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Nonenzymatic Biomimetic Synthesis of Black Tea Pigment Theaflavins

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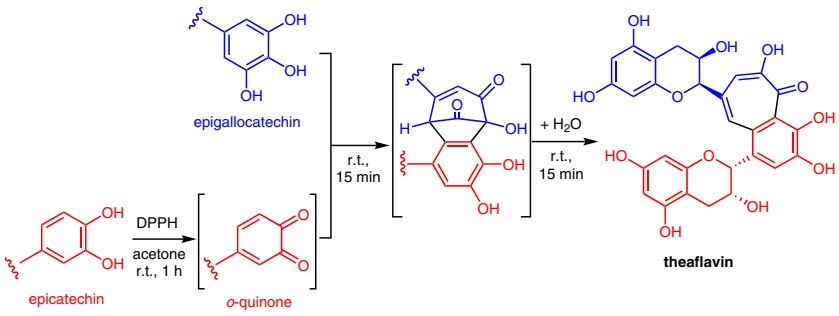

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Abstract Theaflavins are reddish-orange black tea pigments with a benzotropolone chromophore, and their various biological activities have been reported. Theaflavins are produced by oxidative coupling between catechol-type and pyrogallol-type catechins via bicyclo[3.2.1]octane-type intermediates. In this study, a new method for nonenzymatic biomimetic synthesis of theaflavins was developed using the DPPH radical as an oxidizing agent.

Key words theaflavins, quinones, black tea, benzotropolones, bicyclo[3.2.1]octane, biomimetic synthesis

Plant polyphenols have attracted great attention due to their various health benefits,[1] and black tea is one of the most important beverages with abundant polyphenols. Black tea is produced from the leaves of *Camellia sinensis*, which contain tea catechins as major polyphenols.[2] In the process of black tea production, tea catechins are oxidized enzymatically to afford various catechin dimers, such as theaflavins, theasinensins, and theacitrins, along with high-molecular-weight products known as thearubigins.[3] Theaflavins are reddish-orange pigments with a benzotropolone chromophore and are mainly composed of four compounds, theaflavin (**1**), theaflavin-3-*O*-gallate (**2**), theaflavin-3′-*O*-gallate (**3**), and theaflavin-3,3′-di-*O*-gallate (**4**) (Figure 1).[4] Many studies on the biological activities of theaflavins, such as α-amylase inhibition,[5] α-glucosidase inhibition,[6] lipase inhibition,[7] and anti-inflammatory activity,[8] have been reported. Theaflavins are produced by oxidative condensation between catechol-type catechins (epicatechin (**5**) and epicatechin-3-*O*-gallate (**6**)) and pyrogallol-type catechins (epigallocatechin (**7**) and epigallocatechin-3-*O*-gallate (**8**)). Yanase et al. reported that the benzotropolone moiety of theaflavins is formed via a bicyclo[3.2.1]octane-type intermediate produced by coupling of a pyrogallol-type B-ring with an *o*-quinone form of catechol-type B-ring.[9] The bicyclo[3.2.1]octane-type intermediate is stable in aprotic solvents; however, in aqueous solution, the carbonyl group of the intermediate readily reacts with a water molecule and successive oxidation and decarboxylation afford the benzotropolone structure (Scheme 1).[9]

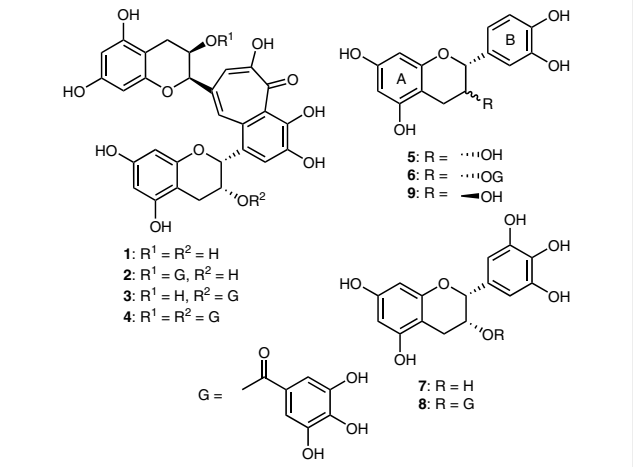
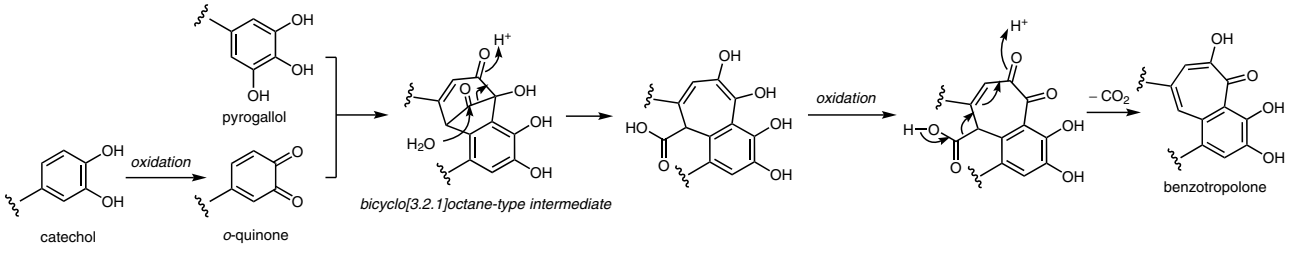


Figure 1 Structures of theaflavins **1**–**4** and tea catechins **5**–**9**

Up to now, several methods of synthesizing theaflavins by enzymatic or nonenzymatic oxidation have been reported. Enzymatic methods were performed with polyphenol oxidase (tyrosinase and laccase),[10] peroxidase,[11] a plant homogenate with high polyphenol oxidase activity,[12] or *C. sinensis* cell culture.[13] Polyphenol oxidase contained in many plants has substrate specificity for catechol rather than pyrogallol,[14] which gives a good yield of theaflavins. However, these enzymatic methods also afford other products, such as dimers of pyrogallol-type catechins. On the other hand, there have been a few studies on nonenzymatic synthesis of



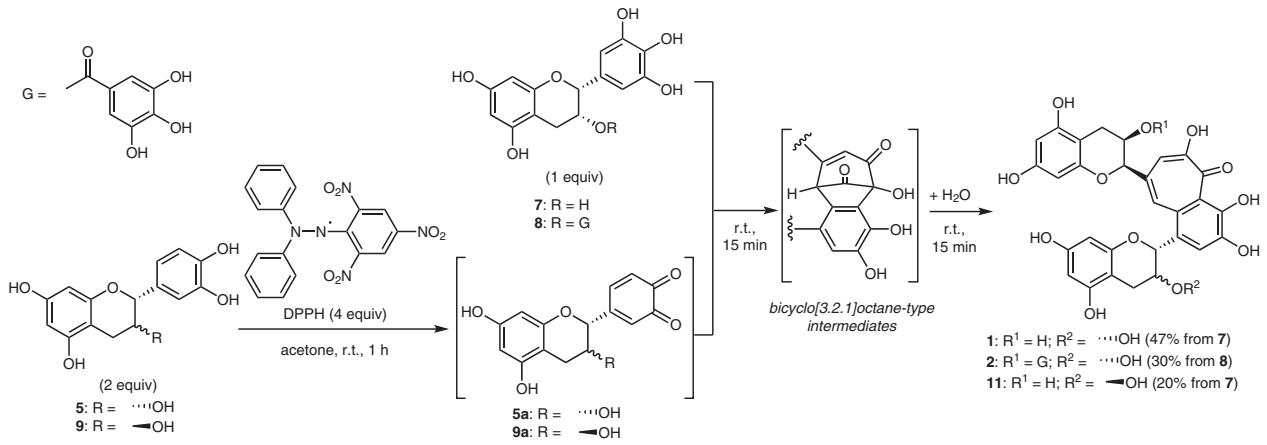
Scheme 1 Proposed production mechanism of benzotropolone moiety in theaflavins

theaflavins. In the 1960–1970s, the synthesis of theaflavins was performed by using $K_3[Fe(CN)_6]$ in water (yield: 5–19%).[4a,d] However, pyrogallol-type catechin dimers are also generated under these conditions, as is the case in the enzymatic oxidation.[15] Recently, several groups have reported the biomimetic synthesis of benzotropolone derivatives including the formation of the bicyclo[3.2.1]octane-type structure as a key intermediate in aprotic solvent. Yanase et al. reported the synthesis of simple benzotropolone derivatives by using the Fetizon reagent (Ag_2CO_3/Celite) as an oxidant.[9,16] Kan et al. reported the synthesis of theaflavins, although their method needs protection and deprotection of hydroxy groups at the catechin A-ring (total yield of **1** from **7**: 5.7%).[17] The protection of hydroxy groups at the catechin A-ring was unavoidable due to an oxidation process using $Pb(OAc)_4$.

If a catechol-type B-ring could be oxidized to the corresponding *o*-quinone selectively without protection of A-ring hydroxy groups, the synthesis of theaflavins will be accomplished by a much simpler procedure. In this study, we developed a new method for nonenzymatic biomimetic synthesis of theaflavins without a protecting group.

Previously, it was reported that the oxidation of epicatechin (**5**) and catechin (**9**) with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical in acetone affords the *o*-quinones **5a** and **9a**, respectively.[18,19] Production of the quinones was confirmed by derivatization with *o*-phenylenediamine as well as ^{13}C NMR spectra of the reaction mixture.[18] Based on this, we proposed the following synthetic route for theaflavins: Firstly, a catechol-type catechin is oxidized with the DPPH radical in acetone to prepare its *o*-quinone without protecting A-ring hydroxy groups. Secondly, a pyrogallol-type catechin is added to form the bicyclo[3.2.1]octane intermediate. Finally, addition of water to the reaction mixture causes ring cleavage followed by spontaneous oxidation and decarboxylation to afford theaflavins.

The optimized reaction conditions for the synthesis of theaflavin (**1**) are as follows: four equivalents of DPPH were added to an acetone solution of epicatechin (**5**, 2 equiv) and stirred for one hour at room temperature to generate the *o*-quinone **5a**. Then, epigallocatechin (**7**, 1 equiv) was added and stirred for 15 min to form a bicyclo[3.2.1]octane-type intermediate.[20] Finally, water was added and stirred for 15 min to afford **1** (47% from **7**, Scheme 2).[21] The ^1H NMR and ^{13}C NMR data of **1** were completely consistent with the literature data.[4c,d,11,22] A small amount of theanaphthoquinone (**10**, 1.6% from **7**), an oxidation product of **1**, was also afforded as a byproduct (Figure 2);[23] however, dimers of **7** were not obtained. A large excess of the DPPH radical of over four equivalents increased the production of **10** from **1**. Direct addition of the DPPH radical to the mixture of **5** and **7** in acetone did not give **1** and only oxidation of **7** was



Scheme 2 Nonenzymatic biomimetic method for the synthesis of theaflavins

observed. This is because the redox potential of pyrogallol-type catechins is much lower than that of catechol-type catechins.[24] Using the same method, theaflavin-3-*O*-gallate (**2**) was synthesized from **5** and epigallocatechin-3-*O*-gallate (**8**) (30% from **8**). However, theaflavin-3′-*O*-gallate (**3**) and theaflavin-3,3′-di-*O*-gallate (**4**) could not be synthesized from epicatechin-3-*O*-gallate (**6**) with **7** or **8**. These results suggest that the presence of the galloyl group in pyrogallol-type catechins does not affect the production of benzotropolone. On the other hand, the presence of the galloyl group in catechol-type catechins prevents the generation of the *o*-quinone of the catechol ring. It is considered that the galloyl group is oxidized by the DPPH radical instead of the catechol ring. Neotheaflavin (**11**), a minor pigment contained in black tea,[4d] was also able to be synthesized from (+)-catechin (**9**) and epigallocatechin (**7**) in the same way (20% from **7**). By using this method, other benzotropolone derivatives could be synthesized from catechol and pyrogallol derivatives. From epicatechin (**5**) and myricitrin (**12**), a non-natural benzotropolone derivative **13** was synthesized by the same method (12% from **12**) (Figure 2).

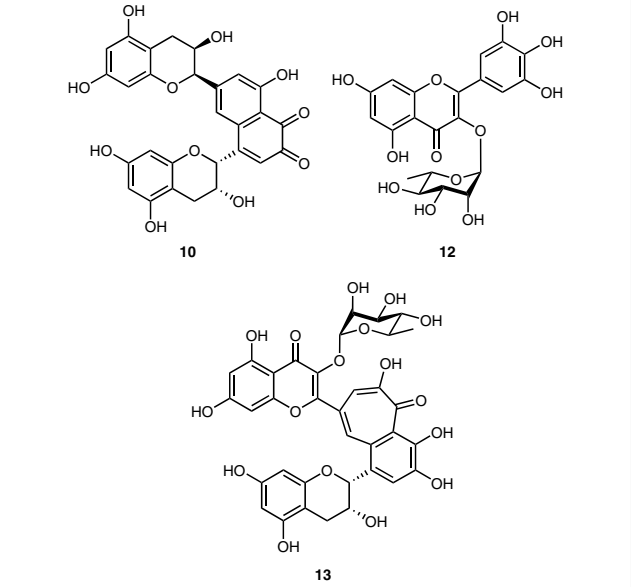


Figure 2 Structures of theanaphthoquinone (**10**), myricitrin (**12**), and **13**

In summary, we have developed a new method for non-enzymatic biomimetic synthesis of black tea pigment theaflavins without a protecting group. Benzotropolone derivatives are rare in nature,[25] and several compounds have been reported, such as purpurogallin glycosides,[26] fomentariol,[27] aurantricholone,[28] crocipodin,[29] and goupiolone A.[30] Our method is considered to be applicable to the synthesis of these benzotropolone derivatives. In addition, there are many catechol and pyrogallol derivatives as natural products;[1] therefore, various non-natural benzotropolone derivatives could be synthesized by applying this method.

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0036-1588529.

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(20) Currently, we are investigating the structure of the bicyclo[3.2.1]octane intermediate by converting it into a stable derivative. The details will be reported in the near future.

(21) **Experimental Procedure for Synthesis of Theaflavin (1)**
DPPH (543 mg, 1.4 mmol) was added to an acetone solution (100 mL) of epicatechin (**5**, 200 mg, 0.69 mmol). After stirring for 1 h at r.t., an acetone solution (50 mL) of epigallocatechin (**7**, 106 mg, 0.35 mmol) was added to the reaction mixture and stirred for 15 min at r.t. Then, water (150 mL) was added to the reaction mixture and stirred for 15 min. The reaction mixture was concentrated in vacuo, and the residue was applied to a column of MCI-gel CHP20P (3.0 × 24 cm; 0–80% aq MeOH, 10% stepwise, each 200 mL) to afford a crude theaflavin (**1**) fraction along with **5** (154 mg, 77%). The crude fraction of **1** was purified with Sephadex LH-20 (3.0 × 20 cm; 40–100% aq MeOH, 10% stepwise, each 200 mL) to afford **1**[4c,d,11,22] (91.3 mg, 0.16 mmol, 47% from **7**) and theanaphthoquinone (**10**)[23] (2.9 mg, 0.0054 mmol, 1.6% from **7**).
Analytical Data for Theaflavin (1)
Reddish-orange amorphous powder, $[\alpha]_D^{25}$ –234 (*c* 0.105, MeOH). FAB-MS: *m*/*z* = 565 [M + H]$^+$, 587 [M + Na]$^+$. HRMS–FAB: *m*/*z* calcd for $C_{29}H_{25}O_{12}$: 565.1346; found: 565.1340 [M + H]$^+$. IR: 3406, 2935, 1627, 1604, 1518, 1470, 1419, 1310, 1227 cm^{-1}. UV (MeOH): λ_{max} (log ε) = 462 (3.49), 378 (3.90), 267 (4.27). ^{1}H NMR (400 MHz, acetone-d_6 + D_2O, 95:5): δ = 8.00 (s, H-g), 7.93 (s, H-e), 7.53 (s, H-c), 6.04 (d, *J* = 2.2 Hz, H-6′), 6.02 (d, *J* = 2.4 Hz, H-6), 5.99 (d, *J* = 2.2 Hz, H-8′), 5.95 (d, *J* = 2.4 Hz, H-8), 5.69 (s, H-2′), 4.98 (s, H-2), 4.45 (m, H-3′), 4.36 (m, H-3), 2.95 (dd, *J* = 16.8, 4.4 Hz, H-4′a), 2.89 (dd, *J* = 16.8, 4.4 Hz, H-4a), 2.80 (dd, *J* = 16.8, 2.3 Hz, H-4b), 2.77 (br d, *J* = 16.8 Hz, H-4′b). ^{13}C NMR (100 MHz, acetone-d_6 + D_2O, 95:5): δ = 185.1 (C-a), 157.8, 157.7, 157.6, 157.5 (C-5, 7, 5′, 7′), 157.0 (C-8′a), 156.5 (C-8a), 154.6 (C-b), 150.4 (C-i), 146.1 (C-h), 135.0 (C-d), 131.7 (C-f), 128.6 (C-k), 126.9 (C-e), 123.9 (C-g), 121.7 (C-j), 118.9 (C-c), 99.8 (C-4′a), 99.3 (C-4a), 96.5 (2 C, C-6, 6′), 95.6 (C-8′), 95.3 (C-8), 81.2 (C-2), 76.6 (C-2′), 66.2 (C-3), 65.0 (C-3′), 29.5 (C-4′), 29.2 (C-4).

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