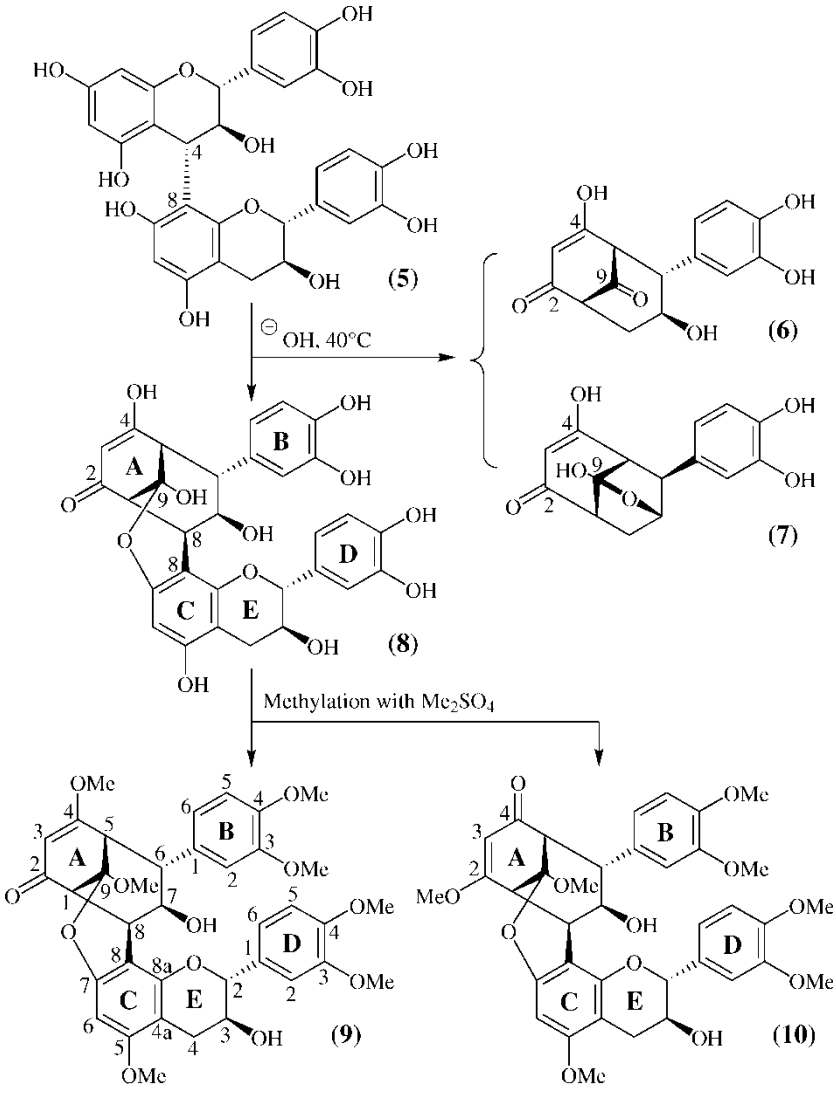

Figure 2. Base-catalyzed reactions of procyanidin B3 (**5**).

NMR experiments (^{13}C-DEPT, ^{1}H-^{1}H COSY, long-range ^{1}H-^{1}H COSY, ^{1}H-^{1}H NOESY, HMQC and HMBC), we identified these compounds as the heptamethyl ethers of catechinic acid-(8β-8, 9-*O*-7)-catechin (**9**) and isocatechinic acid-(8β-8, 9-*O*-7)-catechin (**10**), respectively. The structures of **9** and **10** allowed **8** to be identified as an enolic form of doubly linked CA-catechin dimer, that is, catechinic acid-(8β-8, 9-*O*-7)-catechin.

2D-NMR data for **9** are summarized in Table 3. Details of the NMR assignments for **9** are as follows: The terminal catechin unit was evident in

Table 1. ^{1}H- and ^{13}C-NMR assignments of the upper catechinic acid unit of catechinic acid-catechin dimer (**8**) and its methyl ether derivatives (**9, 10**)

			Methyl ether derivatives of **8**[b]			
	8[a]		**9**		**10**	
Position	^{1}H (ppm, *J* (Hz))	^{13}C (ppm)	^{1}H (ppm, *J* (Hz))	^{13}C (ppm)	^{1}H (ppm, *J* (Hz))	^{13}C (ppm)
1A	3.56 (1H, *m*)	51.35	3.05 (1H, *m*)	47.97	3.07 (1H, *t*, $J = 2.6$)	42.14
2A	—	N.D[c]	—	196.71	—	173.96
CH_3O-2A	—	—	—	—	3.82 (3H, *s*)	56.76
3A	6.11 (1H, *s*)	105.63	5.54 (1H, *s*)	102.78	5.62 (1H, *s*)	104.05
4A	—	N.D.[c]	—	177.09	—	195.00
CH_3O-4A	—	—	3.44 (3H, *s*)	55.76 or 55.78	—	—
5A	3.78 (1H, *m*)	61.91	3.04 (1H, *m*)	55.04	3.09 (1H, *dd*, $J = 4.8, 2.6$)	61.37
6A	3.88 (1H, *dd*, $J = 11.1, 4.8$)	45.78	3.09 (1H, *dd*, $J = 10.5, 4.8$)	43.51	3.01 (1H, *dd*, $J = 11.4, 4.8$)	44.57
7A	5.25 (1H, *dd*, $J = 11.1, 4.8$)	70.05	4.51 (1H, *dd*, $J = 10.5, 4.6$)	69.24	4.59 (1H, *dd*, $J = 11.4, 5.0$)	69.78
8A	4.61 (1H, *t*, $J = 4.8$)	36.75	3.93 (1H, *t*, $J = 4.6$)	35.88	3.88 (3H, *m*)	33.85
9A	—	101.40	—	102.63	—	101.90

(*continued*)

Table 1. Continued

			Methyl ether derivatives of **8**[b]			
	8[a]		**9**		**10**	
Position	^{1}H (ppm, *J* (Hz))	^{13}C (ppm)	^{1}H (ppm, *J* (Hz))	^{13}C (ppm)	^{1}H (ppm, *J* (Hz))	^{13}C (ppm)
CH_3O-9A	—	—	3.35 (3H, *s*)	50.05	3.36 (3H, *s*)	49.97
1B	—	132.37	—	130.63	—	129.78
2B	7.44 (1H, *d*, $J = 2.0$)	117.44	6.61 (1H, *d*, $J = 1.7$)	111.39	6.57 (1H, *d*, $J = 2.0$)	112.28
3B	—	146.75	—	148.92	—	148.83
CH_3O-3B	—	—	3.81 (3H, *s*)	55.76 or 55.78	3.81 (3H, *s*)	55.65 or 55.67
4B	—	145.71	—	148.00	—	147.98
CH_3O-4B	—	—	3.83 (3H, *s*)	55.87	3.80 (3H, *s*)	55.74
5B	7.09 (1H, *d*, $J = 8.1$)	116.01	6.77 (1H, *d*, $J = 8.4$)	111.06	6.75 (1H, *d*, $J = 7.8$)	111.06
6B	6.88 (1H, *dd*, $J = 8.1, 2.0$)	121.38	6.64 (1H, *dd*, $J = 8.4, 1.7$)	119.53	6.58 (1H, *dd*, $J = 7.8, 2.0$)	119.83

[a]Measurement was made in pyridin-d_5.
[b]Measurement was made in $CDCl_3$.
[c]Not detectable.

Table 2. ^{1}H- and ^{13}C-NMR assignments of the terminal catechin unit of catechinic acid-catechin dimer (**8**) and its methyl ether derivatives (**9, 10**)

			Methyl ether derivatives of **8**[b]			
	8[a]		**9**		**10**	
Position	^{1}H (ppm, *J* (Hz))	^{13}C (ppm)	^{1}H (ppm, *J* (Hz))	^{13}C (ppm)	^{1}H (ppm, *J* (Hz))	^{13}C (ppm)
2E	5.01 (1H, *d*, *J* = 9.2)	83.61	4.58 (1H, *d*, *J* = 8.9)	82.00	4.61 (1H, *d*, *J* = 9.0)	82.01
3E	4.56 (1H, *td*, *J* = 9.2, 5.9)	67.99	4.05 (1H, *td*, *J* = 8.9, 5.6)	68.05	4.20 (1H, *td*, *J* = 9.0, 5.8)	67.96
4E		31.24		27.95		27.96
4E ax	3.19 (1H, *dd*, *J* = 16.1, 9.2)		2.61 (1H, *dd*, *J* = 16.3, 8.9)		2.61 (1H, *dd*, *J* = 16.3, 9.0)	
4E eq	3.76 (1H, *dd*, *J* = 16.1, 5.9)		3.11 (1H, *dd*, *J* = 16.3, 5.6)		3.13 (1H, *dd*, *J* = 16.3, 5.8)	
4aC	—	102.94	—	102.29	—	102.27
5C	—	156.92	—	158.10	—	158.20
CH_3O-5C	—	—	3.86 (3H, *s*)	55.61	3.87 (3H, *s*)	55.65 or 55.67
6C	6.76 (1H, *s*)	95.31	6.29 (1H, *s*)	91.63	6.30 (1H, *s*)	91.77
7C	—	155.47	—	154.49	—	154.49
8C	—	102.39	—	101.33	—	100.78
8aC	—	154.95	—	152.15	—	152.17
1D	—	131.65	—	129.29	—	129.46

(*continued*)

Table 2. Continued

			Methyl ether derivatives of **8**[b]			
	8[a]		**9**		**10**	
Position	^{1}H (ppm, *J* (Hz))	^{13}C (ppm)	^{1}H (ppm, *J* (Hz))	^{13}C (ppm)	^{1}H (ppm, *J* (Hz))	^{13}C (ppm)
2D	7.59 (1H, *d*, *J* = 2.0)	116.60	6.87 (1H, *d*, *J* = 2.0)	110.03	6.90 (1H, *d*, *J* = 1.8)	110.21
3D	—	147.08	—	149.30	—	149.31
CH_3O-3D	—	—	3.90 (3H, *s*)	55.99 or 56.02	3.89 (3H, *s*)	55.98
4D	—	147.31	—	149.59	—	149.64
CH_3O-4D	—	—	3.90 (3H, s)	55.99 or 56.02	3.90 (3H, *s*)	55.98
5D	7.16 (1H, *d*, *J* = 7.8)	116.29	6.88 (1H, *d*, *J* = 8.5)	111.34	6.89 (1H, *d*, *J* = 8.4)	111.29
6D	7.19 (1H, *dd*, *J* = 7.8, 2.0)	120.11	6.92 (1H, *dd*, *J* = 8.5, 2.0)	119.99	6.96 (1H, *dd*, *J* = 8.4, 1.8)	120.11

[a]Measurement was made in pyridin-d_5.
[b]Measurement was made in $CDCl_3$.

Table 3. 2D NMR data of the methyl ether of catechinic acid-catechin dimer (**9**)

Position	Cross peaks in 2D NMR spectra[a]			
	^{1}H-^{1}H COSY	Long-range ^{1}H-^{1}H COSY	^{1}H-^{1}H NOESY	HMBC
H-1A	H-8A	H-3A	H-8A, C$\underline{H}_3O$-9A	C-2A, 5A, 7A, 8A, 9A
H-3A	—	H-1A, 5A	H-7A, C$\underline{H}_3O$-4A	C-1A, 2A, 4A, 5A
C$\underline{H}_3O$-4A	—	—	H-3A,5A,2B,6B, C$\underline{H}_3O$-3B	C-4A
H-5A	H-6A	—	H-2B, 6B, C$\underline{H}_3O$-4A, 9A	C-1A, 4A, 6A, 7A, 9A
H-6A	H-5A, 7A	—	H-7A, 2B, 6B	C-4A,5A,7A,8A,1B,2B,6B
H-7A	H-6A, 8A	—	H-3A, 6A, 8A, 2B, 6B	C-6A, 1B, 8C
H-8A	H-1A, 7A	H-6C	H-1A, 7A	C-1A,6A,7A,9A,7C,8C,8aC
C$\underline{H}_3O$-9A	—	—	H-1A, 5A	C-9A
H-2B	—	C$\underline{H}_3O$-3B	H-5A,6A,7A, C$\underline{H}_3O$-4A,3B	C-6A, 1B, 3B, 4B, 6B
C$\underline{H}_3O$-3B	—	H-2B	H-2B, C$\underline{H}_3O$-4A	C-3B
C$\underline{H}_3O$-4B	—	H-5B	H-5B	C-4B
H-5B	H-6B	C$\underline{H}_3O$-4B	C$\underline{H}_3O$-4B	C-1B, 3B, 4B, 6B
H-6B	H-5B	—	H-5A, 6A, 7A, C$\underline{H}_3O$-4A	C-6A, 2B, 4B, 5B
H-2E	H-3E	H-2D, 6D	H-3E, 2D, 6D	C-3E, 4E, 8aC, 1D, 2D, 6D
H-3E	H-4ax,eqE, 2E	—	H-2E, 4ax, eqE, 2D	C-2E, 1D
H-4axE	H-4eqE, 3E	—	H-4eqE, 3E	C-2E, 3E, 4aC, 5C, 8aC
H-4eqE	H-4axE, 3E	—	H-4axE, 3E	C-2E, 3E, 4aC, 5C, 8aC
C$\underline{H}_3O$-5C	—	H-6C	H-6C	C-5C
H-6C	—	H-8A, C$\underline{H}_3O$-5C	C$\underline{H}_3O$-5C	C-8A, 4E, 4aC, 5C, 7C, 8C
H-2D	—	H-2E, C$\underline{H}_3O$-3&4D	H-2E, 3E, C$\underline{H}_3O$-3&4D	C-2E, 1D, 3D, 4D, 6D
C$\underline{H}_3O$-3&4D	—	H-2D, 5D	H-2D, 5D	C-3D, 4D
H-5D	H-6D	C$\underline{H}_3O$-3&4D	C$\underline{H}_3O$-3&4D	C-1D, 3D, 4D, 6D
H-6D	H-5D	H-2E	H-2E	C-2E, 2D, 4D

[a]Cross peaks indicate ^{1}H- and ^{13}C-signals, which are correlated with each proton of **9**.

the ^{1}H-NMR spectrum (H-2E, 3E, 4axE, 4eqE, 6C, 2D, 5D, 6D). This interpretation was supported by the ^{1}H-^{1}H COSY and long-range ^{1}H-^{1}H COSY spectra. The cross peaks in the HMQC spectrum permitted assignments of the methylene (C-4E) and tertiary carbons (C-2E, 3E, 6C, 2D, 5D, and 6D), which were connected to each proton, respectively. The quaternary carbon signals (C-4aC, 5C, 7C, 8C, 8aC, 1D, 3D, 4D) were assigned by consideration of the HMBC spectrum. Because the H-2E, H-4axE and H-4eqE were correlated with the quaternary carbon signal at 152.15 ppm in the HMBC spectrum, this carbon signal was assigned to C-8aC. The quaternary carbon signal at 158.10 ppm was also assigned to C-5C because of the correlation with H-4axE and H-4eqE in the HMBC spectrum. Assignment of the singlet proton signal at 6.29 ppm to H-6C was confirmed by the fact that this signal correlated with C-5C, not with C-8aC in the HMBC spectrum.

Methyl groups were introduced to phenolic hydroxyl groups at 3D and 4D because the ^{1}H-^{1}H NOESY spectrum showed association of the methoxyl proton signals ($C\underline{H}_3O$-3D, 4D) with each adjacent proton signal and because the HMBC spectrum exhibited correlations between these methoxyl protons and the quaternary carbons (C-3D and 4D, respectively). The HMQC spectrum then permitted assignments of the methoxyl carbon signals ($\underline{C}H_3O$-3D, 4D). Other methoxyl proton signal at 3.86 ppm correlated with H-6C in the ^{1}H-^{1}H NOESY spectrum and with C-5C in the HMBC spectrum. Thus, this signal was assigned to $C\underline{H}_3O$-5C, and then the carbon signal at 55.61 ppm was assigned to $\underline{C}H_3O$-5C by connectivity to $C\underline{H}_3O$-5C in the HMQC spectrum. The phenolic hydroxyl group at C-7C was considered to be bonded to the other moiety in the molecule because the methoxyl proton and carbon signals at C-7C were not observed.

The tertiary carbon signal at 35.88 ppm was considered to be C-8A because of the correlation with the H-6C in the HMBC spectrum. The HMQC experiment then permitted assignment of the proton signal at 3.93 ppm (H-8A). The H-8A was correlated with the H-6C in the long-range ^{1}H-^{1}H COSY spectrum; the couplings of the H-8A to the carbon signals due to C-7C, 8C, and 8aC was evident in the HMBC spectrum. These results indicate that C-8A is bonded to C-8C of the terminal catechin unit. The ^{1}H-^{1}H COSY experiment showed association of H-8A with the proton signals at 3.05 (H-1A) and 4.51 ppm (H-7A). This experiment also revealed that H-7A was coupled with the proton signal at 3.09 ppm (H-6A) and that H-6A was coupled with the other proton signal at 3.04 ppm (H-5A). The singlet proton signal at 5.54 ppm was assigned to H-3A by connectivity to H-1A and H-5A in the long-range ^{1}H-^{1}H COSY spectrum. Assignments of the tertiary carbons (C-1A, 3A, 5A, 6A, 7A, 8A) were then possible by consideration of the HMQC spectrum. The quaternary carbon signal at 102.63 ppm showed cross peaks with H-1A and H-5A in the HMBC spectrum; therefore, this carbon was assigned to C-9A. The HMBC spectrum also showed correlations between H-3A and the quaternary carbon signals at 177.09 and 196.71 ppm, between H-1A and the carbon signal at

196.71 ppm, and between H-5A and the carbon signal at 177.09 ppm. Thus, the carbons at 177.09 and 196.71 ppm could be assigned to C-4A and C-2A, respectively.

The aromatic portion of the ^{1}H-NMR spectrum showed three proton signals that could be assigned to H-2B, H-5B, and H-6B of another catechol ring. The assignments of the tertiary (C-2B, 5B, 6B) and the quaternary (C-1B, 3B, 4B) carbons of this catechol ring were accomplished by consideration of the HMQC and the HMBC spectra. The H-6A signal was correlated with C-1B, C-2B, and C-6B, and the H-2B, and the H-6B signals were correlated with C-6A in the HMBC spectrum. These results indicate that the catechol ring is linked to the C-6A position.

Besides three groups of methoxyl protons in the terminal catechin unit, four methoxyl groups were evident in the ^{1}H-NMR spectrum. Considering the ^{1}H-^{1}H NOESY and HMBC spectra, which exhibited cross peaks of these methoxyl proton signals with each adjacent proton and with each quaternary carbon attached to the methoxyl groups, these methoxyl protons were assigned to C$\underline{H}_3O$-4A, 9A, 3B, and 4B. Assignment of the methoxyl carbons ($\underline{C}H_3O$-4A, 9A, 3B, and 4B) was then possible through correlation with each methoxyl proton in the HMQC spectrum.

The aforementioned ^{13}C-NMR data of the upper unit of **9** were similar to those of the methyl ether derivative of CA except for the quaternary C-9A. The C-9A signal was shifted far up field compared to the carbonyl carbon of CA (C-9 of **6**), indicating that the quaternary C-9A was bonded to the other moiety in the molecule similar to CAS.[2] As described earlier, the C-7C was also considered to be bonded to the other moiety in the molecule. Therefore, it has been assumed that C-9A is bonded to C-7C by an ether linkage.

In the same manner, the structure of **10** could be confirmed to be the heptamethyl ether of isocatechinic acid-(8β-8, 9-*O*-7)-catechin. Therefore, it is assumed that **8** is a mixture of keto and enol tautomers similar to CA and CAS,[2,4] because methylation of **8** gives **9** and **10**.

The stereochemistry of the upper unit of **8** is discussed on the basis of the proton spin-spin coupling constants.[16] The coupling constants of **8** were similar to those of the methyl ether derivatives (**9**, **10**), meaning that their conformations resembled each other. The proton spin-spin coupling constants of the upper unit of **8** are shown in Figure 3. The coupling constants of $J_{1,8}$, $J_{7,8}$, and $J_{5,6}$ are 4.8 Hz, suggesting that these vicinal protons are in the equatorial-equatorial or axial-equatorial position in the cyclohexane ring. The large coupling constant of $J_{6,7}$ (11.1 Hz) indicates that these protons are in the axial-axial position. The proposed configuration and conformation of the upper unit of **8** is depicted in Figure 3. Theoretical analysis of the stereostructure for **8** was done using the MMFF and the semi-empirical AM-1 method of MacSpartan Pro software to obtain the lowest-energy conformation and the equilibrium geometry (Figure 4). The optimized stereostructure showed dihedral angles of 61.5°, 56.8°, 177.9°, and 59.1° for H-1A/H-8A,

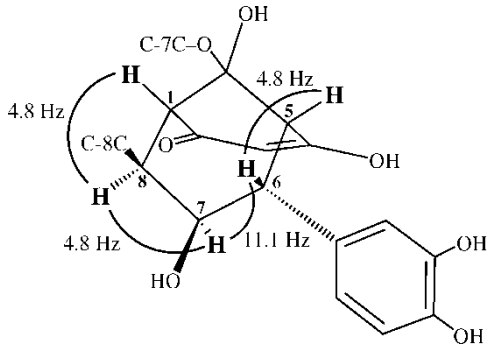

Figure 3. The proton spin-spin coupling constants of the upper unit of catechinic acid-catechin dimer (**8**).

H-7A/H-8A, H-6A/H-7A, H-5A/H-6A, respectively, and these values were approximately equal to those calculated from the observed coupling constants by the vicinal Karplus correlation.

Proposed mechanisms for the base-catalyzed reactions of PC-B3 are shown in Figure 5. Considering the formation of CA (**6**) and CAS (**7**), it is assumed that at first, the interflavanoid bond of PC-B3 (**5**) is cleaved to give the A-ring quinone methide intermediate (**11**) from the upper unit and (+)-catechin (**1**) from the terminal unit.[11,12] Then, the catechin is converted to the B-ring quinone methide intermediate through opening of the pyran ring, followed by the intramolecular rearrangements to form CA (**6**) and CAS (**7**). The novel catechinic acid-catechin dimer (**8**) had an unexpected 8β-8 interunit linkage contrary to the 4α-8 one of the starting material (**5**). Thus, it is assumed that catechin-(4β-8)-catechin (**12**), a stereoisomer of PC-B3 at the C-4 position of the upper unit, is formed as an intermediate

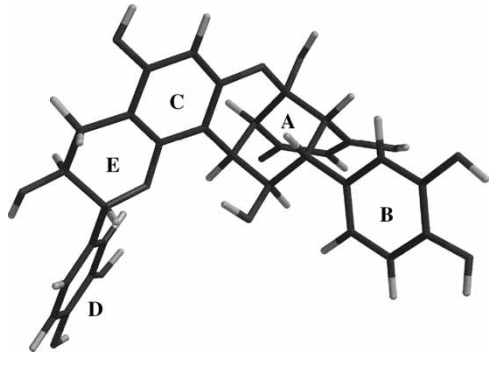

Figure 4. The optimized stereostructure of catechinic acid-catechin dimer (**8**) calculated by the MMFF and the semi-empirical AM-1 method.

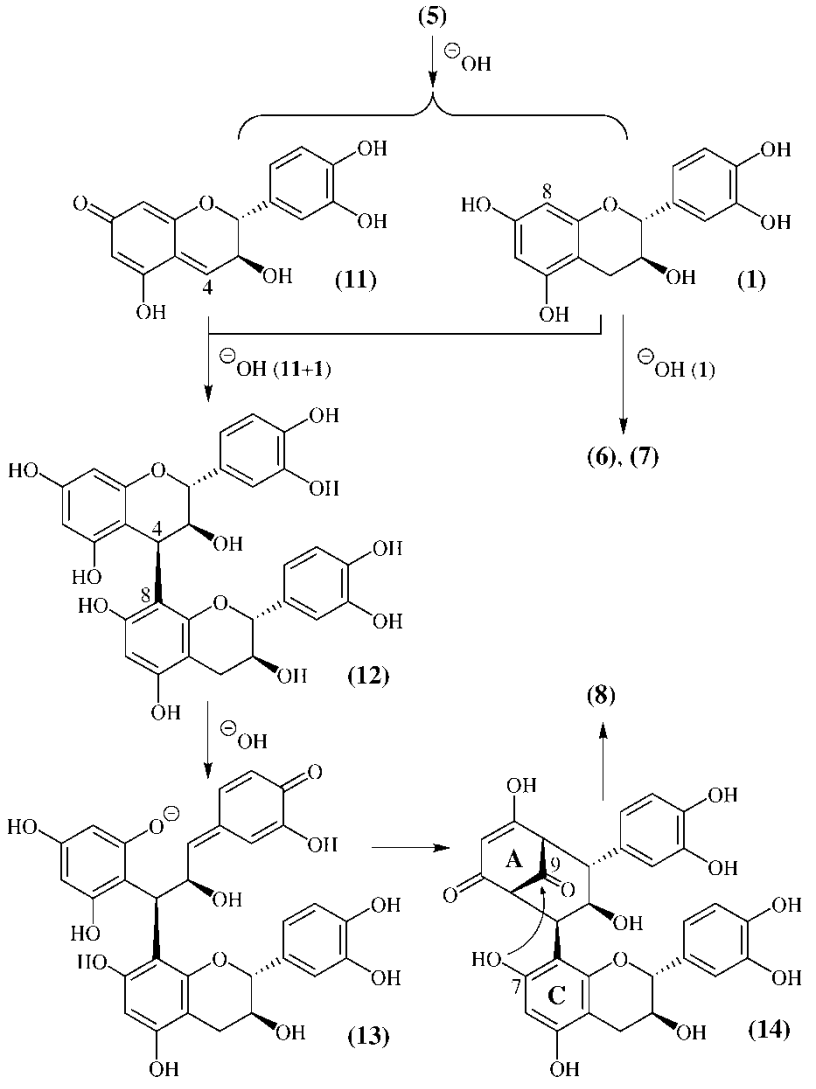

Figure 5. Proposed mechanisms for the base-catalyzed reactions of procyanidin B3.

product through the cleavage of interflavanoid bond and the subsequent recondensation between C-8 of **1** and another face at C-4 of **11**. Furthermore, the quinone methide structure (**13**) is formed at alkaline pH, followed by the rearrangement to **14** in manner similar to base-catalyzed reactions of (+)-catechin. In **14**, it is possible for the hydroxyl group at C-7C to get close to the carbonyl carbon at C-9A. Therefore, the hemiacetal linkage between C-9A and C-7C is formed in analogy with that of CAS[2] to give the

doubly linked catechinic acid-catechin dimer (**8**). The proposed reaction mechanisms for the base-catalyzed reactions of PC-B3 show that cleavage of both the interflavanoid bond and the pyran ring occurred and that the interflavanoid bond cleaved prior to the pyran ring, as is the case for the base-catalyzed reactions of polymeric procyanidins with nucleophiles reported by Laks et al.[11,12]

EXPERIMENTAL

NMR spectra were recorded on a JEOL ALPHA-500 spectrometer. FAB-MS spectra were obtained using a JEOL HX-110A spectrometer.

Synthesis of Procyanidin B3 (5)

PC-B3 was synthesized as described by Botha et al.[17] (+)-Taxifolin (1,014.0 mg) was treated with $NaBH_4$ and then reacted with (+)-catechin (4,013.0 mg) under acidic conditions. The reaction product was purified by Sephadex LH-20 column chromatography eluted with EtOH to obtain PC-B3 (1039.8 mg). FAB-MS m/z: 579 $[M+H]^+$. ^{1}H- and ^{13}C-NMR spectral data (in acetone-d_6) were in agreement with the literature.[18]

Base-Catalyzed Reaction of Procyanidin B3 (5)

The base-catalyzed reaction of PC-B3 (500.4 mg) at pH 12 and 40°C was carried out with the same procedure used previously for (+)-catechin.[2,5] The reaction products (442.9 mg) were applied to a Sephadex LH-20 column and eluted with EtOH to give two fractions (fraction I, II). Fraction I was further separated by Sephadex LH-20 column chromatography eluted with MeOH-H_2O (1:1, v/v) to give catechinic acid (**6**, 92.4 mg) and catechinic acid stereoisomer (**7**, 3.4 mg). These compounds were identified by comparison of FAB-MS, ^{1}H- and ^{13}C-NMR spectra with those of authentic catechinic acid and catechinic acid stereoisomer.[2] Fraction II was further purified by Sephadex LH-20 column chromatography eluted with MeOH-H_2O (1:1, v/v) to give 12.5 mg of **8**.

Methylation of Catechinic Acid-Catechin Dimer (8)

Methylation of catechinic acid-catechin dimer (11.6 mg) was done by the same method as described previously.[2] Two main components were detected by silica gel TLC developed with benzene-EtOH-H_2O-AcOH

(200:47:15:1, v/v/v/v, upper phase). They were separated by preparative TLC to give heptamethyl ether of catechinic acid-catechin dimer (**9**) (4.5 mg, more mobile on TLC) and heptamethyl ether of isocatechinic acid-catechin dimer (**10**) (2.5 mg).

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