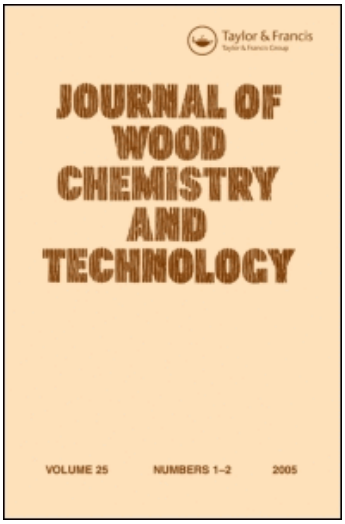

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

FORMATION OF A NOVEL CATECHINIC ACID STEREOISOMER FROM BASE-CATALYZED REACTIONS OF ( )-CATECHIN

Koh Hashida[a]; Seiji Ohara[a]
[a] Forestry and Forest Products Research Institute, Ibaraki, Japan

Online publication date: 06 December 2002

To cite this Article Hashida, Koh and Ohara, Seiji(2002) 'FORMATION OF A NOVEL CATECHINIC ACID STEREOISOMER FROM BASE-CATALYZED REACTIONS OF ( )-CATECHIN', Journal of Wood Chemistry and Technology, 22: 1, 11 — 23
To link to this Article: DOI: 10.1081/WCT-120004431
URL: http://dx.doi.org/10.1081/WCT-120004431

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY, 22(1), 11–23 (2002)

FORMATION OF A NOVEL CATECHINIC ACID STEREOISOMER FROM BASE-CATALYZED REACTIONS OF (+)-CATECHIN

Koh Hashida* and Seiji Ohara

Forestry and Forest Products Research Institute
Tsukuba Norin Kenkyu Danchi-Nai,
Ibaraki 305-8687, Japan

ABSTRACT

A novel catechinic acid stereoisomer, 6-(3,4-dihydroxyphenyl)-7,9-epoxy-9-hydroxy-bicyclo[3.3.1]nona-2,4-dione was isolated from base-catalyzed reaction products of (+)-catechin at pH 12 and 40°C. The 6S absolute configuration of this compound has been confirmed from its ^{1}H-NMR coupling constants, while the 6R configuration was previously reported for catechinic acid. The formation of a novel catechinic acid stereoisomer shows that the rearrangement of (+)-catechin to catechinic acid through the quinone–methide intermediate is not stereospecific but stereoselective.

Key Words: (+)-Catechin; Base-catalyzed reaction; Catechinic acid; Catechinic acid stereoisomer

*Corresponding author. Fax: +81-0298-73-3797; E-mail: koh@ffpri.affrc.go.jp

INTRODUCTION

It is well known that vegetable tannins have various functions such as protein-adsorbing capacity,[1] antioxidative,[2] antifungal[3,4] and antiviral[5] properties and inhibitory effects on enzymes.[6,7] From a structural point of view, they are divided into two major groups, condensed tannins and hydrolyzable tannins. Condensed tannins are of widespread existence in plants and present in large concentrations in the bark of timber species such as conifers, *Salix* species, and *Acacia* species.[8–10] However, protein-adsorbing capacity of condensed tannins are generally not as high as hydrolyzable tannins because their rotational degree of freedom is smaller due to the presence of pyran-rings in the molecules.[1] Therefore, it can be assumed that the development of the method for opening the pyran-rings leads to an improvement of the protein-adsorbing capacity and the various functions of condensed tannins.

We have been studying base-catalyzed reactions of (+)-catechin, which is an abundant monomer unit of condensed tannins, in order to obtain the pyran-ring opened products. On the base-catalyzed reactions of (+)-catechin **(1)**, several investigations have been reported (Figure 1). In the reactions at 25°C, *ent*-epicatechin **(3)** is formed by epimerization at the C-2 position of pyran-ring, whereas catechinic acid **(4)** is obtained in high yield in the reactions at 100°C.[11,12] These reactions are supposed to proceed through opening of the pyran-ring to give the quinone–methide intermediate **(2)**, followed by reclosure of the pyran-ring or by nucleophilic attack of carbanion at the C-8 position on the C-2 position of **(2)**. On the other hand, a radical reaction mechanism is also proposed because these reactions are inhibited by the exclusion of oxygen.[13] We previously showed that at intermediate temperature (40°C), diarylpropanol-catechinic acid dimer **(5)** is produced together with catechinic acid.[14] Although several other compounds are produced in low yield on that condition, we have not yet identified them. In this study, the reactions of (+)-catechin at pH 12 and 40°C were investigated further to clarify the reaction mechanisms.

RESULTS AND DISCUSSION

Treatment of (+)-catechin at pH 12 and 40°C for 24 h gave several compounds. One of the main products was identical with catechinic acid **(4)**. The ^{1}H-NMR spectra of its methyl ether derivatives were in agreement with those of 4,3′,4′-tri-*O*-methylcatechinic acid and 2,3′,4′-tri-*O*-methylisocatechinic acid, as reported by Sears et al.[12] This identification has made possible the assignments of the ^{1}H- and ^{13}C-NMR data of catechinic acid

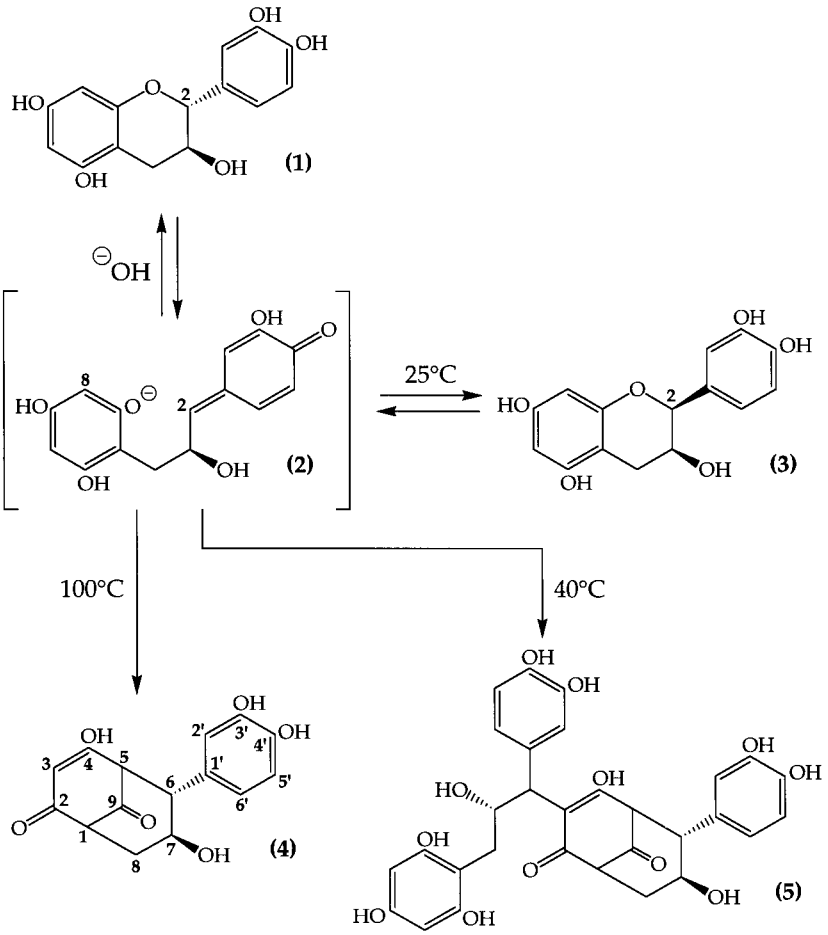

Figure 1. Base catalyzed reactions of (+)-catechin at pH 12.

in prydine-d_6, shown here for the first time. Another main product was identified as diarylpropanol-catechinic acid dimer by comparison of the NMR and FAB-MS spectra of its methyl ether derivative with those in the literature.[14]

In addition to these two main products, a small amount of a novel compound was isolated. On two-dimensional cellulose TLC, this compound gave little color initially after spraying vanillin-HCl reagent but it then slowly changed to an intense yellow, which was similar to the phenomenon observed for catechinic acid by Laks.[15] The FAB-MS spectrum showed

$[M + H]^+ = 291$, indicating this compound has the same molecular weight as catechinic acid. The ^{1}H- and ^{13}C-NMR spectra of this compound showed 10 proton and 15 carbon signals, and the chemical shifts were partially similar to those of catechinic acid. The NMR chemical shift assignments (Table 1), determined by ^{13}C-DEPT and a variety of two-dimensional NMR experiments (^{1}H–^{1}H COSY, long-range ^{1}H–^{1}H COSY, HSQC and HMBC), allowed this compound to be identified as a novel catechinic acid stereoisomer, 6-(3,4-dihydroxyphenyl)-7,9-epoxy-9-hydroxy-bicyclo[3.3.1]nona-2,4-dione **(6,7)** (Figure 2). Details of the interpretation of NMR results are as follows:

The ^{13}C-DEPT spectrum showed only one methylene carbon signal at 39.65 ppm (C-8). Two proton signals at δ 2.04 and 2.63 ppm were assigned to H-8a and H-8b, respectively, by connectivity to the methylene carbon in the HSQC experiment. The proton signal (H-1) appeared as a multiplet at 3.29 ppm with $J = 12.7$, 4.2 Hz from coupling to H-8b, H-8a and a small coupling of 2.1 Hz due to long-range coupling to H-5 at 3.49 ppm. The ^{1}H–^{1}H COSY experiment then permitted assignment of the proton resonances at 3.46 (H-6) and 4.60 ppm (H-7). The singlet proton at 5.83 ppm could be assigned to H-3 because of the association of this proton with the resonances at 3.29 (H-1) and 3.49 ppm (H-5) observed in the long-range ^{1}H–^{1}H COSY spectrum. Assignment of the tertiary carbons, i.e., C-1, C-3, C-5, C-6 and C-7, was then possible by consideration of the HSQC spectrum. For the assignments of the quaternary carbons, the HMBC experiment was essential. In the HMBC spectrum, the H-1 and H-5 signals exhibited cross peaks to the carbon signal at 111.60 ppm, therefore, this carbon signal was assigned to C-9. Also the H-3 signal at 5.83 ppm showed cross peaks with the broad carbon signals at 191.3 and 189.0 ppm. The signal at 191.3 ppm was assigned to C-2 by consideration of the HMBC spectrum showing correlation between this carbon signal and H-8a, H-8b. Another broad signal at 189.0 ppm was assigned to C-4 by connectivity to H-6 in the same spectrum.

Although the ^{1}H–^{1}H COSY experiment did not show correlation between H-6 and H-7, correlation between H-6 and C-7 and between H-8a, H-8b and C-6 was observed in the HMBC experiment. The HMBC spectrum also showed correlation between H-7 and C-9 indicating the formation of an ether linkage between C-7 and C-9. This interpretation is furthermore confirmed by the results of the ^{13}C-NMR chemical shifts. The C-7 signal (79.82 ppm) shifted about 13 ppm downfield compared to the corresponding carbon signal of catechinic acid (66.24 ppm), and the C-9 signal was observed at 111.60 ppm instead of the carbonyl carbon at 206.16 ppm observed for catechinic acid.

Table 1. ^{1}H- and ^{13}C-NMR Chemical Shifts and the Results of Two Dimensional NMR Experiments of Catechinic Acid Stereoisomer (**6**, **7**)

	Chemical Shifts			Cross Peaks in Two Dimensional NMR Spectra[a]		
	^{1}H		^{13}C			
Position	δ (ppm)	*J* (Hz)[b]	δ (ppm)	^{1}H–^{1}H COSY[b]	Long-Range ^{1}H–^{1}H COSY	HMBC
1	3.29 (*m*)	12.7, 4.2, 2.1	48.78	H-8b, 8a, 5	H-3, 7	C-8, 9
2	–	–	191.3	–	–	–
3	5.83 (*s*)	–	99.88	–	H-1, 5	C-1, 2, 4, 5
4	–	–	189.0	–	–	–
5	3.49 (*dd*)	4.5, 2.1	59.57	H-6, 1	H-3	C-1, 3, 6, 9, 1′
6	3.46 (*d*)	4.5	57.54	H-5	H-8a, 8b, 2′, 6′	C-4, 5, 7, 8, 9, 1′, 2′, 6′
7	4.60 (*d*)	5.7	79.82	H-8b	H-1	C-1, 5, 8, 9, 1′
8a	2.04 (*dd*)	12.7, 4.2	39.65	H-8b, 1	H-6, 7	C-1, 2, 6, 7, 9
8b	2.63 (*td*)	12.7, 5.7	39.65	H-8a, 1, 7	H-6	C-1, 2, 6, 7, 9
9	–	–	111.60	–	–	–
1′	–	–	137.52	–	–	–
2′	7.67 (*d*)	2.0	115.91	H-6′	H-6, 5′	C-6, 1′, 3′, 4′, 6′
3′	–	–	147.37	–	–	–
4′	–	–	146.06	–	–	–
5′	7.16 (*d*)	8.0	116.42	H-6′	H-2′	C-1′, 3′, 4′
6′	7.11 (*dd*)	8.0, 2.0	119.00	H-5′, 2′	H-6	C-6, 2′, 4’

[a]Cross peaks indicate ^{1}H- and ^{13}C-signals which are correlated with each proton of (**6**, **7**).
[b]The *J* values are related to the ^{1}H–^{1}H COSY cross peaks given on the same line, respectively.

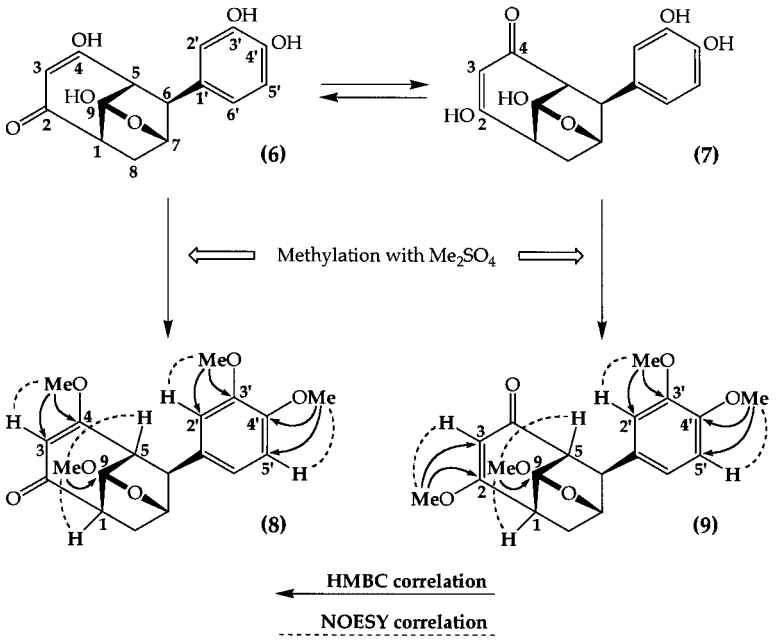

Figure 2. Proposed structures of catechinic acid stereoisomer and its methyl ether derivatives. HMBC- and NOESY-correlations from methoxyl protons are shown for the methyl ether derivatives.

One catechol ring was evident in the aromatic portion of the ^{1}H-NMR spectrum. Three proton signals, the doublet at 7.67 ppm with $J = 2.0$ Hz, the doublet at 7.16 ppm with $J = 8.0$ Hz and the double doublet at 7.11 ppm with $J = 8.0$, 2.0 Hz, were assigned to H-2′, H-5′ and H-6′, respectively. The HSQC experiment then permitted assignment of the carbon resonances at 115.91 (C-2′), 116.42 (C-5′) and 119.00 (C-6′) ppm. The quaternary carbon signal at 137.52 ppm was assigned to C-1′ by connectivity to the aliphatic protons (H-5, 6 and 7) as well as to H-5′ in the HMBC spectrum. The HMBC experiment also permitted assignment of the other quaternary carbons, C-3′ (147.37 ppm) and C-4′ (146.06 ppm), through the association of these carbon signals with H-5′ and H-6′, respectively. The H-2′ and H-6′ signals were correlated with H-6 in the long-range ^{1}H–^{1}H COSY spectrum, and the H-6 signal was correlated with C-1′ in the HMBC spectrum. These results indicate that the catechol ring is linked to the C-6 position.

The stereochemistry of catechinic acid stereoisomer is discussed on the basis of the proton spin-spin coupling constants[16] (Figure 3). The values of

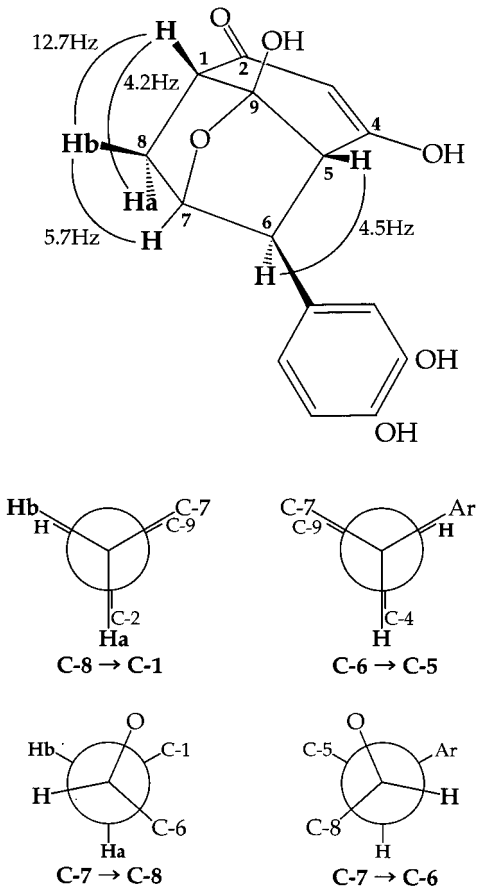

Figure 3. The proton spin-spin coupling constants of catechinic acid stereoisomer and its newman projections.

$J_{1,8a}$ (4.2 Hz), $J_{7,8b}$ (5.7 Hz) and $J_{5,6}$ (4.5 Hz) suggest that each dihedral angles are around $30 \sim 60^\circ$ or $110 \sim 140^\circ$. The large coupling constant of $J_{1,8b}$ (12.7 Hz) indicates that the corresponding dihedral angle is close to 0 or 180°. Furthermore, the dihedral angles between H-6 and H-7, and between H-7 and H-8a are estimated to be $80 \sim 90^\circ$ because both values of $J_{6,7}$ and $J_{7,8a}$ are about 0 Hz. These results permit a conclusion that the configuration of catechinic acid stereoisomer is as shown in Figure 3. The 6S configuration for this compound differs from the 6R configuration for catechinic acid reported by Sears.[12]

To confirm the proposed structure, catechinic acid stereoisomer was methylated to give two methyl ether derivatives. The FAB-MS spectra of both derivatives showed $[M + H]^+ = 347$ indicating tetramethyl ether derivatives. From the data of ^{1}H- and ^{13}C-NMR of these derivatives, they were identified as 4,9,3′,4′-tetra-*O*-methylcatechinic acid stereoisomer **(8)** and 2,9,3′,4′-tetra-*O*-methylcatechinic acid stereoisomer **(9)**, respectively (Figure 2)**.** The basis for these assignments is as follows:

In the ^{1}H-NMR spectra of **(8)** and **(9)**, four signals due to methoxyl protons were observed. Assignment of the methoxyl protons of **(8)** was accomplished by consideration of the ^{1}H–^{1}H NOESY spectrum exhibiting association of the methoxyl proton signals with each adjacent proton signal as shown in Figure 2. The HMBC spectrum also provided the evidence for assignment of the methoxyl protons, that is, these methoxyl protons were correlated with the quaternary carbons to which the methoxyl groups are attached. Assignment of the methoxyl protons of **(9)** was possible in the same manner as was done for **(8)**. It is assumed that **(8)** and **(9)** are derived from catechinic acid stereoisomer **(6, 7)**, and that the tautomerism between **(6)** and **(7)** occurs in a similar manner to catechinic acid.[12] This is supported by the fact that the broadening of the carbon resonances due to C-2 and C-4 is not observed in the ^{13}C-NMR spectra of **(8)** and **(9)**, whereas it is observed for the phenolic form of **(6**, **7)**.

Proposed reaction mechanisms for the formation of catechinic acid and its stereoisomer are shown in Figure 4. At first, quinone-methide intermediate **(2)** is formed through opening of the pyran-ring of (+)-catechin **(1)** in alkaline medium. Then, the nucleophilic attack of C-8 carbanion upon the C-2 carbon takes place to form catechinic acid **(4)** and the intermediate product with 6S configuration **(10)**. In this rearrangement reaction, there could be two reaction-faces, i.e., the *si*- and *re*-face, at C-2. Therefore, it is possible for the rearrangement reaction to form two stereoisomers. The reaction between C-8 and *re*-face at C-2 results in the formation of catechinic acid **(4)**, the absolute configuration of which has already established by the single-crystal X-ray structure analysis of the methyl ether derivative.[12,17] On the other hand, the intermediate product **(10)** is formed by the reaction between C-8 and *si*-face at C-2. The cyclohexanone ring of **(10)** may take several conformations, but it is expected that this ring prefers a boat conformation in which the catechol B-ring is equatorial. When the cyclohexanone ring adopts the boat conformation, the hydroxyl group at C-7 and the carbonyl carbon at C-9 are close to each other. Thus the subsequent formation of ether linkage between C-7 and C-9 takes place to give catechinic acid stereoisomer **(6**, **7)**.

On the base-catalyzed reactions of catechin, the formation of catechinic acid seemed to be stereospecific because any stereoisomers had not

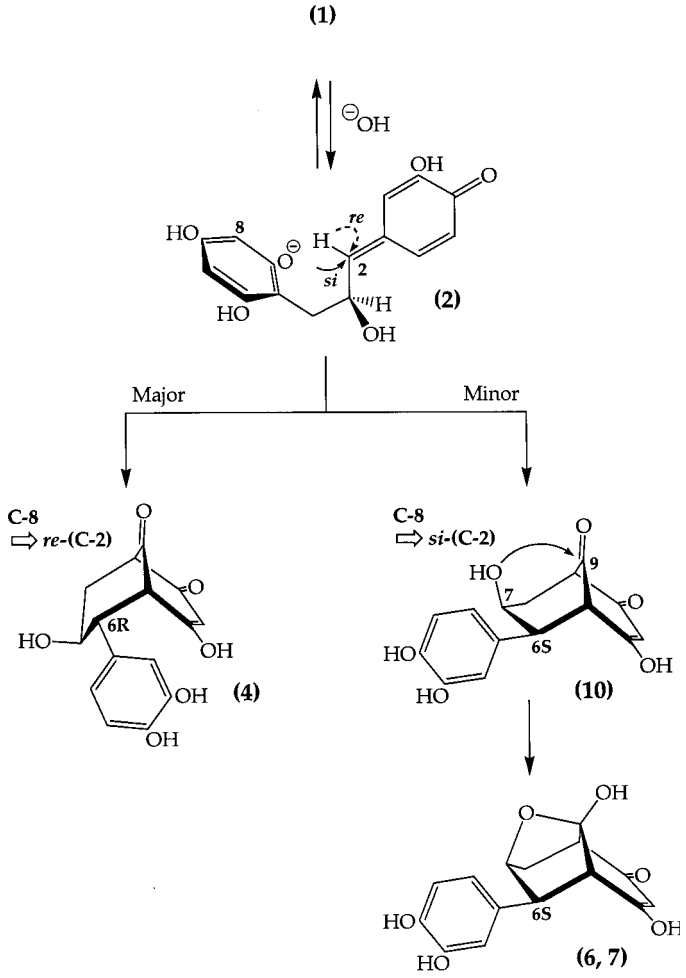

Figure 4. Proposed reaction mechanisms for the formation of catechinic acid (**4**) and its stereoisomer (**6**, **7**).

found. But it was a long-standing problem to explain why this reaction was stereospecific. Our finding of the formation of a novel catechinic acid stereoisomer and the proposed reaction mechanisms proved that the base-catalyzed rearrangement reaction of catechin to form catechinic acid is not stereospecific but stereoselective.

EXPERIMENTAL

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

Two-dimensional cellulose TLC (HPTLC plates cellulose F, Merck) was used to examine the reaction products by developing the plates first with *tert*-butyl alcohol–AcOH–H_2O (3 : 1 : 1, v/v/v) and then in the second dimension with 6% AcOH. Compounds were visualized by spraying the plates with diazotized sulphanilic acid or vanillin-HCl regent. Separations of the reaction products were made with Sephadex LH-20 using EtOH or MeOH–H_2O (1 : 1, v/v) as the eluting solvent. TLC and preparative TLC of the methylated derivatives were performed using TLC- or PLC-plates silica gel 60 F_{254}, Merck (thickness 0.25 and 2 mm, respectively) developed with benzene–EtOH–H_2O–AcOH (200 : 47 : 15 : 1, v/v/v/v, upper phase).

Base-Catalyzed Reaction of (+)-Catechin at pH 12 and 40°C

(+)-Catechin (1.0 g) was combined with 40 ml of H_2O and the pH was adjusted to 12.0 by adding solid NaOH with constant stirring. The resulting solution was bubbled with N_2 for 1 h, sealed in a reaction vial, and kept in a 40°C water-bath for 24 h. After neutralization of the reaction solution by passing it through an AG 50W-X4 ion-exchange resin (H^+ form, 50 g), the eluate was freeze–dried to afford a brown amorphous powder (712.3 mg). This reaction product was applied to a Sephadex LH-20 column (2.5 × 60 cm) eluted with EtOH. The elution of compounds was monitored by cellulose TLC developed in one dimension with 6% AcOH, and tubes 2–14 and 15–22 were combined to give fraction I and II, respectively.

Catechinic Acid (4)

Fraction I was evaporated to dryness to give **(4)** (408.7 mg). FAB-MS *m/z*: 291 $[M+H]^+$. ^{1}H-NMR (in pyridine-d_5 at 25°C): δ 2.32 (1H, *m*, $J = 12.9$, 10.9, 4.0 Hz, H-8a), 2.99 (1H, *m*, $J = 12.9$, 5.2, 3.4 Hz, H-8b), 3.52 (1H, *dd*, $J = 10.9$, 3.9 Hz, H-6), 3.65 (1H, *m*, H-1), 3.84 (1H, *dd*, $J = 3.9$, 1.5 Hz, H-5), 5.03 (1H, *td*, $J = 10.9$, 5.2 Hz, H-7), 6.13 (1H, *s*, H-3), 6.94 (1H, *dd*, $J = 8.1$, 2.1 Hz, H-6′), 7.11 (1H, *d*, $J = 8.1$ Hz, H-5′), 7.41 (1H, *d*, $J = 2.1$ Hz, H-2′). ^{13}C-NMR (in pyridine-d_5 at 25°C): δ

38.50 (C-8), 55.01 (C-6), 58.43 (C-1), 64.05 (C-5), 66.24 (C-7), 107.37 (C-3), 116.23 (C-5′), 117.15 (C-2′), 120.81 (C-6′), 131.31 (C-1′), 146.32 (C-4′), 146.96 (C-3′), 182.08 (C-4), 189.21 (C-2), 206.16 (C-9).

Catechinic Acid Stereoisomer (6, 7)

Fraction II was further purified by Sephadex LH-20 column chromatography eluted with MeOH–H_2O (1 : 1, v/v) to give **(6, 7)** (11.3 mg). FAB-MS m/z: 291 $[M+H]^+$. NMR data (in pyridine-d_5 at 25°C) were shown in Table 1.

Methylation of Catechinic Acid

Catechinic acid (100 mg), acetone (12 ml), dimethyl sulfate (0.28 ml) and K_2CO_3 (1600 mg) were refluxed overnight. Silica gel TLC showed mainly two spots and they were separated by preparative TLC to give 4,3′,4′-tri-*O*-methylcatechinic acid (52.7 mg, more mobile on TLC) and 2,3′,4′-tri-*O*-methylisocatechinic acid (26.6 mg).

4,3′,4′-tri-*O*-methylcatechinic acid. FAB-MS *m/z*: 333 $[M+H]^+$. ^{1}H-NMR (in $CDCl_3$ at 25°C): δ 1.98 (1H, *m*, *J* = 13.0, 11.4, 4.1 Hz, H-8a), 2.64 (1H, *m*, *J* = 13.0, 5.4, 3.4 Hz, H-8b), 3.11 (1H, *dd*, *J* = 10.5, 4.0 Hz, H-6), 3.34 (2H, *m*, H-1 and H-5), 3.60 (3H, *s*, O$\underline{CH_3}$-4), 3.88 (3H, *s*, O$\underline{CH_3}$-3′), 3.89 (3H, *s*, O$\underline{CH_3}$-4′), 4.45 (1H, *m*, H-7), 5.76 (1H, *s*, H-3), 6.68 (1H, *d*, *J* = 2.0 Hz, H-2′), 6.72 (1H, *dd*, *J* = 8.0, 2.0 Hz, H-6′), 6.87 (1H, *d*, *J* = 8.0 Hz, H-5′). ^{13}C-NMR (in $CDCl_3$ at 25°C): δ 37.11 (C-8), 53.32 (C-6), 55.91 (O$\underline{C}H_3$-3′ or O$\underline{C}H_3$-4′), 55.93 (O$\underline{C}H_3$-3′ or O$\underline{C}H_3$-4′), 56.47 (O$\underline{C}H_3$-4), 58.85 (C-5), 59.46 (C-1), 65.91 (C-7), 105.40 (C-3), 111.33 (C-2′), 111.42 (C-5′), 119.59 (C-6′), 129.55 (C-1′), 148.93 (C-3′), 149.43 (C-4′), 174.33 (C-4), 194.74 (C-2), 203.82 (C-9).

2,3′,4′-tri-*O*-methylisocatechinic acid. FAB-MS *m/z*: 333 $[M+H]^+$. ^{1}H-NMR (in $CDCl_3$ at 25°C): δ 1.99 (1H, *m*, *J* = 13.3, 11.0, 4.1 Hz, H-8a), 2.62 (1H, *m*, *J* = 13.3, 5.5, 3.3 Hz, H-8b), 3.08 (1H, *dd*, *J* = 11.0, 4.0 Hz, H-6), 3.34 (1H, *m*, H-1), 3.38 (1H, *dd*, *J* = 4.0, 1.5 Hz, H-5), 3.85 (3H, *s*, O$\underline{CH_3}$-2), 3.86 (3H, *s*, O$\underline{CH_3}$-4′), 3.87 (3H, *s*, O$\underline{CH_3}$-3′), 4.45 (1H, *td*, *J* = 11.0, 5.5 Hz, H-7), 5.78 (1H, *s*, H-3), 6.67 (1H, *d*, *J* = 2.1 Hz, H-2′), 6.69 (1H, *dd*, *J* = 8.1, 2.1 Hz, H-6′), 6.85 (1H, *d*, *J* = 8.1 Hz, H-5′). ^{13}C-NMR (in $CDCl_3$ at 25°C): δ 35.21 (C-8), 51.62 (C-1), 55.28 (C-6), 55.81 (O$\underline{C}H_3$-3′ or O$\underline{C}H_3$-4′), 55.90 (O$\underline{C}H_3$-3′ or O$\underline{C}H_3$-4′), 57.08 (O$\underline{C}H_3$-2), 66.47 (C-7), 66.84 (C-5), 105.30 (C-3), 111.33 (C-5′), 112.16

(C-2′), 120.14 (C-6′), 128.42 (C-1′), 148.96 (C-3′), 149.30 (C-4′), 175.49 (C-2), 191.95 (C-4), 203.83 (C-9).

These ^{1}H-NMR data of the methyl ether derivatives were in agreement with those of the literature.[12]

Methylation of Catechinic Acid Stereoisomer

Methylation of catechinic acid stereoisomer (7.3 mg) was performed by the same method as above. Two main spots were detected on silica gel TLC and they were separated by preparative TLC to give 4,9,3′,4′-tetra-*O*-methyl-catechinic acid stereoisomer **(8)** (3.2 mg) and 2,9,3′,4′-tetra-*O*-methylcatechinic acid stereoisomer **(9)** (3.0 mg, more mobile on TLC).

4,9,3′,4′-Tetra-*O*-methylcatechinic acid stereoisomer (8). FAB-MS *m/z*: 347 $[M+H]^+$. ^{1}H-NMR (in $CDCl_3$ at 25°C) δ 1.86 (1H, *dd*, $J=13.0$, 4.4 Hz, H-8a), 2.60 (1H, *td*, $J=13.0$, 5.8 Hz, H-8b), 2.90 (1H, *dd*, $J=4.0$, 2.3 Hz, H-5), 2.99 (1H, *m*, $J=13.0$, 4.4, 2.3 Hz, H-1), 3.03 (1H, *d*, $J=4.0$ Hz, H-6), 3.57 (3H, *s*, OC$\underline{H_3}$-9), 3.75 (3H, *s*, OC$\underline{H_3}$-4), 3.88 (3H, *s*, OC$\underline{H_3}$-4′), 3.90 (3H, *s*, H-OC$\underline{H_3}$-3′), 4.46 (1H, *d*, $J=5.8$ Hz, H-7), 5.29 (1H, *s*, H-3), 6.83 (1H, *d*, $J=8.4$ Hz, H-5′), 6.89 (1H, *dd*, $J=8.4$, 2.1 Hz, H-6′), 6.95 (1H, *d*, $J=2.1$ Hz, H-2′). ^{13}C-NMR (in $CDCl_3$ at 25°C): δ 37.89 (C-8), 46.67 (C-1), 51.84 (C-5), 52.70 (O$\underline{C}H_3$-9), 55.88 (O$\underline{C}H_3$-3′ or O$\underline{C}H_3$-4′), 55.89 (O$\underline{C}H_3$-3′ or O$\underline{C}H_3$-4′), 56.34 (O$\underline{C}H_3$-4), 57.92 (C-6), 79.16 (C-7), 97.31 (C-3), 110.30 (C-2′), 111.18 (C-5′), 113.23 (C-9), 119.20 (C-6′), 136.55 (C-1′), 148.11 (C-4′), 149.12 (C-3′), 179.04 (C-4), 199.13 (C-2).

2,9,3′,4′-Tetra-*O*-methylcatechinic acid stereoisomer (9). FAB-MS *m/z*: 347 $[M+H]^+$. ^{1}H-NMR (in $CDCl_3$ at 25°C): δ 1.85 (1H, *dd*, $J=12.5$, 3.5 Hz, H-8a), 2.58 (1H, *td*, $J=12.5$, 5.3 Hz, H-8b), 2.86 (1H, *m*, $J=12.5$, 3.5, 2.4 Hz, H-1), 3.00 (1H, *dd*, $J=4.9$, 2.4 Hz, H-5), 3.04 (1H, *d*, $J=4.9$ Hz, H-6), 3.56 (3H, *s*, OC$\underline{H_3}$-9), 3.78 (3H, *s*, OC$\underline{H_3}$-2), 3.86 (3H, *s*, OC$\underline{H_3}$-4′), 3.88 (3H, *s*, OC$\underline{H_3}$-3′), 4.51 (1H, *d*, $J=5.3$ Hz, H-7), 5.31 (1H, *s*, H-3), 6.80 (1H, *d*, $J=8.1$ Hz, H-5′), 6.86 (1H, *dd*, $J=8.1$, 2.1 Hz, H-6′), 6.90 (1H, *d*, $J=2.1$ Hz, H-2′). ^{13}C-NMR (in $CDCl_3$ at 25°C): δ 40.02 (C-8), 41.58 (C-1), 52.66 (O$\underline{C}H_3$-9), 55.18 (C-6), 55.84 (O$\underline{C}H_3$-3′), 55.94 (O$\underline{C}H_3$-4′), 56.30 (O$\underline{C}H_3$-2), 57.44 (C-5), 79.31 (C-7), 97.57 (C-3), 110.22 (C-2′), 111.33 (C-5′), 113.21 (C-9), 119.01 (C-6′), 136.70 (C-1′), 148.06 (C-4′), 149.18 (C-3′), 179.41 (C-2), 198.02 (C-4).

REFERENCES

1. Haslam, E. In *Plant Polyphenols: Vegetable Tannins Revisited*. Chap. 4, Cambridge University Press: New York, 1988.
2. Ricardo da Silva, J.M.; Darmon, N.; Fernandez, Y.; Mitjavila, S. J. Agric. Food Chem. **1991**, *39*(9), 1549.
3. Laks, P.E. In *Chemistry and Significance of Condensed Tannins*, Hemingway, R.W.; Karchesy, J.J., Ed.; Plenum Press: New York, 1989; 503–515.
4. Eberhardt, T.L.; Young, R.A. J. Agric. Food Chem. **1994**, *42*(8), 1704.
5. Kakiuchi, N.; Hattori, M.; Namba, T.; Nishizawa, M.; Yamagishi, T.; Okuda, T. J. Nat. Prod. **1985**, *48*, 614.
6. Mitsunaga, T.; Abe, I.; Kotani, M.; Ono, H.; Tanaka, T. J. Wood Chem. Technol. **1997**, *17*(3), 327.
7. Helsper, J.P.F.G.; Kolodziej, H.; Hoogendijk, J.M.; Norel, A.V. Phytochemistry **1993**, *34*(5), 1255.
8. Samejima, M.; Yoshimoto, T. Mokuzai Gakkaishi **1981**, *27*(6), 491.
9. Ohara, S.; Yanagi, K. Mokuzai Gakkaishi **1995**, *41*(4), 406.
10. Yazaki, Y. Australian Forestry **1997**, *53*, 148.
11. Kiatgrajai, P.; Wellons, J.D.; Gollob, L.; White, J.D. J. Org. Chem. **1982**, *47*(15), 2910.
12. Sears, K.D.; Casebier, R.L.; Herget, H.L. J. Org. Chem. **1974**, *39*(22), 3244.
13. Kennedy, J.A.; Munro, M.H.G.; Powell, H.K.J.; Porter, L.J.; Foo, L.Y. Aust. J. Chem. **1984**, *37*, 885.
14. Ohara, S.; Hemingway, R.W. J. Wood Chem. Technol. **1991**, *11*(2), 195.
15. Laks, P.E.; Hemingway, R.W. J. Chem. Soc. Perkin Trans. I **1987**, 1875.
16. Silverstein, R.M.; Bassler, G.C.; Morrill, T.C. In *Spectrometric Identification of Organic Compounds,* Fifth Edition, Chap. 4, John Wiley & Sons Inc.: New York, 1991.
17. McCandlish, L.E.; Hanson, J.C.; Stout, G.H. Acta Cryst. **1976**, *B32*, 1793.

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016