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Isolation of key intermediates during formation of oolongtheanins

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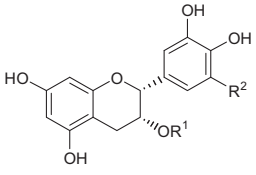
ABSTRACT

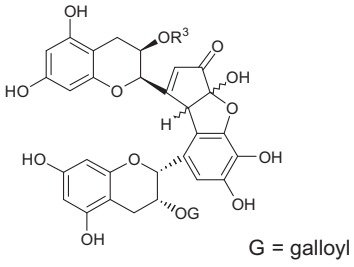
Oolongtheanin-3′-*O*-gallate (**2b**) was obtained by treatment of (−)-EGCg (**1d**) with $CuCl_2$. This transformation was achieved over three steps, with the isolation of two intermediates; their chemical structures were determined through derivatization reactions, MS, and 1D/2D NMR techniques. One intermediate was identified as dehydrotheasinensin A (**3**); the other was identified as the novel dimer pro-oolongtheanin-3′-*O*-gallate (**6**). Compound **3** was converted to **6** by heating in aprotic solvent, and compound **6** was converted to **2b** by addition of water.

Tea (*Camellia sinensis*) is the most widely consumed beverage in the world, and different types of tea can be classified by differences in the processing methods, as green tea (unfermented tea), oolong tea (semi-fermented tea), and black tea (fermented tea). Catechins (Fig. 1), some of the major polyphenols in tea leaves, have attracted attention because of their various health benefits, including antioxidant activity[1a,b] and antitumor activity.[2a,b] Catechins in tea leaves are oxidized during the fermentation process, triggering the formation of dimers such as theaflavins, theasinensins, oolongtheanins, and other polymers. Oolongtheanin (**2a**)[3] is the characteristic dimer found in oolong tea, and is expected to have various bioactivities[4] (Fig. 1); however, detailed studies have thus far been limited by the fact that such components are found as complicated mixtures in oolong tea and are difficult to isolate.

Many studies on the oxidation mechanisms of tea catechins have been reported to date. Mechanistic studies on the formation of theaflavins—pigments found in black tea, which are formed by the dimerization of catechins—have been reported by Takino et al.[5] and Salfeld.[6] Previously, we also reported the isolation of a bicyclo[3.2.1]-type intermediate in the formation of benzotropolone, which is used as a model to study theaflavin formation.[7] While the formation mechanism for theasinensin A (**3**) via



1a: $R^1 = R^2 = H$, Epicatechin
1b: R^1 = galloyl, R^2 = H, Epicatechin gallate
1c: R^1 = H, R^2 = OH, Epigallocatechin
1d: R^1 = galloyl, R^2 = OH, Epigallocatechin gallate



2a: $R^3 = H$, Oolongtheanin
2b: R^3 = galloyl, Oolongtheanin-3'-*O*-gallate

Figure 1. Chemical structures of tea polyphenols.

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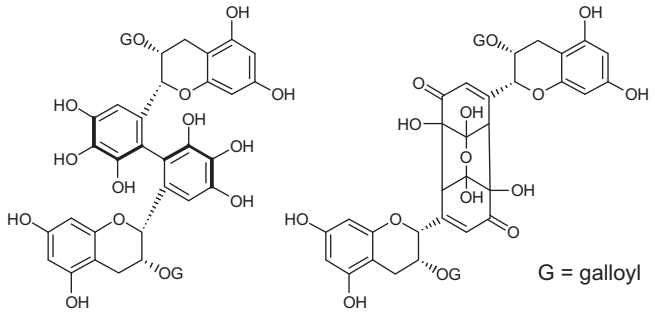

3 4

Figure 2. Chemical structures of **3** and **4**.

dehydrotheasinensin A (**5**) has been reported by Tanaka et al.,[8] analogous studies on oolongtheanin formation are scarce.[9a,b] Therefore, we have initiated oxidation studies of catechins and isolation of the reaction intermediates for the purpose of clarifying the formation mechanism of oolongtheanins.

The transformation of (−)-epigallocatechin gallate ((−)-EGCg; **1d**) has been conducted under various oxidative conditions, including $K_3[Fe(CN)_6]$,[10] Pd/C,[11] and $CuCl_2$.[12] When **1d** was treated with $K_3[Fe(CN)_6]$ or Pd/C, theasinensin A (**3**) and compound **4**[13] were generated (Fig. 2). In contrast, treatment of **1d** with $CuCl_2$, which was previously reported to effect the formation of **3**,[12] yielded only small amounts of **3**; generation of **4** was not observed. Although these dimers are composed of identical catechin units, the bonds connecting the dimeric species differ; moreover, dimers **3** and **2a** possess similar connectivities. Thus, $CuCl_2$ was suggested as a potential reagent for oolongtheanin synthesis. This reaction was examined in greater detail, revealing that oolongtheanin-3′-*O*-gallate (**2b**) could be formed in three steps via two intermediates, called intermediates I and II.

The reaction solution of **1d** after treatment with $CuCl_2$ was extracted with EtOAc/H_2O. The aqueous layer was subjected to column chromatography using a Diaion HP20SS resin (Mitsubishi Chemical Co.). The column was eluted with acetonitrile after washing with water to remove $CuCl_2$, providing intermediate I in very high purity. The matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) measurements of intermediate I showed a molecular ion peak at *m*/*z* 953 $[M+Na]^+$. The ^1H and ^{13}C NMR, HMQC, and HMBC spectra were obtained and assigned; accordingly, its chemical structure was presumed as dehydrotheasinensin A (**5**), which has been previously reported by Tanaka et al. as the intermediate during formation of **3** by reaction with ascorbic acid.[8,12] The identity of intermediate I was further confirmed by treatment with ascorbic acid, resulting in immediate conversion to **3**; thus, intermediate I was established as **5**.

Intermediate II was obtained by heating **5** in 1,4-dioxane. The MALDI-TOF-MS of intermediate II showed a molecular ion peak at *m*/*z* 935 $[M+Na]^+$. A comparison of IR spectra indicated the

Table 1
^1H and ^{13}C NMR data for compounds **6**–**8** (600/150 MHz)

Position	Compound **6** (d_6-acetone)		Major of **7** (d_6-acetone)		Compound **8** (CD_3OD)	
	^1H (*J*, Hz)	^{13}C	^1H (*J*, Hz)	^{13}C	^1H (*J*, Hz)	^{13}C
2	5.82 (s)	74.3	5.89 (s)	73.6	5.00 (s)	76.4
3	5.55 (b s)r	68.4	5.63 (br s)	68.0	5.68 (br s)	69.0
4	3.13 (dd, 4.1, 17.0)	26.9	3.17 (dd, 4.8, 17.1)	25.5	2.90 (dd, 4.1, 17.1)	27.7
	2.94 (d, 17.0)		2.93–2.96 (d)		2.85 (d, 17.1)	
4a		98.7		98.0		99.7
5		157.6		156.4		158.0
6	6.00 (br s)	96.6	6.02 (d, 2.0)	95.8	5.78 (d, 2.0)	96.6
7		157.6		156.9		158.0
8	5.97 (d, 2.0)	95.7	5.99 (d, 2.0)	94.9	5.85 (d, 2.0)	95.7
8a		157.0		156.1		157.5
1′		128.0		126.2		128.0
2′		108.0		112.4		117.4
3′		140.4		138.8		148.9
4′		134.6		136.5		131.0
5′		147.3		145.1		148.5
6′	7.32 (s)	113.3	7.08 (s)	110.3	6.63 (s)	109.5
2″	5.02 (s)	75.7	4.94 (s)	74.6	4.02 (s)	76.4
3″	4.50 (br s)	65.9	4.47 (br s)	65.5	5.88 (br s)	65.6
4″	2.74 (dd, 4.1, 17.1)	26.1	2.80-2.84 (m)	25.5	2.68 (dd, 4.1, 17.1)	27.0
	2.84 (d, 17.0)				2.83 (d. 17.1)	
4″a		98.2		97.9		99.2
5″		157.6		156.5		157.8
6″	6.00 (br s)	96.9	5.99 (d, 2.0)	96.1	5.84 (br s)	97.1
7″		157.6		156.5		157.8
8″	5.94 (d, 2.1)	95.5	5.93 (d, 2.0)	94.8	5.83 (br s)	95.8
8″a		155.6		155.1		156.0
1‴		171.6		174.3		91.8
2‴	6.52 (s)	128.6	6.43 (s)	128.6	3.43 (d, 19.9)	43.5
					2.97 (d, 19.9)	
3‴		198.2		206.1		213.5
4‴		164.3	5.26 (s)	94.9		171.1
5‴		81.1		79.8		86.6
6‴	4.93 (s)	47.2	4.56 (s)	47.8	4.14 (s)	59.8
–OMe		—		—	3.30 (s)	53.0
Galloyl–COO–		165.3, 165.9		165.4, 164.4		167.5, 167.7
Galloyl-1		121.0, 121.4		120.6		121.1, 121.7
Galloyl-2,6	6.90 (s), 7.07 (s)	109.8, 109.9	7.03 (s), 6.94 (s)	109.1	6.95 (s), 6.94 (s)	110.1
Galloyl-3,5		138.8		138.0		139.9, 139.8
Galloyl-4		145.9, 145.7		145.0		146.6, 146.3

presence of a carbonyl group (1767 cm^{-1}) in compound **6**; ^{13}C NMR exhibited the presence of an ester moiety (δ_C 164.3 ppm) (Table 1). To establish its identity, intermediate II was subsequently treated with $NaBH_4$, generating compound **7**. The ^1H NMR spectrum of **7** indicated the presence of two isomeric mixtures at a ratio of 1:2. The ^{13}C NMR spectrum lacked an ester signal; instead, the appearance of a new signal at 94.9 ppm indicated the presence of an acetal moiety, suggesting a transformation of the ester moiety to an acetal under reductive conditions. Furthermore, the appearance of one additional proton signal was observed in ^1H NMR, and the MALDI-TOF-MS showed a peak at *m*/*z* 937 $[M+Na]^+$; namely, compound **7** was generated by the one-step reduction of the ester moiety in intermediate II. On the basis of these results and 2D NMR spectral analysis, the chemical structure of **7** was determined as shown in Figure 3. Therefore, intermediate II was identified based on the above results as **6** (Fig. 3), and the relative stereochemistry of **6** was determined by ROESY correlations (Fig. 4). We have named **6** pro-oolongtheanin-3′-*O*-gallate. Further confirmation that **6** was the reaction intermediate was established by conversion of **6** to **2b** in water.

The chemoselectivity of $NaBH_4$-mediated carbonyl reductions toward aldehydes and ketones is well known; the reduction of esters and lactones by $NaBH_4$, however, requires a large excess of the reagent, and/or high reaction temperature conditions.[14a,b] Compound **6** has a hydroxyl group at the α-position of lactone. Therefore, it was suggested that $NaBH_4$ might form a complex between the α-hydroxy group and lactone carbonyl, thus facilitating nucleophilic attack by hydride on the lactone carbonyl group to generate a lactol. Although the hydroxyl group of **6** is also situated in an α-position to the adjacent ketone, the nearly coplanar orientation of the hydroxyl group with the lactone carbonyl results in preferential complex formation with the lactone instead of the ketone (Fig. 5).[15a,b] When **6** was reduced with an excess of $NaBH_4$ or a more reactive reducing agent such as $LiAlH_4$, a complex mixture of products was generated, which was attributed to the reduction

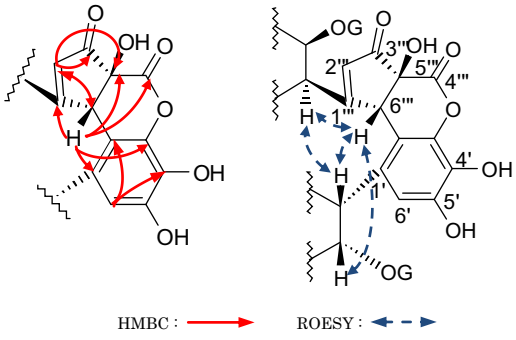

Figure 4. Key HMBC and ROESY correlations of **6**.

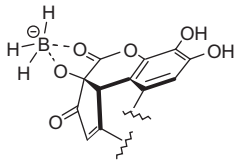

Figure 5. Formation of complex between α-hydroxy and lactone carbonyl group.

of the α,β-unsaturated ketone and/or transformation to the alcohol by lactone ring-opening.

When **6** was reacted with methanol instead of water, compound **8** was observed. Electrospray ionization mass spectrometry (ESI-MS) of **6** showed a molecular ion peak at *m*/*z* 943 $[M-H]^-$. Moreover, ^1H and ^{13}C NMR data revealed the presence of a methyl ester moiety; thus the addition of a methoxy unit was suggested (Table 1). As a result, the chemical structure of **8** was determined as shown in Figure 3. Compound **8** was formed via recyclization

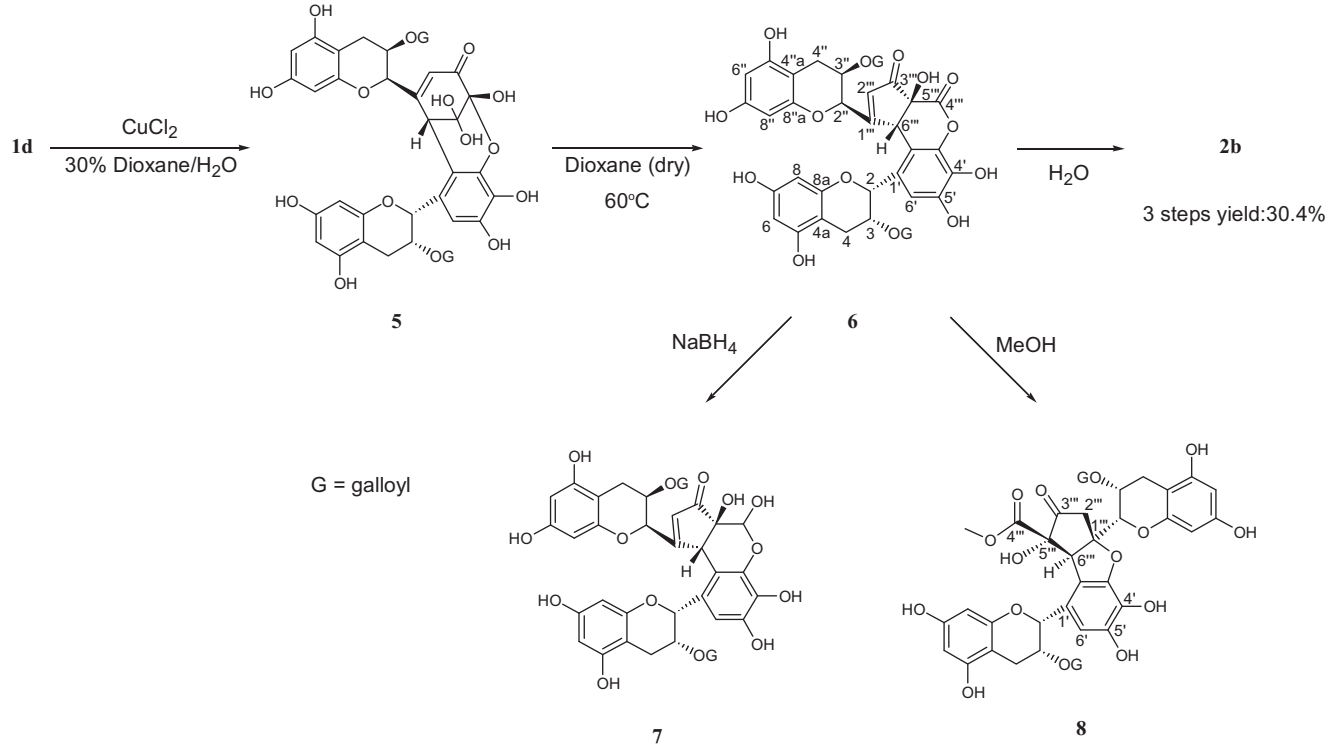

Figure 3. Preparation of **5**–**8** and **2b** from **1d**.

after a nucleophilic ring-opening reaction by the attack of methanol on the lactone moiety. The stereochemistry of **6** was predicted from the mechanism of formation.

In conclusion, we have succeeded in the synthesis of oolongtheanin-3′-*O*-gallate (**2b**), and we clarified that this reaction proceeds over three steps. Furthermore, dehydrotheasinensin A (**5**) and novel catechin dimer, pro-oolongtheanin-3′-*O*-gallate (**6**), were isolated as the key intermediates of this reaction sequence. Identification of these intermediates is anticipated to aid in clarification of the formation mechanism of complex oolong tea polyphenols. Further mechanistic studies are now in progress.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.10.069.

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