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Synthesis of oolongtheanins and their inhibitory activity on micellar cholesterol solubility in vitro

Kazuki Ogawa, Sayumi Hirose, Hitomi Yamamoto, Masaya Shimada, Satoshi Nagaoka, Emiko Yanase *

Faculty of Applied Biological Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

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ABSTRACT

The synthesis of oolongtheanins (**1a–d**) was accomplished from EGC and/or EGCg in three steps. Oolongtheanin-3′-*O*-gallate (**1b**) showed more potent inhibitory activity on micellar cholesterol solubility than did EGCg.

Tea (*Camellia sinensis*) is a globally popular beverage. It is classified as green tea, oolong tea and black tea according to the fermentation conditions of the tea leaves. Oolong tea has attracted attention because of its health benefits, such as its anti-obesity effects.[1] Obesity and hyperlipidemia increases the risk of various diseases, such as heart disease and cerebral arterial disease.[2] Oolong tea is traditionally reported to be effective for the prevention of obesity and improvement of lipid metabolism. Recently, anti-hypocholesterolemic activity has been reported in the green tea catechins and the polymerized polyphenols of black tea and oolong tea.[3–9] Oolongtheanins (**1a–1d**; Fig. 1)[10] are one of the characteristic polyphenols found in oolong tea and are also predicted to exert various bioactive effects.[11,12] Recently their chemical structures were revised by Tanaka et al.[2] However, detailed studies on their activity have thus far been limited because the numerous components found in the extract leads to a complicated mixture and thus, preparative scale isolation is difficult. A few synthetic studies towards **1a** and **1b** have been reported using enzymatic oxidation.[13] The generation of oolongtheanin-3′-*O*-gallate (**1b**) was reported as a byproduct during the synthesis of theasinensin A (**2**) using $CuCl_2$ as an oxidizing agent.[14,15] However, an efficient synthetic method for oolongtheanins (**1a–d**) has not been reported. In a previous study, we proved the mechanism for the formation of **1a** and **1b** from EGC (**3**) or EGCg (**4**).[16] In this study, we investigated effective synthetic methods towards **1a** and **1b**, which are homodimers of **3** and **4**, and the syntheses of **1c** and **1d**, which are heterodimers of **3** and **4**.

We also examined the ability of synthetic oolongtheanins to decrease the micellar solubility of cholesterol using a model micelle system in vitro.[17]

First, the synthesis of **1a** and **1b**, which are homodimers of **3** and **4**, was investigated (Scheme 1). Previously, we achieved the transformation of **4** to **1b** in three steps *via* two intermediates. Therefore, we now moved on to optimizing each step of the reaction to inhibit formation of byproducts and to increase the yields of oolongtheanins as follows: for the first step, a solution of **4** was treated with $CuCl_2{\cdot}2H_2O$ in 30% aq MeOH under optimal conditions. The reaction was monitored by HPLC and three new peaks were observed (data not shown). The chemical structures corresponding to these peaks were determined by LC/MS and NMR to be **2**, **5b**, and the MeOH adduct of **5** (**5MeOH**), respectively. The transformation of **5MeOH** to **5b** was not observed. Hence, the same reaction was carried out in 30% aq 1,4-dioxane, which would not form an adduct, and the yield of **5b** was improved. The resulting solution was subjected to HP20SS column chromatography, and was eluted with CH_3CN after washing with water to remove $CuCl_2{\cdot}2H_2O$, providing crude product **5b**, which was used directly in the next reaction. The transformation of **3** to **5a** was performed in the same manner.

Crude **5b** was transformed to **6b** by a dehydration and rearrangement reaction. The reaction was performed under anhydrous

Abbreviations: EC, (−)-epicatechin; EGC, (−)-epigallocatechin; ECg, (−)-epicatechin-3-*O*-gallate; EGCg, (−)-epigallocatechin-3-*O*-gallate.

* Corresponding author. Tel./fax: +81 08 58 293 2914.
E-mail address: e-yanase@gifu-u.ac.jp (E. Yanase).

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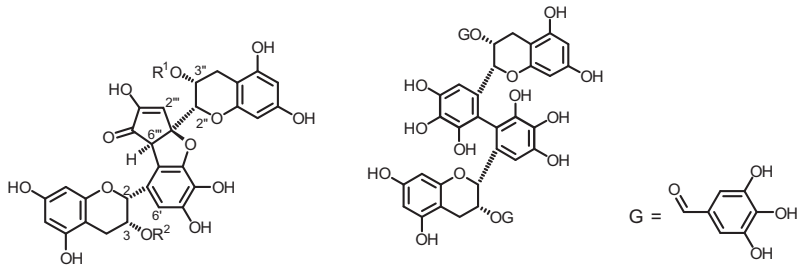
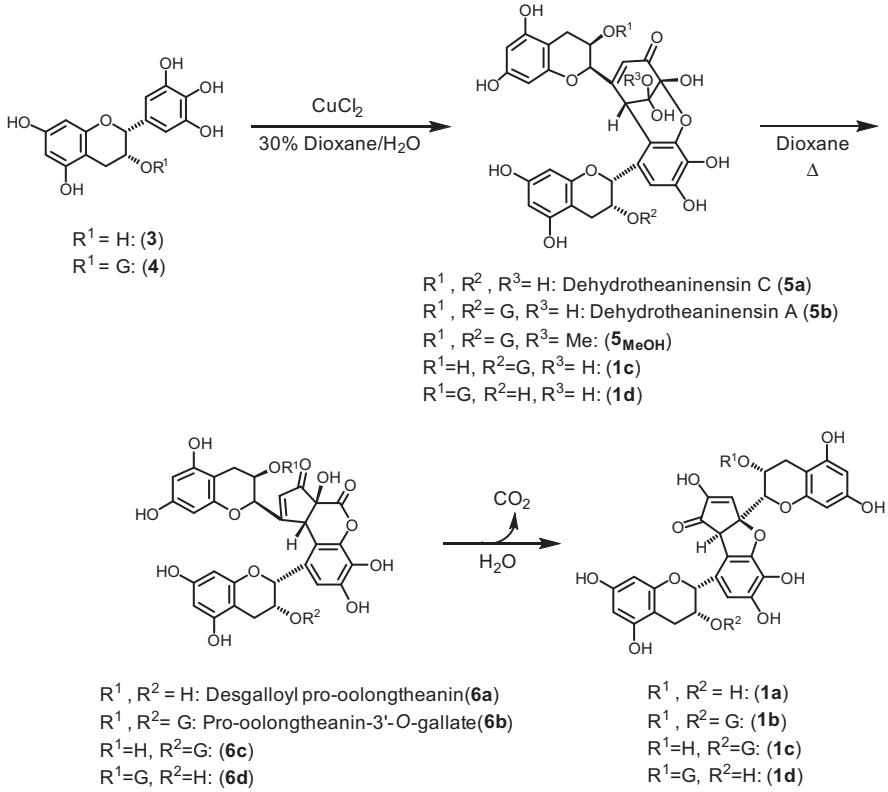


1a : R^1, R^2 = H, Desgaloyl oolongtheanin
1b : R^1, R^2 = G, Oolongtheanin-3'- *O*-gallate
1c : R^1 = H, R^2 = G, Oolongtheanin
1d : R^1 = G, R^2 = H

2 : theasinensin A

Figure 1. Structure of tea polyphenols.



R^1 = H: (**3**)
R^1 = G: (**4**)

R^1, R^2, R^3= H: Dehydrotheaninensin C (**5a**)
R^1, R^2= G, R^3= H: Dehydrotheaninensin A (**5b**)
R^1, R^2= G, R^3= Me: (**5_{MeOH}**)
R^1=H, R^2=G, R^3= H: (**1c**)
R^1=G, R^2=H, R^3= H: (**1d**)

R^1, R^2 = H: Desgalloyl pro-oolongtheanin(**6a**)
R^1, R^2= G: Pro-oolongtheanin-3'-*O*-gallate(**6b**)
R^1=H, R^2=G: (**6c**)
R^1=G, R^2=H: (**6d**)

R^1, R^2 = H: (**1a**)
R^1, R^2= G: (**1b**)
R^1=H, R^2=G: (**1c**)
R^1=G, R^2=H: (**1d**)

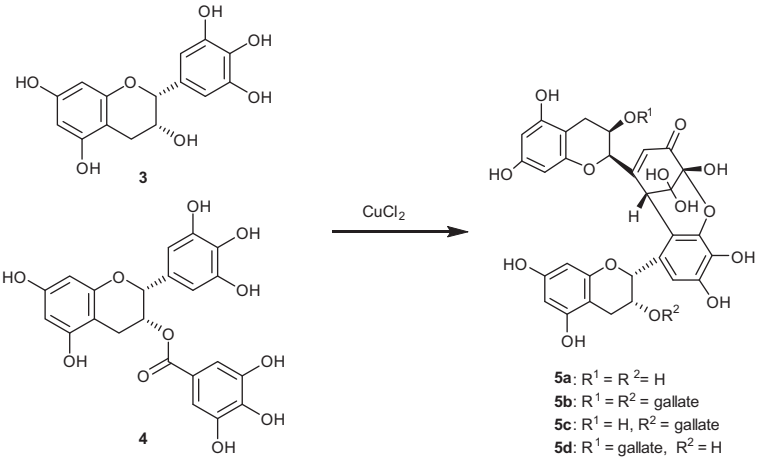
Scheme 1. Synthesis of oolongtheanins (**1a**–**d**).

conditions for efficient conversion. A solution of **5b** in anhydrous 1,4-dioxane was heated at 60 °C to give crude **6b**. The same procedure could be used for the conversion of **5a** to **6a**.

The transformation of **6b** to **1b** was carried out using water as the solvent, for hydrolysis of the lactone moiety. The synthesis of **1b** was completed in 39% yield from **4**. Although the transformation of **6a** to **1a** was performed in the same manner, the yield of **1a** was significantly lower than **1b** due to the generation of an unidentified byproduct. No byproduct was observed when using 50% aq CH_3CN instead of water and the synthesis of **1a** was completed in 40% yield from **3**.

Next, the synthesis of **1c** and **1d**, which are heterodimers of **3** and **4**, were investigated. When oxidation of the mixture of **3** and **4** was performed in the same manner, **1a**–**d** were prepared in almost equal amounts. It is assumed that the initial step for the formation mechanism of oolongtheanin involves a nucleophilic attack of either **3** or **4** on *o*-quinone, which is formed from another molecule of **3** or **4**.[16] Hence, the upper unit of oolongtheanin is derived from the oxidant of **3** or **4**, while the lower unit is derived from nucleophilic attack of **3** or **4**. For the preparation of each homodimer, the reaction rate from **3** was slightly faster than that from **4**. This result suggests that **3** is more susceptible to oxidation or is a better nucleophile than **4.** Therefore, the selective synthesis of either one of the heterotype dimers could be possible. We optimized the reaction conditions for the transformation of **3** and **4** to **5a**–**d**. The results are shown in Table 1. The product ratio of the reaction mixture under each set of conditions were calculated on the basis of an integrated value of peaks in the ^{1}H NMR spectrum. The results indicate that the ratio for generation of the analogues of **5** was influenced by the nature of the solvent. When the mixture of

Table 1
Oxidative coupling reaction of **3** and **4**



Entry	Solvent	Product ratio			
		5a	**5b**	**5c**	**5d**
1	30% aq dioxane	1	1	1	1
2	50% aq dioxane	1	1	1	1
3	10% aq dioxane	1	1	1	1
4	H_2O	2	1	2	1

The product ratio of the reaction mixture was calculated on the basis of the integrals of the peaks in the ^{1}H NMR spectrum.

Table 2
^{1}H NMR (500 MHz) data for oolongtheanins in d_6-acetone (ppm)

Position	**1a**	**1b**	**1c**	**1d**
2	5.16	5.44	5.42	5.19
3	4.00	5.44	5.40	4.02
2″	4.35	4.68	4.38	4.67
3″	4.38	5.75	4.42	5.77
6'	6.90	6.88	6.84	6.93
2‴	6.61	6.56	6.65	6.54
6‴	4.58	4.67	4.67	4.55
A ring	5.97	6.06	6.05	6.02
	5.96	6.01	5.99	6.00
	5.83	5.99	5.98	5.88
	5.75	5.89	5.78	5.87
Galloyl 2,6		7.09	7.08	7.08
		7.06		

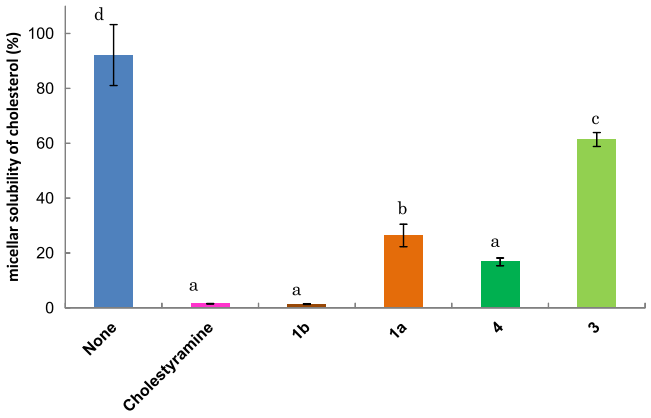


Figure 2. Inhibitory activity on micellar cholesterol solubility. Values are expressed as means ± SEM ($n = 4$). Means with different superscript letters are significantly different ($P < 0.05$) by Tukey's test.

3 and **4** was treated with $CuCl_2 \cdot 2H_2O$ in ~10–50% aq 1,4-dioxane, compounds **5a–d** were generated in almost equal amounts (entries 1–3). On the other hand, when this treatment was carried out in water, generation of compounds **5b** and **5d** was suppressed and **5a** and **5c** were generated as the major products (entry 4).

The mixture of **5a–d** was treated in the same manner as previously to afford a mixture of **1a–d** (Scheme 1). Compounds **1a–d** were separated by preparative HPLC and their chemical structures were determined through ^{1}H NMR spectroscopy (Table 2). Compound **5d**, which was difficult to obtain from this method, was successfully generated from **5b** by selective hydrolysis of the gallate group using the enzymatic reaction of tannase (data not shown).

The micellar solubility of cholesterol with **1a**, **1b**, **3**, **4**, and cholestyramine, which is currently used as a medicine, was measured in vitro by a previously described method (Fig. 2).[17] As a result, **1b** showed a strong inhibitory ability on the micellar solubility of cholesterol (1.40 ± 0.14%), which was comparable to the positive control, cholestyramine (1.53 ± 0.08%). In addition, catechins and oolongtheanins with a galloyl moiety showed stronger inhibitory effects than those without the galloyl moiety. This result was in good agreement with previous reports which had shown that catechins with a galloyl moiety have a higher inhibitory ability than free catechins.[3,9,12] Intriguingly, although **1a** does not contain a galloyl moiety, a relatively strong inhibitory activity was shown (26.39 ± 4.10%). These data suggested that not only the presence of the galloyl moiety, but also the presence of the 5,5,6 ring system within **1a** and **1b** contributed to the enhanced inhibitory ability.

In conclusion, we have succeeded in the effective synthesis of oolongtheanins **1a–d** based on their formation via oxidative coupling. Moreover, **1b** showed strong inhibitory ability, comparable to cholestyramine, for the micellar solubility of cholesterol in vitro.

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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.bmcl.2015.01.002.

References and notes

1. Han, L.-K.; Takaku, T.; Li, J.; Kimura, Y.; Okuda, H. *Int. J. Obes.* **1999**, *23*, 98.
2. Tanaka, T.; Yan, Li.; Matsuo, Y.; Shibahara, A.; Kouno, I. *Abstract of Paper*, XXVth International Conference on Polyphenols **2010**; 165–166.
3. Kajiya, K.; Kumazawa, S.; Nakayama, T. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 2638.
4. Yang, H.-M.; Wang, H.-C.; Chen, H.-L. *J. Nutr. Biochem.* **2001**, *12*, 14.
5. Ikeda, I.; Kobayashi, M.; Hamada, T.; Tsuda, T.; Goto, H.; Imaizumi, K.; Nozawa, A.; Sugimoto, A.; Kakuda, T. *J. Agric. Food Chem.* **2003**, *51*, 7303.
6. Hara, Y.; Moriguchi, S.; Kusumoto, A.; Nakai, M.; Ono, Y.; Abe, K.; Ohta, H.; Shibata, H.; Kiso, Y. *Jpn. Pharmacol. Ther.* **2004**, *32*, 335.
7. Toyoda-Ono, Y.; Yoshimura, M.; Nakai, M.; Fukui, Y.; Asami, S.; Shibata, H.; Kiso, Y.; Ikeda, I. *Biosci. Biotechnol. Biochem.* **2007**, *71*, 971.
8. Ikeda, I.; Yamahira, T.; Kato, M.; Ishikawa, A. *J. Agric. Food Chem.* **2010**, *58*, 8591.
9. Kobayashi, M.; Nishizawa, M.; Inoue, N.; Hosoya, T.; Yoshida, M.; Ukawa, Y.; Sagesaka, Y. M.; Doi, T.; Nakayama, T.; Kumazawa, S.; Ikeda, I. *J. Agric. Food Chem.* **2014**, *62*, 2881.
10. Hashimoto, F.; Nonaka, G.; Nishioka, I. *Chem. Pharm. Bull.* **1988**, *36*, 1676.
11. Akizawa, T.; Yahara, S.; Hashimoto, F.; Yamada, M.; Suma, S.; Kono, T.; Uchida, K.; Oshiba, Y. Jpn. Kokai Tokkyo Koho JP 2000226329 A, 2000.
12. Nakai, M.; Fukui, Y.; Asami, S.; Toyoda-Ono, Y.; Iwashita, T.; Shibata, H.; Mitsunaga, T.; Hashimoto, F.; Kiso, Y. *J. Agric. Food. Chem.* **2005**, *53*, 4593.
13. Tanaka, T.; Matsuo, Y.; Kouno, I. *J. Agric. Food Chem.* **2005**, *53*, 7571.
14. Matsuo, Y.; Tanaka, T.; Kouno, I. *Tetrahedron* **2006**, *62*, 4774.
15. Tanaka, T.; Watarumi, S.; Matsuo, Y.; Kamei, M.; Kouno, I. *Tetrahedron* **2003**, *59*, 7939.
16. Hirose, S.; Tomatsu, K.; Yanase, E. *Tetrahedron Lett.* **2013**, *54*, 7040.
17. Nagaoka, S.; Nakamura, A.; Shibata, H.; Kanamaru, Y. *Biosci. Biotechnol. Biochem.* **2010**, *74*, 1738.