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Tannins and Related Compounds. LXIX.[1] Isolation and Structure Elucidation of B,B'-Linked Bisflavanoids, Theasinensins D—G and Oolongtheanin from Oolong Tea. (2)

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Chemical examination of the aqueous acetone extract of commercial oolong tea has led to the isolation of four new theasinensins D—G, and a new type of dimeric flavan-3-ol named oolongtheanin, together with previously reported theasinensins A, B, and C. On the basis of chemical and spectroscopic evidence, theasinensins D and E were characterized as atropisomers (**4** and **5**) of theasinensins A (**1**) and C (**3**), respectively, while theasinensins F and G were shown to be B,B'-linked bisflavanoids (**6** and **7**) consisting of (−)-epicatechin 3-*O*-gallate and (−)-epigallocatechin 3-*O*-gallate units, in which the biphenyl bonds possess *S*- and *R*-chiralities, respectively. Similarly, oolongtheanin was characterized as a novel metabolite (**8**), probably derived from theasinensins by oxidation of one of the B-rings.

Keywords——oolong tea; *Camellia sinensis* var. *viridis*; Theaceae; polyphenol; theasinensin; oolongtheanin; oxidative metabolite; bisflavanoid; flavan-3-ol; fermentation

In an attempt to clarify the mechanism of oxidation of tea leaf polyphenols in the fermentation process, we have been examining the chemical composition of polyphenols in a variety of beverage teas at different stages of fermentation. We previously studied those in green tea[2] and black tea,[3] as well as in fresh green tea leaf.[4] In addition, preliminary examination of an oolong tea extract resulted in the isolation and structural elucidation of several acylated flavan-3-ols.[5] The present paper describes a further chemical examination of oolong tea polyphenols, which led to the isolation and characterization of a series of new B,B'-linked bisflavanoids, theasinensins D—G (**4**—**7**) and oolongtheanin (**8**), together with previously reported theasinensins A—C (**1**—**3**).

Commercial oolong tea (commercial name; shiraore) was extracted with 80% aqueous acetone, and the extract was repeatedly chromatographed over Sephadex LH-20 dextran and reversed-phase gels as shown in Chart 1 to yield compounds **1**—**8**. Among them, compounds **1** and **2** were found to be identical with theasinensins A (**1**) and B (**2**), respectively,[4] while the physical and spectral data of compound **3** (named theasinensin C) coincided with those of a sample previously obtained by tannase hydrolysis of **1** and **2**.[4]

Formerly, we presumed the atropisomerism of the biphenyl bond in **1**, **2** and **3** to be in the *S*-series based on analysis of the circular dichroism (CD) spectra.[4] Since in this study, **1**, **2** and **3** were obtained in large amounts sufficient for chemical examination, we attempted to confirm the atropisomerism by degradation of **3** to a compound with known absolute stereochemistry (Chart 3). The decamethyl ether (**3a**), prepared by methylation of **3** with dimethyl sulfate and potassium carbonate in dry acetone, was treated with *p*-toluenesulfonyl chloride in pyridine to afford the di-tosylate (**3b**). On alkaline treatment, followed by potassium permanganate and sodium periodate oxidation,[6] the tosylate (**3b**) yielded dimethyl hexamethoxydiphenoate (**3c**), whose specific optical rotation [+12.3° (MeOH)], although the value was rather small, clearly indicated it to have *R*-configuration.[7] Thus, the atropisom-

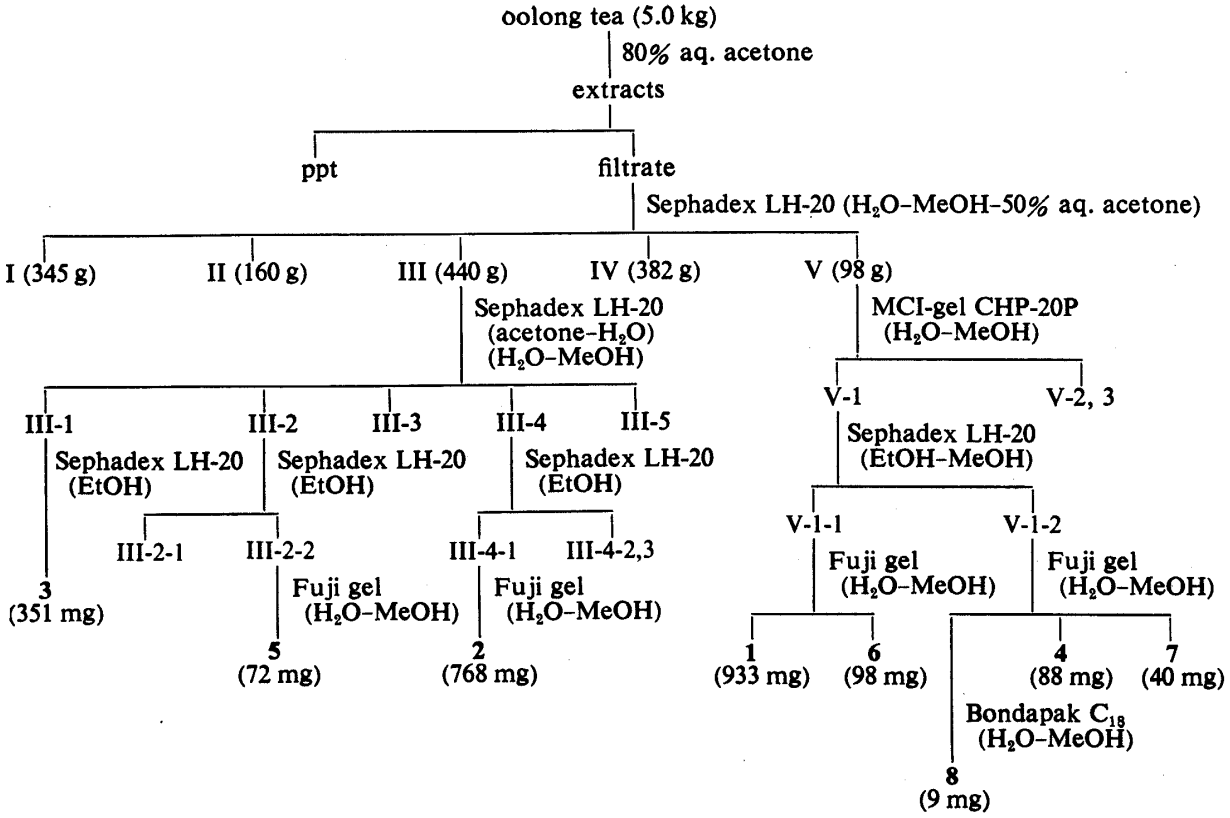
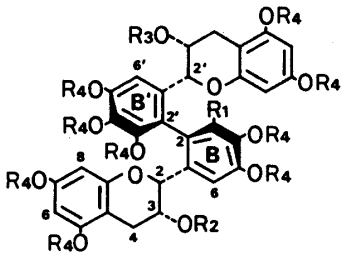
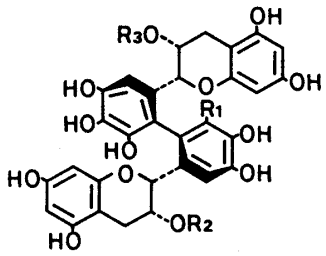


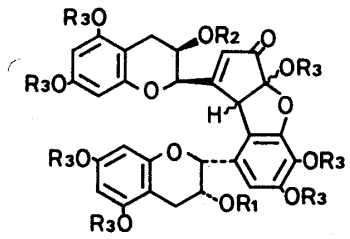
Chart 1



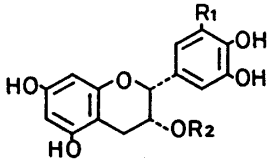
	R1	R2	R3	R4
1	OH	G	G	H
2	OH	G	H	H
3	OH	H	H	H
3a	OMe	H	H	Me
3b	OMe	Ts	Ts	Me
6	H	G	G	H
6a	H	H	H	H



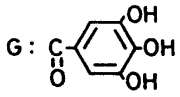
	R1	R2	R3
4	OH	G	G
5	OH	H	H
7	H	G	G
7a	H	H	H
12	OH	G	H

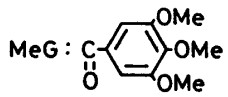


	R1	R2	R3
8	G	H	H
8a	H	H	H
8b	MeG	H	Me
8c	G	G	H



	R1	R2
9	OH	G
10	OH	H
11	H	G





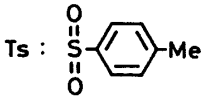
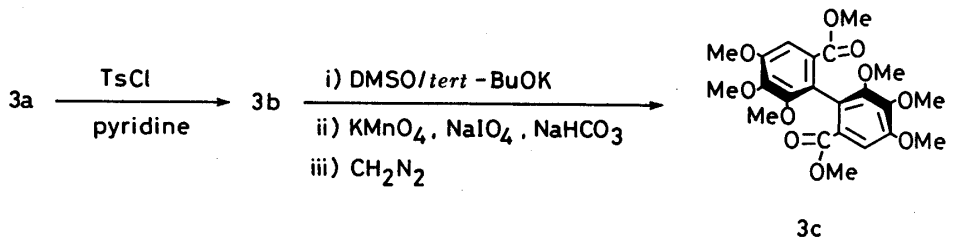


Chart 2



3c

Chart 3

erism in **1**, **2** and **3** was confirmed unambiguously to be in the *R*-series, in disagreement with the previous observation.[4)]

Compounds **4** and **5** showed blue and orange colorations with the ferric chloride and anisaldehyde–sulfuric acid reagents, respectively, suggesting the presence of a pyrogallol ring and a flavan-3-ol moiety in the molecule. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra (Table II) of **4** and **5** were almost indistinguishable from those of theasinensins A (**1**) and C (**3**), showing the presence of seemingly twenty-two and fifteen carbons, respectively. Although the ^{13}C-NMR spectra were also correlated closely with those of (−)-epigallocatechin 3-*O*-gallate (**9**) and (−)-epigallocatechin (**10**), the observation of an unsymmetrical signal pattern of the B-ring in **4** and **5** indicated the presence of a substituent in the B-ring. This finding was consistent with the proton nuclear magnetic resonance (^{1}H-NMR) spectra (Table I) of **4** and **5**, which exhibited a seemingly one-proton singlet arising from the B-ring. In addition, the fact that the molecular masses measured by fast atom bombardment mass spectroscopy (FAB-MS) of **4** and **5** coincided exactly with those of **1** and **3**, respectively, clearly indicated that **4** and **5** possess symmetrical dimeric flavan-3-ol structures in which two units are linked at the B,B′-rings through a carbon–carbon bond. Furthermore, **4** and **5** were structurally related in that, on enzymatic hydrolysis with tannase, **4** afforded **5**, together with gallic acid. Since the ^{1}H-NMR spectrum of **4** showed the flavan 3-H signal to be shifted downfield (δ 5.66) as compared with that of **5**, the galloyl group was considered to be located at the C-3 position. To confirm unambiguously the structures of these compounds, derivation of **4** by oxidative coupling of 2 mol of **9** with potassium ferricyanide in a weakly alkaline medium was attempted, and successfully afforded, among others, compound **4**, thus establishing the structures except for the atropisomerism of the biphenyl bond. In the circular dichroism (CD) spectra of **3** and **5**, the signs of the Cotton effects, especially those of two bands around 220—230 and 230—240 nm, were opposite (Fig. 1). This fact indicates that **5** possesses *S*-configuration. Thus, theasinensins D and E were characterized as **4** and **5**, which are atropisomers of **1** and **3**, respectively.

Compounds **6** and **7** were also positive to the ferric chloride and anisaldehyde–sulfuric acid tests. The presence of two flavan-3-ol moieties in **6** and **7** was readily deduced from the observation of each pair of ^{1}H-NMR signals, especially of two H-2 and H-3 signals (Table I). In addition, the small coupling constants between the H-2 and H-3 signals indicated them to possess the 2,3-*cis* configuration, while the lowfield shifts of the H-3 signals, as well as aromatic resonances at δ 7.04 (4H, s) in **6** and δ 7.07 (2H, s) and δ 7.09 (2H, s) in **7**, showed the presence of two galloyl groups at the flavan C-3 positions in each case. The most characteristic features in the ^{1}H-NMR spectra of **6** and **7** were three well-resolved singlets (δ 6.77, 6.93 and 7.31 in **6**; δ 6.70, 6.99 and 7.30 in **7**). Taking the chemical shifts into account, these signals were assignable to the protons on the B-ring having vicinal di- and/or tri-hydroxy groups. Further information as to the substitution systems in the B-rings was provided by ^{13}C-NMR analysis (Table II). Of the twelve carbon resonances from the B-rings, the chemical shifts (δ 107.6, 117.7, 129.5, 133.2, 144.1 and 145.4 in **6**; δ 108.5, 115.8, 130.4, 133.6, 145.3 and 145.5 in **7**) of six were almost in line with those found in theasinensins A—E (**1**—**5**), while the remaining six

TABLE I. ^{1}H-NMR Spectral Data for Theasinensins and Oolongtheanin[a)]

	1	2	3	4	12	5	6	7	8	8a
C-Ring 2-H	4.80 (s)	4.80 (s)	4.62 (s)	4.85 (s)	4.92 (s)	4.76 (s)	4.83 (s)	4.88 (s)	5.40 (s)	5.18 (s)
3-H	5.35 (m)	5.35 (m)	4.04 (m)	5.66 (m)	5.14 (m)	4.02 (m)	5.43 (m)	5.48 (m)	5.39 (m)	4.05 (m)
4-H	2.57 (dd, J=18, 4 Hz)	2.57 (dd, J=18, 4 Hz)	2.37 (dd, J=18, 4 Hz)	2.86 (m)	2.64 (m)	2.42 (dd, J=16, 4 Hz)	2.39—3.03 (m)	2.74—2.93 (m)	2.96 (d, J=17 Hz)	2.79 (d, J=17 Hz)
	2.91 (d, J=18 Hz)	2.91 (d, J=18 Hz)	2.72 (d, J=18 Hz)			2.69 (d, J=16 Hz)			3.26 (dd, J=17, 4 Hz)	3.12 (dd, J=17, 3 Hz)
C′-Ring 2′-H		4.62 (s)			4.59 (s)		4.71 (s)	4.99 (s)	4.37 (s)	4.36 (s)
3′-H		4.07 (m)			4.26 (m)		5.43 (m)	5.71 (m)	4.38 (m)	4.39 (m)
4′-H		2.37 (dd, J=18, 4 Hz)			2.50 (dd, J=17, 4 Hz)		2.39—3.03 (m)	2.74—2.93 (m)	2.70 (d, J=12 Hz)	2.69 (d, J=10 Hz)
		2.72 (d, J=18 Hz)			2.84 (d, J=17 Hz)				2.84 (d, J=12 Hz)	2.83 (d, J=10 Hz)
B-Ring 3-H							7.31 (s)	7.30 (s)		
6-H	6.88 (s)	6.90 (s)	6.85 (s)	6.99 (s)	7.03 (s)	7.11 (s)	6.77 (s)	6.70 (s)	6.90 (s)	6.94 (s)
B′-Ring 2′-H									6.64 (s)	6.63 (s)
5′-H									4.62 (s)	4.56 (s)
6′-H		6.81 (s)			7.39 (s)		6.93 (s)	6.99 (s)		
A,A′-Ring 6,6′,8,8′-H	5.95 (d, J=2 Hz)	5.84 (d, J=2 Hz)	5.83 (d, J=2 Hz)	6.03 (s)	5.95 (2H, s)	5.91 (d, J=2 Hz)	5.88—6.04 (m)	5.94—6.09 (m)	5.77 (d, J=2 Hz)	5.76 (d, J=2 Hz)
	6.03 (d, J=2 Hz)	5.95 (d, J=2 Hz)	5.94 (d, J=2 Hz)		5.89 (d, J=2 Hz)	5.99 (d, J=2 Hz)			5.98 (d, J=2 Hz)	5.86 (d, J=2 Hz)
		5.96 (d, J=2 Hz)			5.96 (d, J=2 Hz)				5.99 (d, J=2 Hz)	6.00 (d, J=2 Hz)
		6.01 (d, J=2 Hz)							6.06 (d, J=2 Hz)	6.04 (d, J=2 Hz)
Galloyl 2,6-H	7.00 (4H, s)	6.96 (s)		7.08 (4H, s)	7.09 (s)		7.04 (4H, s)	7.07 (s)	7.07 (s)	
								7.09 (s)		

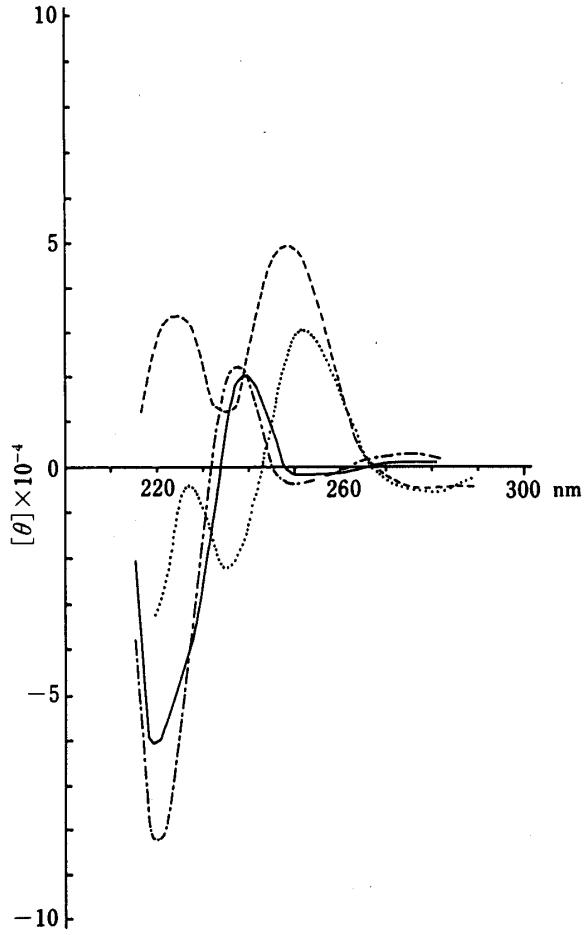
a) Spectra were measured in acetone-d_6 + D_2O at 100 MHz.

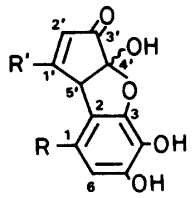
TABLE II. ^{13}C-NMR Spectral Data for Theasinensins and Oolongtheanin[a]

	1	2	3	4	12	5	6	7	8	8a
C-Ring										
2-C	76.0	76.1	77.1	76.2	76.3	77.4	76.0	75.3[b]	78.7	78.9
3-C	68.9	68.6	64.9	68.4	68.3	64.7	68.9[b]	68.7[c]	69.5	65.8
4-C	26.8	26.8	28.7	27.7	27.4	30.0	26.9	27.4[d]	26.7	29.6[b]
C′-Ring										
2′-C		77.2			77.4		76.0	76.2[b]	75.7	76.6
3′-C		64.9			65.1		68.4[b]	68.4[c]	62.8	62.8
4′-C		28.6			30.2		26.9	27.9[d]	29.6	29.7[b]
A,A′-Ring										
4a,4′a-C	98.3	98.5	99.1	98.8	98.5	99.6	98.3	98.7	98.9	99.3
		99.4			99.7		98.7	98.9	99.3	99.8
6,6′,8,8′-C	95.5	95.7	95.5	95.7	95.7	95.7	95.7	95.7	95.4	95.4
	96.4	95.9	96.1	96.6	96.1	96.3	96.5	96.5	96.5	95.5
		96.1			96.4					96.4
		96.5								
5,5′,7,7′,8a,8′a-C	157.0	157.3	157.0	157.1	157.1	157.0	157.1	157.3	156.3	156.3
	157.3	157.7	157.1		157.2	157.1	157.4	157.4	157.0	157.0
			157.4		157.4	157.5	157.5		157.2	157.1
					157.6				157.4	157.5
									158.1	157.7
B-Ring										
1-C	129.0	129.1	129.9	129.8	129.6	130.5	127.9[c]	128.9[e]	130.4	130.3
2-C	112.7	111.8	111.9	114.1	113.8	114.1	125.4[c]	126.2[e]	115.7	115.7
3-C	145.6[b]	144.9[b]	144.9[b]	143.8[b]	143.5[b]	143.3[b]	117.7[d]	118.8[f]	147.0[b]	146.3[c]
4-C	133.3	133.3[c]	133.0	134.0	134.0	133.5	144.1[e]	143.1[g]	127.2	128.3
5-C	145.9[b]	146.1[b]	145.8[b]	145.8[b]	145.8[b]	145.4[b]	145.4[e]	145.1[g]	147.9[b]	147.8[c]
6-C	108.0	107.9	108.2	108.4	108.6	108.8	119.2	121.6	108.8	109.5
B′-Ring										
1′-C		130.3			130.9		129.5	130.4	155.9	155.9
2′-C		111.3			114.4		116.0[d]	115.8[f]	125.1	124.9
3′-C		144.9[b]			145.5[b]		144.1[e]	145.3[g]	200.8	200.6
4′-C		133.6[c]			134.0		133.2	133.6	94.6	94.4
5′-C		146.3[b]			145.8[b]		145.4[e]	145.5[g]	53.5	53.6
6′-C		108.8			109.8		107.6	108.5		
Galloyl										
–COO–	167.0	166.7		166.0	166.8		166.8	166.6	166.8	
							167.0			
1-C	121.3	121.6		121.5	121.5		121.4	121.6	121.3	
2,6-C	110.0	110.0		110.1	110.0		110.0	110.1	110.0	
3,5-C	145.8	145.8		145.8	145.9		145.8	145.9	145.6	
4-C	139.0	138.9		139.0	139.0		139.1	139.1	139.1	

a) Spectra were measured in acetone-d_6 + D_2O at 25.05 MHz. *b*—*g*) Assignments may be interchanged in each column.

carbon resonances consisting of two doublets and four singlets in the off-resonance spectra indicated the presence of a catechol ring with a 1,2,4,5-tetra-substitution system. These spectroscopic findings, coupled with the fact that (−)-epicatechin 3-*O*-gallate (**11**) and (−)-epigallocatechin 3-*O*-gallate (**9**) exist as major components in fresh tea leaf, suggested that **6** and **7** consist of (−)-epigallocatechin 3-*O*-gallate and (−)-epicatechin 3-*O*-gallate units linked at each C-2′ position through a carbon–carbon bond. This was supported by FAB-MS analysis of **6** and **7**, which showed the same $(M+H)^+$ peak at *m/z* 899, sixteen mass units less than that found in the cases of **1** and **4**. Although the NMR spectra of **6** and **7** were closely correlated with each other as mentioned above, CD measurements clearly indicated that they





A

Fig. 1. CD Spectra of Compounds **3**, **5**, **6a** and **7a**

3, ——; **5**, ------; **6a**, – – – –; **7a**, – – – –.

are mutually atropisomeric. Thus, as shown in Fig. 1, the CD curves of the desgalloyl derivatives **6a** and **7a**, obtained by tannase hydrolysis of **6** and **7**, respectively, showed opposite Cotton effects. In addition, the signs of the Cotton effects in **6a** and **7a** corresponded well to those found in **3** and **5**, respectively, thus establishing their chiralities to be in the *S*- and *R*-series. From the findings described above, the structures of **6** and **7** were concluded to be as shown in Chart 2.

Compound **8** exhibited in the ^{1}H-NMR spectrum two pairs of *meta*-coupled aromatic doublets and of aliphatic signals (Table I), characteristic of the respective A- and C-rings in 5,7-substituted flavan-3-ols. In addition, the appearance of an aromatic two-proton singlet at δ 7.07, as well as the lowfield shift (δ 5.39) of one of the methine signals, suggested the presence of one galloyl group in the molecule. These ^{1}H-NMR observations were consistent with the ^{13}C-NMR data, which showed, together with seven galloyl signals, the presence of two phloroglucinol rings, four oxygen-bearing methines and two methylenes (Table II). As regards the B-ring, the ^{13}C-NMR spectrum showed six aromatic carbon signals (Table II), the chemical shifts being closely correlated with those of theasinensins. Furthermore, ^{13}C-NMR analysis suggested the presence of a methine (δ 53.5, d), a hemiacetal (δ 94.6, s), a tri-substituted double bond (δ 125.1, d and δ 155.9, s) and a carbonyl group (δ 200.8, s). Taking into account the remarkable chemical shift difference of the double bond signals, the carbonyl group is considered to be conjugated. The modest lowfield shifts (*ca.* +2 ppm) of the C-3 and C-5 signals in the above-mentioned aromatic B-ring, as compared with those of theasinensins, suggested that the C-3 oxygen atom participates in forming a hemiacetal ring. Thus, for the possible B-ring structure, only one form (A) was considered. On enzymatic hydrolysis with tannase, **8** yielded gallic acid and a hydrolysate (**8a**). The ^{13}C-NMR spectrum of **8a** showed,

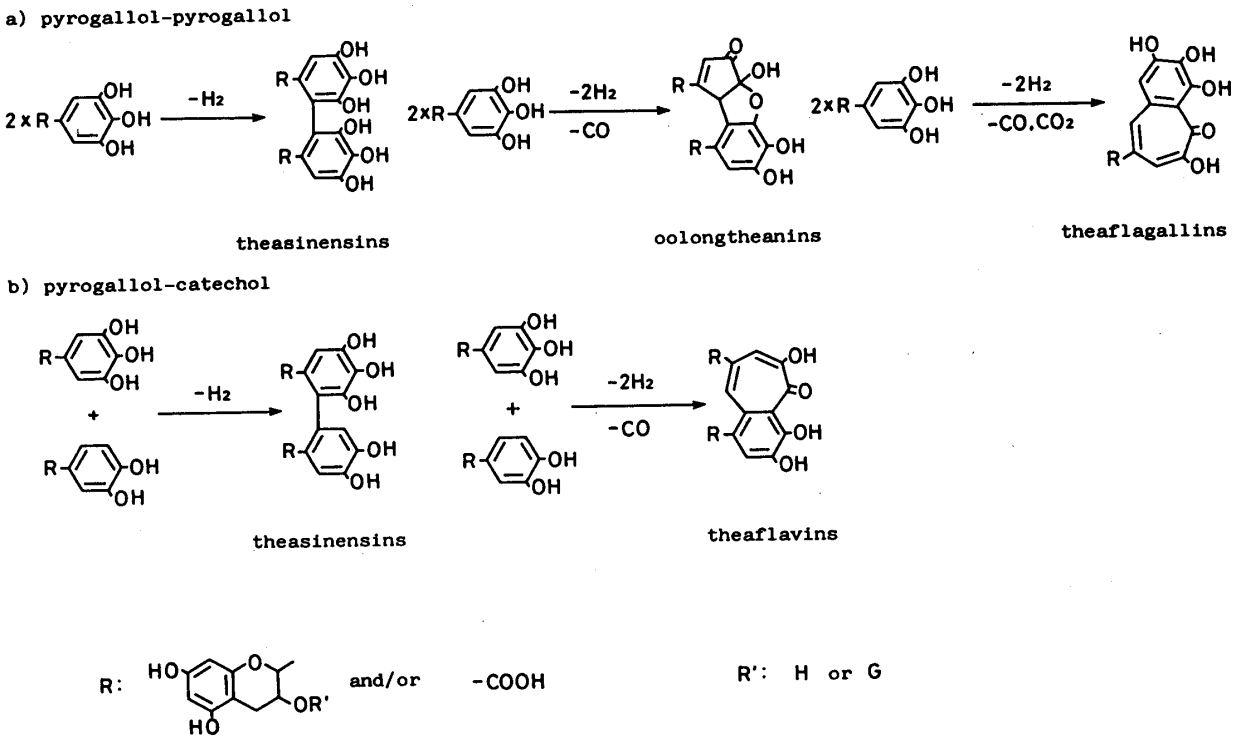


Chart 4. Possible Oxidation Pathways of Tea Catechins during Fermentation

among others, a flavan C-3 signal at δ 65.8, whose chemical shift was analogous to those of (−)-epigallocatechin (**10**) and theasinensins C (**3**) and E (**5**). On comparison of the ^{13}C-NMR spectra of **8** and **8a**, the significant upfield shift (−3.7 ppm) of the C-3 signal clearly indicated the location of the galloyl group at the C-3 position in the epigallocatechin part. The positive and negative FAB-MS of **8** showed prominent $(M+H)^+$ and $(M-H)^-$ peaks at *m/z* 733 and 731, respectively, which were consistent with the proposed structure (**8**). Furthermore, the fact that on treatment with diazomethane, **8** formed the decamethyl ether (**8b**), in which one aliphatic (δ 3.35) and nine aromatic methoxyls were found to exist by ^{1}H-NMR spectroscopy, also supported the structure (**8**). In order to establish the structure more definitively, an attempt was made to prepare **8** by oxidative coupling of **9** and **10**, and treatment of a 1 : 1 mixture of **9** and **10** with potassium ferricyanide successfully afforded a product found to be identical with **8**. Thus, the structure of **8**, including the absolute stereochemistry of the C-ring carbons, was confirmed, though the absolute configuration of the methine and hemiacetal carbons in the cyclopentenone moiety still remains to be solved.

Although considerable attention has been drawn to the chemistry of tea leaf polyphenols, little is known about the mechanism of the oxidation of the polyphenols during the fermentation. In a series of chemical studies, we have shown that enzymatic oxidation occurs invariably at the catechol and pyrogallol rings, especially at the B-ring of flavan-3-ol derivatives, and that there seem to be several oxidation systems, as shown in Chart 4.

Experimental

Details of the instruments and chromatographic conditions used in this study were essentially the same as described in the previous paper,[5] except for the following: negative FAB-MS was measured on a JEOL DX-300 spectrometer and CD spectra with a JASCO J-20 spectropolarimeter in methanol.

Isolation——Fractions III and IV, previously obtained from the 80% aqueous acetone extract of commerical oolong tea (commercial name: shiraore),[5] were separated as shown in Chart 1 to furnish theasinensins A—G (**1**—**7**) and oolongtheanin (**8**).

Methylation of 3——A mixture of **3** (800 mg), Me_2SO_4 (5 ml) and anhydrous K_2CO_3 (5 g) in dry acetone (200 ml) was refluxed for 6 h with stirring. After removal of inorganic salts by filtration, the filtrate was concentrated to a syrup, which was chromatographed over silica gel. Elution with benzene–acetone (9 : 1, v/v) gave **3a** (480 mg) as a white amorphous powder, $[\alpha]_D^{23}$ −10.3° (c=0.9, $CHCl_3$). *Anal.* Calcd for $C_{40}H_{46}O_{14}\cdot 1/2H_2O$: C, 63.23; H, 6.24. Found: C, 63.05; H, 6.21. ^{1}H-NMR ($CHCl_3$) δ: 3.42 (2H, dd, J=16, 4 Hz, 4-H), 3.80 (2H, d, J=16 Hz, 4-H), 3.67, 3.71, 3.84, 3.91 (30H in total, each s, 10×OCH_3), 4.16 (2H, m, 3-H), 4.54 (2H, br s, 2-H), 6.03 (4H, s, 6,8-H), 7.12 (2H, s, 6′-H).

Tosylation of 3a——A solution of **3a** (425 mg) in dry pyridine (20 ml) containing *p*-toluenesulfonyl chloride (850 mg) was refluxed for 5 h. Excess reagent was decomposed by addition of ice-water, and the resulting precipitates were collected by filtration. Purification by silica gel chromatography with benzene–acetone (30 : 1—15 : 1, v/v) yielded the tosylate (**3b**) (350 mg) as a white amorphous powder, $[\alpha]_D^{28}$ +16.0° (c=0.2, $CHCl_3$). *Anal.* Calcd for $C_{54}H_{58}O_{18}\cdot 1/2H_2O$: C, 60.72; H, 5.57. Found: C, 60.96; H, 5.83. ^{1}H-NMR ($CHCl_3$) δ: 2.38 (2H, dd, J=18, 4 Hz, 4-H), 2.41 (6H, s, 2×CH_3), 2.99 (2H, d, J=18 Hz, 4-H), 3.63, 3.66, 3.81, 3.87 (30H in total, each s, 10×OCH_3), 4.33 (2H, s, 2-H), 5.35 (2H, m, 3-H), 5.91, 5.98 (each 2H, d, J=2 Hz, 6,8-H), 6.78 (2H, s, 6′-H), 7.11, 7.47 (each 4H, d, J=8 Hz, arom-H).

Degradation of 3b——A solution of **3b** (350 mg) and potassium *tert*-butoxide (2.5 g) in dimethyl sulfoxide–benzene (1 : 5, v/v) (150 ml) was left standing at room temperature for 20 min. The reaction mixture was acidified with 1 N HCl, diluted with H_2O (50 ml) and extracted with Et_2O. The organic layer thus separated was washed with H_2O, dried over Na_2SO_4 and concentrated to dryness to give a residue (30 mg), which was oxidized with $KMnO_4$ (0.5 g) and $NaIO_4$ (0.5 g) in *tert*-BuOH–H_2O (1 : 1, v/v) (10 ml) containing $NaHCO_3$ (0.3 g) under reflux for 12 h. After cooling, the reaction mixture was treated with $NaHSO_3$, and acidified with 50% H_2SO_4. The acidic solution was extracted with ether five times, and the organic layer was washed with H_2O, dried over Na_2SO_4, and concentrated to dryness. The residue in MeOH was treated with ethereal diazomethane at room temperature for 10 min, and after evaporation of the solvent, the residue was chromatographed over silica gel with benzene–ethyl acetate (15 : 1, v/v) to yield (*R*)-dimethyl hexamethoxydiphenoate (**3c**) (2 mg) as a colorless syrup, $[\alpha]_D^{24}$ +12.3° (c=0.2, MeOH), CD (c=0.0023, MeOH) $[\theta]^{18}$ (nm): −34600 (225), 0 (238), 23900 (230), 0 (267), −7100 (277).

Theasinensin D (4)——An off-white amorphous powder, $[\alpha]_D^{28}$ −158.6° (c=1.0, acetone). *Anal.* Calcd for $C_{44}H_{34}O_{22}\cdot 5H_2O$: C, 52.59; H, 4.41. Found: C, 52.31; H, 4.40. FAB-MS m/z: 915 [(M+H)$^+$, 21%]. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II. CD (c=0.0005, MeOH) $[\theta]^{18}$ (nm): −191000 (217), 0 (250), −90500 (275).

Tannase Hydrolysis of 4——A solution of **4** (50 mg) in H_2O (5 ml) was shaken with tannase at room temperature for 10 min. The reaction mixture was worked up as described above to give gallic acid and **6a** (8 mg) as an off-white increasing amounts of MeOH, gave gallic acid and **5** (16 mg).

Theasinensin E (5)——An off-white amorphous powder, $[\alpha]_D^{23}$ +22.6° (c=1.0, acetone). *Anal.* Calcd for $C_{30}H_{26}O_{14}\cdot 2/3H_2O$: C, 57.87; H, 4.43. Found: C, 58.04; H, 4.91. Negative FAB-MS m/z: 609 [(M−H)$^-$, 8%]. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II. CD (c=0.0023, MeOH) $[\theta]^{18}$ (nm): −31600 (219), −36000 (227), −22100 (235), 0 (243), 30500 (252), 0 (267), −6300 (280).

Theasinensin F (6)——An off-white amorphous powder, $[\alpha]_D^{26}$ −263.6° (c=1.0, acetone). *Anal.* Calcd for $C_{44}H_{34}O_{21}\cdot 5H_2O$: C, 53.44; H, 4.49. Found: C, 53.33; H, 4.32. FAB-MS m/z: 899 [(M+H)$^+$, 3%]. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II. CD (c=0.0031, MeOH) $[\theta]^{18}$ (nm): −267000 (218), −7900 (240), −28200 (253), −22500 (262), −52600 (282).

Tannase Hydrolysis of 6——A solution of **6** (15 mg) in H_2O (3 ml) was shaken with tannase at room temperature for 10 min. The reaction mixture was worked up as described above to give gallic acid and **6a** (8 mg) as an off-white amorphous powder, $[\alpha]_D^{26}$ −48.5° (c=0.5, acetone). *Anal.* Calcd for $C_{30}H_{26}O_{13}\cdot 2H_2O$: C, 57.14; H, 4.79. Found: C, 57.24; H, 4.84. FAB-MS m/z: 595 [(M+H)$^+$, 1%]. ^{1}H-NMR (acetone-d_6+D_2O) δ: 2.32, 2.41 (each 1H, dd, J=16, 4 Hz, 4,4′-H), 2.71 (2H, d-like, J=16 Hz, 4,4′-H), 4.04, 4.12 (each 1H, m, 3,3′-H), 4.56, 4.70 (each 1H, s, 2,2′-H), 5.80 (2H), 5.95, 5.96 (each 1H, d, J=2.5 Hz, 6,6′,8,8′-H), 6.67, 6.88, 7.27 (each 1H, s, B,B′-ring-H). ^{13}C-NMR (acetone-d_6+D_2O) δ: 28.9, 29.5 (C-4,4′), 65.1, 65.3 (C-3,3′), 77.1 (C-2,2′), 95.8, 96.2 (C-6,6′,8,8′), 99.3 (C-4a,4′a), 108.0 (B′-ring C-6), 116.5, 117.6 (B-ring C-3, B′-ring C-2′), 119.5 (B-ring C-6), 125.4 (B-ring C-2), 128.1 (B-ring C-1), 131.1, 132.0 (B′-ring C-1′,4′), 143.9, 145.3 (B-ring C-4,5, B′-ring C-3′,5′), 157.4 (C-5,5′,7,7′,8a,8′a). CD (c=0.0028, MeOH) $[\theta]^{18}$ (nm): −82300 (220), 0 (232), 22900 (238), 0 (245), −4200 (249), 0 (262), 2800 (276).

Theasinensin G (7)——An off-white amorphous powder, $[\alpha]_D^{26}$ −103.0° (c=1.0, acetone). *Anal.* Calcd for $C_{44}H_{34}O_{21}\cdot 5H_2O$: C, 53.44; H, 4.49. Found: C, 53.33; H, 4.37. FAB-MS m/z: 899 [(M+H)$^+$, 1%]. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II. CD (c=0.0014, MeOH) $[\theta]^{18}$ (nm): −69500 (214), −52500 (217), −72000 (221), 0 (238), 13200 (245), 0 (255), −34800 (280).

Tannase Hydrolysis of 7——A solution of **7** (10 mg) in H_2O (2 ml) was shaken with tannase at room temperature for 10 min. The reaction mixture was worked up as described above to give gallic acid and **7a** (3 mg) as an off-white amorphous powder, $[\alpha]_D^{25}$ +104.5° (c=0.2, acetone). *Anal.* Calcd for $C_{30}H_{26}O_{13}\cdot 5/2H_2O$: C, 56.34; H, 4.89. Found: C, 56.33; H, 4.94. Negative FAB-MS m/z: 593 [(M−H)$^-$, 3%]. ^{1}H-NMR (acetone-d_6+D_2O) δ: 2.45 (2H, dd, J=17, 4 Hz, 4,4′-H), 2.70 (2H, d, J=17 Hz, 4,4′-H), 4.01 (2H, m, 3,3′-H), 4.81, 4.86 (each 1H, s, 2,2′-H), 5.90, 5.91, 5.98, 5.99 (each 1H, d, J=2 Hz, 6,6′,8,8′-H), 6.67, 7.18, 7.45 (each 1H, s, B,B′-ring-H). ^{13}C-NMR (acetone-d_6+D_2O) δ:

29.8 (C-4,4'), 64.9, 65.0 (C-3,3'), 76.4, 77.4 (C-2,2'), 95.5, 95.6, 96.2 (C-6,6',8,8'), 99.5, 99.6 (C-4a,4'a), 108.9 (B'-ring C-6'), 116.4, 118.6 (B-ring C-3, B'-ring C-2'), 120.1 (B-ring C-6), 126.5 (B-ring C-2), 129.4 (B-ring C-1), 131.1 (B'-ring C-1',4'), 144.8, 145.0, 145.1 (B-ring C-4,5, B'-ring C-3',5'), 157.0, 157.2, 157.3, 157.4 (C-5,5',7,7',8a,8'a). CD (c= 0.0016, MeOH) $[\theta]^{18}$ (nm): 33400 (224), 12400 (235), 49500 (249), 0 (268).

Oolongtheanin (8)——An off-white amorphous powder, $[\alpha]_D^{21}$ −112.9° (c=0.9, acetone). *Anal.* Calcd for $C_{36}H_{28}O_{17}\cdot 2H_2O$: C, 56.25; H, 4.20. Found: C, 56.48; H, 4.24. FAB-MS m/z: 733 $[(M+H)^+$, 8%], negative FAB-MS m/z: 731 $[(M-H)^-$, 1%]. IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1715 (C=O), 1618 (C=C). UV λ_{max}^{MeOH} nm (log ε): 272 (4.16). ^{1}H-NMR: Table I. ^{13}C-NMR: Table II. CD (c=0.0016, MeOH) $[\theta]^{20}$ (nm): −132000 (214), 0 (230), +48100 (239), 0 (280).

Oxidative Condensation of 9——A mixture of $K_3Fe(CN)_6$ (10 g) and $NaHCO_3$ (6 g) in H_2O (100 ml) was added stepwise to an ice-cooled solution of **9** (10 g) in H_2O (500 ml). After stirring for 1 h, the reaction mixture was directly applied to a column of MCI-gel CHP-20P. Elution with H_2O containing increasing amounts of MeOH gave crude **1**, **4** and **8c**, which were purified on Sephadex LH-20, Fuji gel and Bondapak C_{18} with various solvent systems to afford pure samples of **1** (1.18 g) and **4** (174 mg), together with **8c** (39 mg) as a pale brown amorphous powder, $[\alpha]_D^{28}$ −111.1° (c=0.6, MeOH). *Anal.* Calcd for $C_{43}H_{32}O_{21}\cdot 7H_2O$: C, 51.09; H, 4.59. Found: C, 50.98; H, 4.42. Negative FAB-MS m/z: 883 $[(M-H)^-$, 1%]. ^{1}H-NMR (acetone-d_6 + D_2O) δ: 2.66—3.20 (4H, m, 4,4'-H), 4.64 (1H, s, B'-ring 5'-H), 4.71 (1H, s, 2'-H), 5.40 (1H, m, 3-H), 5.45 (1H, s, 2-H), 5.76 (1H, m, 3'-H), 5.88, 6.00, 6.03, 6.08 (each 1H, d, J=2 Hz, 6,6',8,8'-H), 6.58 (1H, s, B'-ring 2'-H), 6.92 (B-ring 6-H), 7.08 (4H, s, 2 × galloyl-H). ^{13}C-NMR (acetone-d_6 + D_2O) δ: 26.6 (C-4,4'), 53.9 (B'-ring C-5'), 66.2, 69.3 (C-3',3), 75.6, 77.9 (C-2',2), 93.7 (B'-ring C-4'), 95.5, 96.5, 96.8 (C-6,6',8,8'), 98.5, 99.0 (C-4a,4'a), 108.5 (B-ring C-6), 110.0 (2 × galloyl C-2,6), 115.8 (B-ring C-2), 120.1, 121.2 (each galloyl C-1), 124.4 (B'-ring C-2'), 130.4 (B-ring C-1), 133.6 (B-ring C-4), 139.1, 139.4 (each galloyl C-4), 145.3, 145.5, 145.9 (B-ring C-3,5, 2 × galloyl C-3,5), 155.9 (B'-ring C-1'), 156.3, 157.1, 157.3 (C-5,5',7,7',8a,8'a), 166.5, 166.8 (each COO), 199.4 (CO). CD (c=0.0025, MeOH) $[\theta]^{20}$ (nm): −85200 (224), 0 (234), +40600 (240), 0 (271), −16200 (285).

Oxidative Condensation of 9 and 10——A mixture of $K_3Fe(CN)_6$ (6 g) and $NaHCO_3$ (3.5 g) in H_2O (50 ml) was added stepwise to an ice-cooled solution of **9** (2.5 g) and **10** (2.5 g) in H_2O (500 ml). After 1 h, the reaction mixture was treated in the same way as described above to yield compounds **1** (57 mg), **2** (195 mg), **3** (184 mg), **4** (12 mg) and **8** (16 mg), together with compound **12** (16 mg) as an off-white amorphous powder, $[\alpha]_D^{28}$ −52.6° (c=0.7, acetone). *Anal.* Calcd for $C_{37}H_{30}O_{18}\cdot 3H_2O$: C, 54.41; H, 4.44. Found: C, 54.41; H, 4.47. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II. CD (c=0.0016, MeOH) $[\theta]^{20}$ (nm): −64700 (219), 0 (250), −17800 (276).

Methylation of 8——A solution of **8** (10 mg) in MeOH (5 ml) was treated overnight with ethereal diazomethane. Usual work-up afforded the decamethyl ether (**8b**) (7 mg) as a white amorphous powder, $[\alpha]_D^{28}$ −71.7° (c=0.7, acetone). *Anal.* Calcd for $C_{46}H_{48}O_{17}\cdot 1/2H_2O$: C, 62.65; H, 5.60. Found: C, 62.67; H, 5.80. FD-MS m/z: 872 $[(M)^+$, 100%]. ^{1}H-NMR ($CDCl_3$) δ: 2.83 (2H, m, 4'-H), 3.00 (1H, d, J=16 Hz, 4-H), 3.40 (1H, dd, J=16, 4 Hz, 4-H), 3.35, 3.73, 3.76, 3.80, 3.81, 3.86, 3.89 (30H in total, each s, 10 × OCH_3), 4.23 (1H, s, 2'-H), 4.46 (1H, s, B'-ring 5'-H), 4.49 (1H, m, 3'-H), 5.51 (1H, s, 2-H), 5.62 (1H, m, 3-H), 6.01, 6.08, 6.12, 6.19 (each 1H, d, J=2 Hz, 6,6',8,8'-H), 6.67, 6.78 (each 1H, s, B'-ring 2'-H, B-ring 6-H), 7.26 (2H, s, galloyl 2,6-H).

Tannase Hydrolysis of 8——A solution of **8** (15 mg) in H_2O (2 ml) was treated with tannase for 10 min. Work-up as described above gave gallic acid and **8a** (7 mg) as an off-white amorphous powder, $[\alpha]_D^{28}$ +17.9° (c=0.6, MeOH). *Anal.* Calcd for $C_{29}H_{24}O_{13}\cdot 4H_2O$: C, 53.37; H, 4.94. Found: C, 53.61; H, 4.78. FAB-MS m/z: 581 $[(M+H)^+$, 5%]. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II. CD (c=0.0020, MeOH) $[\theta]^{18}$ (nm): 0 (205), −130000 (212), 0 (229), +77300 (239), 0 (279), −4600 (284).

Tannase Hydrolysis of 8c——A solution of **8c** (10 mg) in H_2O (2 ml) was incubated with tannase for 10 min. Work-up as described above gave gallic acid and **8a** (3 mg).

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References

1) Part LXVIII: Y. Kashiwada, G. Nonaka, and I. Nishioka, *Phytochemistry*, submitted.
2) G. Nonaka, R. Sakai, and I. Nishioka, *Phytochemistry*, **23**, 1753 (1984).
3) G. Nonaka, F. Hashimoto, and I. Nishioka, *Chem. Pharm. Bull.*, **34**, 61 (1986).
4) G. Nonaka, O. Kawahara, and I. Nishioka, *Chem. Pharm. Bull.*, **31**, 3906 (1983).
5) F. Hashimoto, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **35**, 611 (1987).
6) D. Jacques, C. T. Opei, L. J. Porter, and E. Haslam, *J. Chem. Soc., Perkin Trans. 1*, **1977**, 1637.
7) Y. Ikeya, H. Taguchi, I. Yosioka, and H. Kobayashi, *Chem. Pharm. Bull.*, **27**, 1383 (1979).