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Production and degradation mechanism of theacitrin C, a black tea pigment derived from epigallocatechin-3-O-gallate via a bicyclo[3.2.1]octane-type intermediate

Yosuke Matsuo, Yan Li, Sayaka Watarumi, Takashi Tanaka*, Isao Kouno

Graduate School of Biomedical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan

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ABSTRACT

Black tea is rich in polyphenols and has been shown to have various health benefits; however, its components have not yet been clarified in detail. Enzymatic oxidation of epigallocatechin-3-O-gallate, the most abundant polyphenol in tea, is thought to contribute significantly to the production of black tea polyphenols. We identified theacitrin C, an unstable black tea pigment, as an enzymatic oxidation product of epigallocatechin-3-O-gallate. Degradation of theacitrin C afforded theacitrinin A and 2,3,5,7-tetrahydroxychroman-3-O-gallate. Furthermore, theacitrinin B, which was isolated from black tea, is deduced to be a degradation product of theacitrin A, the desgalloyl analogue of theacitrin C. The structures of theacitrinins A and B were elucidated based on spectroscopic data. This is the first time that a degradation product of theacitrin has been isolated from black tea. We also examined the influence of esterification of the epigallocatechin C-3 hydroxyl group on the decomposition of bicyclo[3.2.1]octane-type intermediates.

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1. Introduction

Plant polyphenols have recently attracted much attention because of their health-promoting effects. Black tea, which is one of the most popular beverages worldwide, contains abundant polyphenols, has various health benefits,¹ and is an important source of plant polyphenols for humans. Black tea is manufactured by the fermentation of fresh tea leaves (*Camellia sinensis*). During this process, green tea catechins, (–)-epicatechin (**17**), (–)-epigallocatechin (**15**), (–)-epicatechin-3-O-gallate (**16**), and (–)-epigallocatechin-3-O-gallate (**1**), are enzymatically oxidized to afford complex mixtures of products.²

(–)-Epigallocatechin-3-*O*-gallate (1), which is a catechin present in abundance in tea, is known to have a wide range of biological activities.³ Enzymatic oxidation of 1 is thought to contribute significantly to the production of black tea polyphenols. Our previous results revealed the structures of various enzymatic oxidation products of 1.⁴ However, some of the products were found to be unstable and were insufficiently characterized. In this paper, we report the isolation and identification of theacitrin C (5), an unstable black tea pigment, as a major enzymatic oxidation product of

1. We then examined the degradation of **5**. We also describe the isolation of theacitrinin B (**9**), which is likely produced by degradation of theacitrin A (**8**), found in black tea. Furthermore, we examined the enzymatic oxidation products of (-)-epigallocatechin-3-*O*-acetate (**10**), to clarify the influence of the hydroxyl group at the C-3 position of the C-ring on the oxidation of epigallocatechin derivatives.

2. Results and discussion

2.1. Production of theacitrin C by enzymatic oxidation of epigallocatechin-3-O-gallate

An HPLC profile of the enzymatic oxidation reaction mixture of (-)-epigallocatechin-3-O-gallate (1) is shown in Fig. 1. Previously, we characterized the structures of the three major products, corresponding to peaks 2–4, as dehydrotheasinensin A (2),^{4a} EGCg quinone dimer A (3),^{4a} and EGCg quinone dimer B (4),^{4b} respectively (Scheme 1). However, until now, the product corresponding to peak 5 could not be isolated due to its instability. Here we report our successful efforts to isolate this unstable product.

An aqueous solution of **1** was stirred vigorously with Japanese pear fruit homogenate, which has a high polyphenol oxidase activity.⁵ After the reaction was observed to reach equilibrium, the mixture was acidified with trifluoroacetic acid (TFA) to inactivate





^{*} Corresponding author. Tel.: +81 95 819 2433; fax: +81 95 819 2477; e-mail address: t-tanaka@nagasaki-u.ac.jp (T. Tanaka).

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Fig. 1. HPLC profile of the enzymatic oxidation products of epigallocatechin-3-O-gallate (1).



Scheme 1. Enzymatic oxidation of epigallocatechin-3-O-gallate (1).

enzyme activity and to suppress the degradation of **2**. After filtration, the filtrate was applied to MCI-gel CHP20P and eluted to afford five fractions. Fraction 3 containing product **5** was further purified using Cosmosil 40C₁₈-PREP and YMC-Pack ODS-AQ.

The molecular formula of **5** was determined to be $C_{44}H_{32}O_{22}$ by HRFABMS (m/z 935.1302 [M+Na]⁺, +1.9 mmu). The ¹H and ¹³C NMR spectra indicated the presence of two sets of flavan A- and C-rings, along with two galloyl groups. The remaining 12 carbon signals in the ¹³C NMR spectrum were attributable to the B-rings and consisted of three carbonyl carbons (δ_C 200.2, 194.5, 177.8), four sp² quaternary carbons (δ_C 173.3, 150.8, 149.5, 118.4), two sp² methines (δ_C 130.0, 118.4), two sp³ quaternary carbons (δ_C 87.2, 50.3), and one sp³ methine (δ_C 57.6). ¹H and ¹³C NMR data of the B-ring moiety of **5** indicated its similarity to the B-ring of theacitrin A (**8**).⁶ The molecular formula of **5** ($C_{44}H_{32}O_{22}$) corresponded to that of mono-galloyl substituted theacitrin A. The structure of **5** was therefore presumed to be theacitrin A 3-*O*-gallate, i.e., theacitrin C,⁶ as shown in Scheme 1. The structure of **5** was confirmed using an HMBC experiment, which showed correlations similar to those observed in **8**.⁶

Theacitrins A (**8**), B, and C (**5**) were previously isolated from black tea as yellow pigments; however, **5** has only been characterized using MS and UV spectroscopic data.⁶ In this paper, the structure of **5** has been characterized for the first time by 1D and 2D-NMR spectroscopic analysis. This is also the first report of the isolation of theacitrins from the enzymatic oxidation of epigallocatechins.⁷

A production mechanism of **5** is proposed, as shown in Scheme 2. The B-ring of **1** is oxidized to its *o*-quinone form (**1a**), and 1,4-addition between **1** and **1a** then occurs to generate a C–C bond. Successive oxidation and intramolecular 1,2-addition produces a bicyclo[3.2.1] octane-type intermediate, which subsequently rearranges to afford **5**.

2.2. Degradation of theacitrin C

During the final step of black tea manufacture, tea leaves are heated to deactivate enzymes and to dry the tea. On heating, theacitrin C (**5**) present in the tea leaves is likely to decompose because of its instability. We therefore examined the degradation products of **5**. An aqueous solution of **5** was heated at 80 °C for 60 min and then analyzed by HPLC. Photodiode array detection revealed a decrease in levels of **5**, and the decomposition products were detected as a broad hump on the HPLC baseline. Reaction in McIlvaine buffer at pH 5.0 also led to a similar result. However, heating in aqueous solution containing 0.1% TFA (ca. pH 1.9) gave products that could be detected as sharp peaks by HPLC analysis. The products were purified using Cosmosil 40C₁₈-PREP to afford **6** and **7** (Scheme 3). Based on spectroscopic comparison, compound **7** was identified as 2,3,5,7-tetrahydroxychroman-3-*O*-gallate,^{4a} previously isolated from black tea.

Compound **6** exhibited a $[M+Na]^+$ peak at m/z 603 in MALDI-TOFMS. The ¹H and ¹³C NMR spectra (Table 1) were similar to those of **5**, except for the absence of signals arising from one set of flavan A- and C-rings, and a galloyl group. Comparison of the ¹³C NMR and HSQC spectra of **6** with those of **5** showed the presence of a 4,5-di-*C*-substituted pyrogallol ring (δ_C 156.0, 146.7, 143.2, 133.1, 114.1, 106.8) instead of the 2,5-cyclohexadien-1-one moiety found in **5**. The structure of the B-ring was also confirmed by the appearance of HMBC correlations of H-f (δ_H 6.94) with C-e (δ_C 55.7), C-g (δ_C 156.0), C-h (δ_C 133.1), C-j (δ_C 114.1), and C-i (δ_C 146.7; ⁴J), and the correlations of H-e (δ_H 4.53) with C-f (δ_C 106.8), C-j, and C-k (δ_C 143.2) (Table 1). These observations indicated that one set of galloylated flavan A- and C-rings, originally attached at C-k of **5**, had been



Scheme 2. Proposed mechanism of theacitrin C (5) production.



Scheme 3. Degradation of theacitrin C (5) to afford theacitrinin A (6) and 2,3,5,7-tetrahydroxychroman-3-O-gallate (7).

eliminated. Thus, the structure of **6** was determined as shown in Scheme 3 and named theacitrinin A. The production of **6** and **7** by elimination of the flavan A- and C-rings is thought to be promoted by aromatization of 2,5-cyclohexadien-1-one, yielding the more stable pyrogallol ring (Scheme 3).

The degradation products of theacitrins, such as **6**, are expected to be contained in black tea, and we therefore examined the constituents of black tea in detail. Black tea was extracted with boiling water, and the extract was successively partitioned with CHCl₃, EtOAc, and 1-BuOH. The 1-BuOH fraction was separated using a combination of column chromatography on Sephadex LH-20, MClgel CHP20P, Chromatorex ODS, and Toyopearl HW-40F, to afford **9**, although compound **6** has not been isolated at the present stage.

¹H and ¹³C NMR spectra of **9** were closely related to those of **6** (Table 1), except for the appearance of signals attributable to one set of flavan A- and C-rings and one galloyl group. The presence of two sets of galloylated flavan A- and C-rings was supported by the $[M+H]^+$ peak at m/z 761 in the FABMS spectrum. The additional flavan-3-ol moiety in **9** was concluded to be attached at C-f, based on the absence of an aromatic singlet signal assigned to H-f in **6** (δ_H 6.94) in ¹H NMR and the down-field shift of C-f observed (**9**: δ_C 113.9; **6**: δ_C 106.8) in ¹³C NMR. The HMBC correlations between H-2' (δ_H 6.12) with C-f, C-g (δ_C 154.0), and C-k (δ_C 140.1) also supported the proposed location of the flavan A- and C-rings in **9**. Accordingly, the structure of **9** was determined as shown in Scheme 4 and named theacitrinin B.

It is likely that compound **9** was produced by dienone-phenol rearrangement⁸ of theacitrin A (**8**) (Scheme 4), while compound **6** was produced by the elimination of the flavan-3-ol moiety from theacitrin C (**5**). The degradation mechanisms of theacitrins are similar to those of proepitheaflagallin (**20**), which is produced by the enzymatic oxidation of epigallocatechin (**15**).⁹ Heating of an

aqueous solution of **20** afforded hydroxytheaflavin by dienone–phenol rearrangement, and the black tea pigment epithea-flagallin by elimination of the flavan-3-ol moiety.^{9a} It is anticipated that **6** is also contained in black tea.

This is the first report of the isolation of theacitrin degradation products from black tea. These results show that the production and degradation reactions of theacitrins may be responsible for the complex mixture of black tea polyphenols.

2.3. Enzymatic oxidation of epigallocatechin-3-O-acetate

Enzymatic oxidation of (-)-epigallocatechin (15) and (-)-epigallocatechin-3-O-gallate (1) affords dehydrotheasinensins C^{9a} and A (2), respectively, as major products (Scheme 1), However, enzymatic oxidation of **1** did not afford the proepitheaflagallin-type product corresponding to **20**, which is one of the oxidation products of 15. Conversely, enzymatic oxidation of 15 did not afford the theacitrin-type product corresponding to 5.9 Because proepitheaflagallin (20) and theacitrin C (5) are both thought to be produced from epigallocatechins via bicyclo[3.2.1]octane-type intermediates, we postulated that the presence of a hydroxyl group at C-3 may determine the decomposition routes of bicyclo[3.2.1]octane-type intermediates. The presence of galloyl esters may also be important because the aromatic ester can interact with the adjacent aromatic ring by $\pi - \pi$ interactions. We therefore synthesized (–)-epigallocatechin-3-O-acetate (10), which has neither a hydroxyl group nor an aromatic ester at C-3, and examined the enzymatic oxidation products of **10** and the decomposition of bicyclo[3.2.1]octane-type intermediates.

(–)-Epigallocatechin-3-*O*-acetate (**10**) was synthesized by acetylation of (–)-epigallocatechin (**15**) and subsequent selective hydrolysis of the phenolic acetyl groups. Enzymatic oxidation of **10**

Table 1
¹ H (500 MHz) and ¹³ C (125 MHz) NMR data for theacitrinins A (6) and B (9) (in acetone-d ₆)

Position	6				9			
	¹ H ^a		¹³ C	HMBC (H to C)	¹ H ^a		¹³ C	HMBC (H to C)
2	5.26	(s)	74.1	c, d	4.94	(br s)	76.4	4, c, d, e
3	5.89	(br s)	66.5	4a	4.01	(br s)	64.0	4a
4	2.96	(dd, 2.7, 17.5)	26.0	2, 4a	2.76	(dd, 2.3, 16.9)	29.0	2, 3, 4a, 5, 8a
	3.08	(dd, 4.8, 17.5)		2, 4a, 5, 8a	2.82	(dd, 4.4, 16.9)		3, 4a, 5, 8a
4a			99.3				99.1	
5			157.7				157.3	
6	6.14	(d, 2.3)	97.3	4a, 5, 7, 8	5.94	(d, 2.3)	96.7	4a, 5, 7, 8
7			158.1				157.3	
8	6.24	(d, 2.3)	95.9	4a, 6, 7, 8a	5.84	(d, 2.3)	95.4	4a, 6, 7, 8a
8a			155.6				155.3	
2′					6.12	(br s)	78.1	3′, f, g, k
3′					5.67	(br s)	70.7	4a', galloyl 7
4′					3.05	(br d, 17.6)	26.2	2′, 3′, 4a′, 5′, 8a′, f (⁴ J)
					3.26	(dd, 4.6, 17.6)		4a', 5', 8a'
4a′							98.9	
5′							157.3	
6′					6.09	(d, 2.3)	97.9	4a', 5', 7', 8'
7′							157.6	
8′					6.08	(d, 2.3)	95.6	4a', 6', 7', 8a'
8a'							154.4	
a			85.4				87.4	
b			200.7				199.8	
с	5.97	(br s)	126.1	2, a, b, d, e	6.32	(br s)	130.4	2, a, b, d, e, k (4 <i>J</i>)
d			174.8				174.1	
e	4.53	(s)	55.7	a, c, d, f, j, k	4.76	(br s)	53.8	a, c, d, f, g (⁴ J), j, k, l
f	6.94	(s)	106.8	e, g, h, i (4J), j			113.9	
g			156.0				154.0	
h			133.1				134.2	
i			146.7				147.2	
j			114.1				113.9	
k			143.2				140.1	
1			198.7				199.1	
galloyl 1		(011)	121.1			(011)	120.8	
galloyl 2, 6	7.03	(2H, s)	109.9	galloyl 1, 2, 3, 4, 5, 6, 7	7.10	(2H, s)	110.0	galloyl 1, 2, 3, 4, 5, 6, 7
galloyl 3, 5			146.0				145.8	
galloyl 4			139.2				139.0	
galloyl /	0.50	(1)	166.1				166.4	
5-0H	8.50	(br s)		4a, 5				
a-OH	5.79	(br s)		a, e				
n-OH	8.74	(DFS)		g, n, 1				
galloyl 3, 5-UH	8.28	(2H, DFS)		gailoyi 2, 3, 4, 5, 6				
galloyi 4-OH	8.08	(DFS)		galloyi 3, 5				
7, g, 1-0H	8.15 9.21	(DFS)						
	0.20	(DFS) (brs)						
	9.30	(DFS)						

^a Multiplicity and coupling constants (Hz) were shown in parentheses.



Scheme 4. Proposed mechanism of theacitrinin B (9) production from theacitrin A (8).

using Japanese pear fruit homogenate afforded three main products, **11** (t_R =22.1 min), **12** (t_R =29.5 min), and **13** (t_R =31.2 min), as shown by HPLC (Fig. 2). These products were purified by column chromatography to give **11** (44%), **12** (2.3%) and **13a** (2.9%). Product **13** was unstable, and gradually degraded to **13a** (t_R =26.7 min) during purification process and only **13a** could be isolated. The molecular formula of **11** was determined to be $C_{34}H_{30}O_{17}$ by HRFABMS (m/z 711.1558 [M+H]⁺, -0.3 mmu). The ¹H and ¹³C NMR spectra of **11** were very similar to those of dehydrotheasinensin A (**2**),^{4a} except for the appearance of signals due to two acetyl groups instead of two galloyl groups. Therefore, **11** was proposed to be dehydrotheasinensin C 3,3"-di-O-acetate (Scheme 5), and the



Fig. 2. HPLC profile of the enzymatic oxidation products of epigallocatechin-3-O-acetate (10).



Scheme 5. Enzymatic oxidation of epigallocatechin-3-O-acetate (10).

structure was confirmed using detailed analysis of 2D-NMR spectra. The structure was further evidenced by the reduction of **11** with dithiothreitol to yield **11a** (Fig. 3).^{4a} The absolute configuration at C-2' of **11** was determined to be *S* because acetylation of **11a** gave theasinensin C dodecaacetate (**11b**) (Fig. 3).

The molecular formula of **12** was determined to be $C_{34}H_{30}O_{17}$ by HRFABMS (m/z 711.1570 [M+H]⁺, +0.9 mmu). The ¹H and ¹³C NMR spectra of **12** were very similar to those of **3**,¹⁰ except for the appearance of signals derived from two acetyl groups instead of two



Fig. 3. Structures of 11a, 11b, and 14.

galloyl groups. Based on a detailed analysis of 2D-NMR spectra, the structure of **12** was determined as shown in Scheme 5.

The molecular formula of **13a** was determined to be $C_{23}H_{18}O_{11}$ by HRFABMS (m/z 471.0930 [M+H]⁺, +0.3 mmu). The ¹H and ¹³C NMR spectra of **13a** resembled those of theacitrinin A (**6**), except for the replacement of a galloyl group by an acetyl group. Therefore, the structure of **13a** was concluded to be that shown in Scheme 5 and confirmed by 2D-NMR spectroscopic analysis.

HPLC analysis with photodiode array detection showed that the UV–vis absorption of **13** (λ_{max} : 203, 223 sh, 307 nm, and gradualslope from ca. 350 to 430 nm) was similar to that of theacitrin C (**5**). Furthermore, HRFABMS spectrum of the mixture of **13** and **13a** obtained in the process of purification confirmed a molecular formula of C₃₄H₂₈O₁₆ for **13** [*m*/*z* 693.1422 (calcd for C₃₄H₂₉O₁₆, 693.1456)]. These results implied that **13** is a theacitrin-type structure with two acetyl groups as shown in Scheme 5. It is probable that **13a** was produced from **13** by a degradation mechanism similar to the production of theacitrinin A (**6**) from theacitrin C (**5**). We could not isolate **13** because of its instability, while **5** was sufficiently stable to be isolated. This implies that the B-ring moiety of **5** could be stabilized by intramolecular stacking with the C-3 galloyl group.

Enzymatic oxidation of **10** afforded the theacitrin-type product **13**; however, no proepitheaflagallin-type products were detected. This result confirmed that when the hydroxyl group at C-3 of epigallocatechin was esterified, theacitrin-type products were produced. On the other hand, when the hydroxyl group at C-3 was not esterified, proepitheaflagallin-type products were produced.⁹



Scheme 6. Production pathways of proepitheaflagallin (20), theacitrin C (5), and theaflavins via bicyclo[3.2.1]octane-type intermediates.

On enzymatic oxidation, a pyrogallol quinone produced from epigallocatechin was coupled with the pyrogallol ring of another epigallocatechin molecule to afford a bicyclo[3.2.1]octane-type intermediate (Scheme 6). When the hydroxyl group at C-3 is esterified, theacitrin-type structures are produced from the intermediates by rearrangement. On the other hand, when the hydroxyl group at C-3 is not esterified, proepitheaflagallin-type structures are produced form the intermediate via hemiacetal formation. Theaflavins are also produced by condensation of catechol quinones produced by enzymatic oxidation of epicatechins with pyrogallol rings of epigallocatechins via bicyclo[3.2.1]octane-type intermediates (Scheme 6).¹¹

Some decomposition products of bicyclo[3.2.1]octane-type intermediates, theaflavins, theacitrins, and epitheaflagallin, are known as black tea pigments. The results described here indicate that the formation of the bicyclo[3.2.1]octane-type skeleton by enzymatic oxidation of tea catechins is a key reaction in the production of black tea pigments. We speculate that the production and decomposition of bicyclo[3.2.1]octane-type intermediates from tea catechins also contribute significantly to the production of structurally unknown black tea pigments, including thearubigins.^{2a}

3. Conclusion

In this study, we identified theacitrin C (**5**) as an enzymatic oxidation product of epigallocatechin-3-O-gallate (**1**), and its degradation products were examined. Furthermore, theacitrinin B (**9**), a degradation product of theacitrin A (**8**), was isolated from black tea. We also examined the influence of the hydroxyl group at the C-3 position of the C-ring of epigallocatechin on the decomposition reactions of bicyclo[3.2.1]octane-type intermediates. The results indicate that the formation of the bicyclo[3.2.1]octane-type skeleton by enzymatic oxidation of tea catechins is a key reaction in the production of black tea pigments.

4. Experimental

4.1. General

¹H, ¹³C NMR, ¹H–¹H COSY, HSOC, HMBC spectra were measured on a Varian UNITYplus 500 (500 MHz for ¹H and 125 MHz for ¹³C NMR), and a JEOL JNM-AL400 (400 MHz for ¹H and 100 MHz for ¹³C NMR) spectrometer. Chemical shifts were recorded as parts per million (ppm) with the solvent signal used as a standard (DMSO- d_6 , $\delta_{\rm H}$ 2.49, $\delta_{\rm C}$ 39.5; acetone- d_6 , $\delta_{\rm H}$ 2.04, $\delta_{\rm C}$ 29.8; CDCl₃, $\delta_{\rm H}$ 7.24, $\delta_{\rm C}$ 77.0). Coupling constants are given in hertz. Melting points were determined on a micro melting point hot stage apparatus (Yanagimoto, Japan) and are uncorrected. FABMS spectra were measured on a JEOL JMS-700N spectrometer, and *m*-nitrobenzyl alcohol or glycerol was used as the matrix. MALDI-TOFMS spectra were measured on an Applied Biosystems Voyager-DE PRO, and 2,5dihvdroxvbenzoic acid was used as the matrix. Elemental analyses were measured on a Perkin–Elmer 2400 II analyzer. UV–vis spectra were measured on a JASCO V-560 spectrophotometer. Optical rotations were measured on a JASCO P-1020 digital polarimeter. IR spectra were measured on a JASCO FT/IR-410 spectrophotometer.

Column chromatography was performed with MCI-gel CHP20P (75–150 μ m, Mitsubishi Chemical, Japan), Sephadex LH-20 (25–100 μ m, GE Healthcare, Sweden), Chromatorex ODS (Fuji Silysia Chemical, Japan), and Toyopearl HW-40F (Tosoh, Japan). MPLC was performed with Cosmosil 40C₁₈-PREP (Nacalai Tesque, Japan) and YMC-Pack ODS-AQ (S-50 μ m, 12 nm, YMC, Japan). TLC was performed on precoated Kieselgel 60 F₂₅₄ plates (0.2 mm thick, Merck, Germany) with toluene/ethyl formate/formic acid (1/7/1, v/ v) and spots were detected using ultraviolet illumination and by spraying with 2% ethanolic FeCl₃ or 5% aq H₂SO₄ reagent followed by heating. Analytical HPLC was performed on a Cosmosil 5C₁₈-ARII (Nacalai Tesque, Japan) column (250×4.6 mm i.d.) with gradient elution from 4 to 30% (39 min) and from 30 to 75% (15 min) of

CH₃CN in 50 mM H₃PO₄ (flow rate, 0.8 mL/min; temperature: 35 °C; detection: JASCO photodiode array detector MD-2010). (–)-Epigallocatechin-3-O-gallate and (–)-epigallocatechin were isolated from commercial green tea and recrystallized from water. Theasinensin C was prepared as described previously.^{9a}

4.2. HPLC analysis of the enzymatic oxidation products of (–)-epigallocatechin-3-O-gallate (1)

Japanese pear fruits (50 g) were homogenized with 50 mL of H_2O and filtered through four layers of gauze. The filtrate (1.0 mL) was mixed with an aqueous solution of (–)-epigallocatechin-3-*O*-gallate (1) (10 mg/1.0 mL) and vigorously stirred for 60 min at room temperature. After stirring, EtOH (300 μ L) was poured into the reaction mixture (100 μ L) and the resulting mixture was then filtered through a membrane filter (0.45 μ m). The filtered solution (5 μ L) was analyzed by analytical HPLC.

4.3. Production of theacitrin C (5) by enzymatic oxidation of (–)-epigallocatechin-3-O-gallate (1)

Japanese pear (Pyrus pyrifolia) fruits (150 g) were homogenized with 150 mL of H₂O and filtered through four layers of gauze. The filtrate (200 mL) was mixed with an aqueous solution of (-)-epigallocatechin-3-O-gallate (1) (2.0 g/200 mL) and vigorously stirred for 60 min at room temperature. After stirring, the reaction solution was acidified with trifluoroacetic acid (TFA) (0.5 mL) and filtered. The filtered solution was then directly applied to a column of MCIgel CHP20P (3×29 cm) and eluted with 0–100% ag MeOH containing 0.1% TFA (5% stepwise elution, each 200 mL) to yield five fractions: fr. 1 (430.4 mg), fr. 2 (334.3 mg), fr. 3 (508.1 mg), fr. 4 (463.5 mg), and fr. 5 (228.3 mg). Fr. 1 was identified as pure dehydrotheasinensin A (2) from spectroscopic data.^{4a} Other fractions were analyzed by HPLC-DAD, and the presence of 2, EGCg quinone dimer A (3) and 1 in fr. 2; 1 and 5 in fr. 3; and EGCg quinone dimer B (4) in fr. 4 were detected. Fr. 3 was purified by MPLC on Cosmosil 40C₁₈-PREP (20×300 mm, 0–40% ag CH₃CN containing 0.05% TFA) and YMC-Pack ODS-AQ $(20 \times 300 \text{ mm}, 0-50\% \text{ ag MeOH})$ to give theacitrin C (5) (27.5 mg). At all stages of purification, obtained fractions were evaporated under reduced pressure below 40 °C, then lyophilized.

4.3.1. Theacitrin C (5). A yellow amorphous powder; $[\alpha]_D^{21}$ –34.3 (*c* 0.12, MeOH); Positive FABMS *m*/*z*: 913 [M+H]⁺, 935 [M+Na]⁺; Negative MALDI-TOFMS m/z: 911 [M-H]⁻; HRFABMS m/z: [M+Na]⁺ calcd for C₄₄H₃₂O₂₂Na, 935.1283; found: 935.1302; UV (MeOH) λ_{max} nm (log ε): 275 (4.45), and gradual-slope from ca. 350 to 450 nm; IR (dry film) ν_{max} cm⁻¹: 3381, 1731, 1696, 1607, 1519; ¹H NMR (DMSO-*d*₆-CF₃COOD, 9:1, 500 MHz) δ: 2.45 (1H, br d, J=17.4 Hz, H-4'), 2.66 (2H, m, H-4, 4'), 2.80 (1H, dd, J=17.4, 4.1 Hz, H-4), 4.30 (1H, s, H-e), 4.40 (1H, s, H-2'), 4.86 (1H, br s, H-2), 5.25 (1H, m, H-3'), 5.72 (1H, br s, H-3), 5.97 (1H, s, H-c), 6.01 (1H, br s, H-6'), 6.05 (1H, br s, H-6), 6.11 (1H, br s, H-8'), 6.15 (1H, br s, H-8), 6.68 (1H, s, H-f), 6.72 (2H, s, galloyl H-2', 6'), 6.79 (2H, s, galloyl H-2, 6); ¹³C NMR (DMSO- d_6 -CF₃COOD, 9:1, 125 MHz) δ : 25.7 (C-4), 28.3 (C-4'), 50.3 (C-k), 57.6 (C-e), 63.3 (C-3'), 65.3 (C-3), 74.7 (C-2), 81.2 (C-2'), 87.2 (C-a), 95.1, 96.8 (C-6, 8), 95.1, 96.6 (C-6', 8'), 98.0 (C-4a), 98.2 (C-4a'), 109.1 (2C, galloyl C-2, 6), 109.5 (2C, galloyl C-2', 6'), 118.4 (2C, C-f, j), 119.3 (galloyl C-1), 119.6 (galloyl C-1'), 130.0 (C-c), 139.0 (galloyl C-4'), 139.3 (galloyl C-4), 145.5 (2C, galloyl C-3, 5), 145.9 (2C, galloyl C-3', 5'), 149.5 (C-g), 150.8 (Ci), 155.0, 156.3, 157.1 (4C) (C-5, 7, 8a, 5', 7', 8a'), 165.4 (galloyl C-7'), 165.7 (galloyl C-7), 173.3 (C-d), 177.8 (C-h), 194.5 (C-l), 200.2 (Cb); HMBC correlations (H to C): H-2/C-c, d; H-4/C-2, 4a; H-6/C-4a, 5, 7, 8; H-8/C-4a, 6, 7, 8a; H-2'/C-3', 4', e, f, j, k; H-3'/C-4a', galloyl C-7'; H-4'/C-2', 4a'; H-6'/C-4a', 5', 7', 8'; H-8'/C-4a', 6', 7', 8a'; H-c/ C-2, a, b, d, e; H-e/C-2', a, c, d, f, j, k, l; H-f/C-2', e, g, h, j, l (⁴*J*); galloyl H-3, 5/galloyl C-1, 3, 4, 5, 7; galloyl H-3', 5'/galloyl C-1', 3', 4', 5', 7'.

4.4. HPLC analysis of the degradation products of theacitrin C(5)

An aqueous solution of theacitrin C (**5**) (0.5 mg/0.2 mL) was heated at 80 °C for 60 min, and then 5 μ L of the solution was analyzed by HPLC-DAD. The same procedure was also carried out using McIlvaine buffer at pH 5.0 and an aqueous solution containing 0.1% TFA (ca. pH 1.9).

4.5. Isolation of the degradation products of theacitrin C (5) in acidic conditions

An aqueous acidic solution of theacitrin C (**5**) containing 0.1% TFA (25.5 mg/5.1 mL) was heated at 80 °C for 60 min. After cooling, the solvent was removed under reduced pressure, and the residue was purified by MPLC on Cosmosil $40C_{18}$ -PREP (20×300 mm, 0-30% aq CH₃CN containing 0.1% TFA) to afford **6** (11.0 mg, 68%) and **7** (8.4 mg, 86%).

4.5.1. Theacitrinin A (**6**). A pale yellow powder; mp 198–201 °C; $[\alpha]_{26}^{26}$ +31.3 (*c* 0.05, MeOH); MALDI-TOFMS *m/z*: 603 [M+Na]⁺; Anal. Calcd for C₂₈H₂₀O₁₄•2H₂O: C, 54.55; H, 3.92. Found: C, 54.50; H, 3.75; UV λ_{max} (MeOH) nm (log ε): 277 (4.10), 293 sh (4.04), 345 sh (3.39); IR (dry film) ν_{max} cm⁻¹: 3396, 1717, 1707, 1687 (sh), 1618, 1523, 1510, 1472; ¹H and ¹³C NMR data: see Table 1.

4.6. Isolation of theacitrinin B (9) from black tea

Black tea (600 g), a blend of tea leaves produced in India and Sri Lanka, was extracted with boiling water (5 L×3), and the extract was concentrated. The extract was successively partitioned with CHCl₃, EtOAc, and 1-BuOH. The 1-BuOH fraction (63.4 g) was separated into ten fractions (fr. 1–10) by Sephadex LH-20 column chromatography (5.0 cm×35 cm, H₂O–MeOH–50% aq acetone). Fr. 6 was further separated using a combination of column chromatography with MCI-gel CHP20P (H₂O–MeOH), Chromatorex ODS (0–50% aq MeOH), Toyopearl HW-40F (40–100% aq MeOH), and Sephadex LH-20 (EtOH), to give theacitrinin B (**9**) (57.3 mg).

4.6.1. Theacitrinin B (**9**). A reddish brown amorphous powder, $[\alpha]_D^{21}$ –377.9 (*c* 0.1, MeOH); FABMS *m/z*: 761 [M+H]⁺, 783 [M+Na]⁺, 745 [M-H₂O+H]⁺; Anal. Calcd for C₃₇H₂₈O₁₈•11/2H₂O: C, 51.69; H, 4.57. Found: C, 51.58; H, 4.60; UV (MeOH) λ_{max} nm (log ε): 278 (4.21), 290 sh (4.15), 350 sh (3.45); IR (dry film) ν_{max} cm⁻¹: 3378, 1719, 1632, 1517, 1467; ¹H and ¹³C NMR data: see Table 1.

4.7. Synthesis of (-)-epigallocatechin-3-O-acetate (10)

A solution of (–)-epigallocatechin (**15**) (4.01 g, 13.1 mmol) in a mixture of acetic anhydride (7.5 mL) and pyridine (15 mL) was stirred at room temperature for 8 h. The solution was then poured into ice-cold water and filtered. The filtered residue was purified by column chromatography on silica gel (CHCl₃–MeOH, 100:0–99:1) to give (–)-epigallocatechin pentaacetate (**15a**) (5.30 g, 72%) as a white powder. All physical and spectroscopic data of **15a** were identical to those reported previously.¹² A solution of **15a** (4.01 g, 7.17 mmol) in a mixture of McIlvaine buffer at pH 4.0 (30 mL) and dioxane (30 mL) was heated at reflux for 76 h. After cooling, the solvent was removed under reduced pressure. The residue was purified by column chromatography using MCI-gel CHP20P (0–80% aq MeOH) and Sephadex LH-20 (0–100% aq MeOH), to give (–)-epigallocatechin-3-O-acetate (10) (1.49 g, 60%).

4.7.1. (–)-*Epigallocatechin*-3-O-*acetate* (**10**). A white amorphous powder, $[\alpha]_{D}^{19}$ –62.0 (*c* 0.11, MeOH); HRFABMS *m*/*z*: $[M+H]^+$ calcd for C₁₇H₁₇O₈, 349.0923; found, 349.0910; UV (MeOH) λ_{max} nm (log ε): 270 (3.32); IR (dry film) ν_{max} cm⁻¹: 3340, 1711, 1605, 1513, 1461, 1377; ¹H NMR (acetone-*d*₆, 400 MHz) δ : 1.86 (3H, s, Ac), 2.76 (1H, dd, *J*=17.6, 2.4 Hz, H-4), 2.95 (1H, dd, *J*=17.6, 4.9 Hz, H-4), 4.94 (1H, s, H-2), 5.36 (1H, m, H-3), 5.94, 6.04 (each 1H, d, *J*=2.4 Hz, H-6, 8), 6.54 (2H, s, H-2', 6'), 7.30 (1H, br s, OH at C-4'), 7.87 (2H, s, OH at C-3', 5'), 8.13, 8.34 (each 1H, s, OH at C-5, 7); ¹³C NMR (acetone-*d*₆, 100 MHz) δ : 20.9 (Ac(CH₃)), 26.3 (C-4), 68.9 (C-3), 77.7 (C-2), 95.8, 96.4 (C-6, 8), 98.9 (C-4a), 106.7 (2C, C-2', 6'), 130.5 (C-1'), 133.2 (C-4'), 146.2 (2C, C-3', 5'), 156.9, 157.4, 157.8 (C-5, 7, 8a), 170.3 (Ac(CO)).

4.8. Enzymatic oxidation of (–)-epigallocatechin-3-O-acetate (10)

Japanese pear fruits (100 g) were homogenized with 100 mL of H₂O and filtered through four layers of gauze. The filtrate (93 mL) was mixed with an aqueous solution of (-)-epigallocatechin-3-0acetate (10) (930 mg/93 mL) and vigorously stirred for 60 min at room temperature. After stirring, the reaction solution was acidified with TFA (0.5 mL) and insoluble materials were removed by filtration. The filtrate was directly applied to a column of MCI-gel CHP-20P $(3 \times 29 \text{ cm})$ and eluted with 0–100% ag MeOH containing 0.1% TFA (5% stepwise elution, each 200 mL) to yield five fractions (fr. 1–5). Each fraction was concentrated in vacuo below 40 °C and lyophilized. Fr. 1 (420.1 mg) was pure compound **11**. Fr. 2 (57.2 mg) was subjected to Chromatorex ODS (2×27 cm, 0-60% aq MeOH containing 0.1% TFA) column chromatography to give **12** (10.8 mg). Fr. 3 (217.4 mg) was separated by Cosmosil 40C₁₈-PREP $(20\times300 \text{ mm}, 4-30\% \text{ ag CH}_3\text{CN})$ and the same column (4-60% ag)MeOH) to afford **13a** (18.5 mg) and **12** (10.7 mg).

4.8.1. Compound **11**. A white amorphous powder; $[\alpha]_D^{30}$ –132.4 (c 0.095, MeOH); FABMS m/z: 711 [M+H]⁺, 733 [M+Na]⁺, 749 $[M+K]^+$, 693 $[M-H_2O+H]^+$; HRFABMS m/z: $[M+H]^+$ calcd for $C_{34}H_{31}O_{17}$, 711.1561; found, 711.1558; UV (MeOH) λ_{max} nm (log ε): 269 (3.56), 305 (3.30); IR (dry film) v_{max} cm⁻¹: 3407, 1724, 1633, 1519, 1469, 1379; ¹H NMR (500 MHz, acetone-*d*₆) δ: 1.85 (3H, s, 3"-OAc), 1.88 (3H, s, 3-OAc), 2.73 (1H, br d, J=16.9 Hz, H-4), 2.83 (1H, dd, J=16.9, 4.6 Hz, H-4), 2.86 (1H, br d, J=17.0 Hz, H-4"), 3.02 (1H, dd, J=17.0, 4.5 Hz, H-4"), 4.37 (1H, s, H-2'), 4.87 (1H, br s, H-2), 5.29 (1H, m, H-3), 5.42 (1H, m, H-3"), 5.71 (1H, br s, H-2"), 5.93, 5.96, 5.98, 6.00 (each 1H, d, J=1.6 Hz, H-6, 8, 6", 8"), 6.20 (1H, s, H-6'), 6.85 (1H, s, H-6^{'''}); ¹³C NMR (125 MHz, acetone- d_6) δ : 20.8, 20.9 (3-, 3"-OAc(CH₃)), 26.4 (C-4"), 26.6 (C-4), 45.5 (C-2'), 67.8 (C-3), 68.6 (C-3"), 74.3 (C-2"), 76.9 (C-2), 92.0 (C-3'), 95.7, 95.8, 96.3, 96.8 (C-6, 8, 6", 8"), 96.6 (C-4'), 98.5 (C-4a"), 98.6 (C-4a), 109.2 (C-6'"), 111.9 (C-2""), 122.9 (C-6'), 127.4 (C-1""), 133.4 (C-4""), 142.8 (C-3""), 146.0 (C-5'"), 155.4, 156.7, 157.1 (2C) (C-5, 8a, 5", 8a"), 157.6 (2C, C-7, 7"), 162.0 (C-1'), 169.9 (3-OAc(CO)), 170.6 (3"-OAc(CO)), 191.5 (C-5'); HMBC correlations (H to C): H-2/C-3, 4, 1', 2', 6'; H-3/C-4a, 3-OAc (CO); H-4/C-2, 3, 4a, 5, 8a; H-2'/C-2, 1', 3', 4', 5' (⁴J), 6', 1''', 2''', 3''', 4''' (⁴*J*); H-6'/C-2, 1', 2', 3' (⁴*J*), 4', 2''' (⁴*J*); H-2"/C-3", 4", 1'", 2'", 6'"; H-3"/C-4a", 3"-OAc(CO); H-4"/C-2", 3", 4a", 5", 8a"; H-6""/C-2' (⁴J), 2", 1'", 2'", 3'" (⁴J), 4'", 5'"; H-3-OAc/C-3 (⁴J), 3-OAc(CO); H-3"-OAc/C-3" (⁴*J*), 3"-OAc(CO).

4.8.1.1. Reduction of **11** with dithiothreitol. To a solution of **11** (55.5 mg, 78 μ mol) in H₂O (20 mL) was added dithiothreitol (50 mg) and the mixture was stirred at room temperature for 2 h. The solution was directly applied to MCI-gel CHP20P (2×23 cm) and eluted with 0–70% aq MeOH to give **11a** (31.0 mg, 57%). A pale

brown amorphous powder; $[\alpha]_{D}^{29} - 76.4$ (*c* 0.10, MeOH); FABMS *m*/ *z*: 695 [M+H]⁺; HRFABMS *m*/*z*: [M+H]⁺ calcd for C₃₄H₃₁O₁₆, 695.1612; found: 695.1628; UV (MeOH) λ_{max} nm (log ε): 209 (4.95), 236 sh (4.35), 274 sh (3.49); IR (dry film) ν_{max} cm⁻¹: 3403, 1717, 1631, 1518, 1467, 1378; ¹H NMR (400 MHz, acetone-*d*₆) δ : 1.88 (6H, s, Ac), 2.52 (2H, dd, *J*=17.6, 4.9 Hz, H-4), 2.73 (2H, br d, *J*=17.6 Hz, H-4), 4.69 (2H, s, H-2), 5.13 (2H, m, H-3), 5.86, 5.98 (each 2H, d, *J*=2.2 Hz, H-6, 8), 6.78 (2H, s, H-6'), 7.03, 7.53, 7.90, 8.09, 8.26 (each 2H, br s, OH at C-5, 7, 3', 4', 5'); ¹³C NMR (100 MHz, acetone-*d*₆) δ : 21.0 (Ac(CH₃)), 26.4 (C-4), 68.3 (C-3), 75.5 (C-2), 95.8, 96.5 (C-6, 8), 98.3 (C-4a), 107.9 (C-6'), 111.5 (C-2'), 129.1 (C-1'), 133.9 (C-4'), 144.8, 146.5 (C-3', 5'), 157.0, 157.3, 157.8 (C-5, 7, 8a), 171.3 (Ac(CO)).

4.8.1.2. Acetylation of **11a**. A solution of **11a** (20.3 mg, 29 μmol) in a mixture of acetic anhydride (0.5 mL) and pyridine (0.5 mL) was stirred for 20 h. After stirring, the solution was poured into ice-cold water and filtered. The filtered residue was purified by column chromatography on silica gel (CHCl₃-MeOH, 100:0-98:2) to give 11b (20.5 mg, 63%). By the same procedure, acetylation of theasinensin C(14) (26.9 mg, 44 µmol) also afforded 11b (29.8 mg, 61%). A white amorphous powder; $\left[\alpha\right]_{D}^{33}$ +17.7 (*c* 0.12, CHCl₃); MALDI-TOFMS *m*/*z*: 1137 [M+Na]⁺, 1153 [M+K]⁺; Anal. Calcd for C54H50O26 H2O: C, 57.25; H, 4.63. Found: C, 57.33; H, 4.61; UV (MeOH) λ_{max} nm (log ε): 206 (4.92), 270 (3.38); IR (dry film) *v*_{max} cm⁻¹: 2925, 2853, 1771, 1744, 1624, 1593, 1487, 1438, 1370; ¹H NMR (400 MHz, CDCl₃) δ: 1.978, 1.985, 2.228, 2.235, 2.25, 2.27 (each 6H, s, Ac), 2.72 (4H, br s, H-4), 4.61 (2H, s, H-2), 5.40 (2H, br s, H-3), 6.49, 6.53 (each 2H, d, *J*=2.4 Hz, H-6, 8), 7.62 (2H, s, H-6'); ¹³C NMR (100 MHz, CDCl₃)δ: 19.7, 20.1, 20.7, 20.7, 20.8, 21.0 (Ac(CH₃)), 26.0 (C-4), 65.0 (C-3), 74.1 (C-2), 108.0, 108.7, 109.7 (C-4a, 6, 8), 121.7, 122.9 (C-2', 6'), 134.7, 135.3 (C-1', 4'), 141.7, 143.6 (C-3', 5'), 149.4, 149.5 (C-5, 7), 155.0 (C-8a), 166.5, 167.3, 167.6, 168.5, 169.0, 170.3 (Ac(CO)).

4.8.2. *Compound* **12**. A tan amorphous powder; $[\alpha]_{D}^{16} - 33.7$ (*c* 0.10, MeOH); FABMS m/z: 711 [M+H]⁺; HRFABMS m/z: [M+H]⁺ calcd for C₃₄H₃₁O₁₇, 711.1561; found, 711.1570; UV (MeOH) λ_{max} nm $(\log \epsilon)$: 206 (4.72), 228 sh (4.27), 268 sh (3.60); IR (dry film) *v*_{max} cm⁻¹: 3390, 1719, 1673, 1629, 1607, 1519, 1469; ¹H NMR (500 MHz, acetone- d_6) δ : 1.87 (6H, s, Ac), 2.83 (2H, dd, J=17.5, 2.0 Hz, H-4), 2.93 (2H, dd, J=17.5, 4.7 Hz, H-4), 3.29 (2H, s, H-2'), 4.56 (2H, br s, H-2), 5.63 (2H, m, H-3), 6.01, 6.07 (each 2H, d, J=2.3 Hz, H-6, 8), 6.44 (2H, d, J=1.6 Hz, H-6'); ¹³C NMR (125 MHz, acetone-d₆) δ: 20.7 (Ac(CH₃)), 25.7 (C-4), 59.9 (C-2'), 64.7 (C-3), 76.4 (C-2), 85.4 (C-4'), 95.7, 96.8 (C-6, 8), 98.3 (C-4a), 104.7 (C-3'), 126.9 (C-6'), 155.3, 157.2 (C-5, 8a), 156.6 (C-1'), 157.9 (C-7), 170.3 (Ac(CO)), 196.6 (C-5'); HMBC correlations (H to C): H-2/C-4, 1', 2', 6'; H-3/C-4, 4a, Ac(CO); H-4/C-2, 3, 4a, 5, 6 (⁴J), 8 (⁴J), 8a; H-6, 8/C-4a, 7, 5 or 8a, 8 or 6; H-2'/C-2, 1', 3', 4', 5', 6'; H-6'/C-2, 1', 2', 3' (⁴J), 4'; H-Ac/C-3 (⁴J), Ac(CO).

4.8.3. Compound **13a**. A pale yellow amorphous powder; $[\alpha]_D^{30}$ +61.5 (*c* 0.12, MeOH); FABMS *m*/*z*: 471 [M+H]⁺, 493 [M+Na]⁺, 941 [2M+H]⁺; HRFABMS *m*/*z*: [M+H]⁺ calcd for C₂₃H₁₉O₁₁, 471.0927; found: 471.0930; UV (MeOH) λ_{max} nm (log ε): 277 sh (3.79), 300 (3.82), 346 sh (3.45); IR (dry film) ν_{max} cm⁻¹: 3346, 1711, 1683, 1606, 1509, 1471, 1339; ¹H NMR (500 MHz, acetone- d_6) δ : 1.98 (3H, s, Ac), 2.85 (1H, dd, *J*=17.5, 2.9 Hz, H-4), 3.00 (1H, d, *J*=17.5, 4.9 Hz, H-4), 4.48 (1H, s, H-e), 5.17 (1H, br s, H-2), 5.70 (1H, m, H-3), 5.93 (1H, br s, H-c), 6.14 (1H, d, J=2.3 Hz, H-6), 6.18 (1H, d, J=2.3 Hz, H-8), 6.92 (1H, s, H-f); ¹³C NMR (125 MHz, acetone-*d*₆) δ: 20.9 (Ac(CH₃)), 25.9 (C-4), 55.7 (C-e), 66.5 (C-3), 74.0 (C-2), 85.4 (C-a), 95.9 (C-8), 97.3 (C-6), 99.2 (C-8a), 106.8 (C-f), 114.2 (C-j), 126.0 (C-c), 133.0 (C-h), 143.2 (C-k), 146.6 (C-g), 155.5 (C-8a), 156.1 (C-i), 157.6 (C-5), 158.1 (C-7), 170.7 (Ac(CO)), 174.9 (C-d), 198.7 (C-l), 200.7 (C-b); HMBC correlations (H to C): H-2/C-4, 8a, b (⁴J), c, d; H-3/C-4, 4a, d, Ac(CO); H-4/C-2, 3, 4a, 5, 6 (⁴*J*), 8 (⁴*J*), 8a; H-6/C-4a, 5, 7, 8; H-8/C-4a, 6, 7, 8a; H-c/C-2, a, b, d, e, k (⁴*J*); H-e/C-2, a, c, d, f, j, k, i (⁴*J*), l; H-f/C-d (⁴*J*), e, g, h, i (⁴*J*), j, k, l (⁴*J*); H-Ac/C-3 (⁴*J*), Ac(CO).

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Supplementary data

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References and notes

1. (a) Gardner, E. J.; Ruxton, C. H. S.; Leeds, A. R. Eur. J. Clin. Nutr. 2007, 61, 3-18; (b) Ruxton, C. H. S. BNF Nutr. Bull. 2008, 33, 91-101.

- 2. For reviews of black tea polyphenols, see: (a) Haslam, E. Phytochemistry 2003, 64, 61–73; (b) Tanaka, T.; Matsuo, Y.; Kouno, I. Int. J. Mol. Sci. 2010, 11, 14–40; (c) Drynan, J. W.; Clifford, M. N.; Obuchowicz, J.; Kuhnert, N. Nat. Prod. Rep. 2010, 27, 417-462.
- 3. Nagle, D. G.; Ferreira, D.; Zhou, Y.-D. Phytochemistry 2006, 67, 1849-1855.
- (a) Tanaka, T.; Watarumi, S.; Matsuo, Y.; Kamei, M.; Kouno, I. Tetrahedron 2003, 59, 4 7939–7947; (b) Tanaka, T.; Matsuo, Y.; Kouno, I. J. Agric. Food Chem. 2005, 53, 7571–7578; (c) Li, Y.; Tanaka, T.; Kouno, I. Phytochemistry 2007, 68, 1081–1088.
- 5. Tanaka, T.; Mine, C.; Inoue, K.; Matsuda, M.; Kouno, I. J. Agric. Food Chem. 2002, 50, 2142-2148.
- 6. Davis, A. L.: Lewis, I. R.: Cai, Y.: Powell, C.: Davis, A. P.: Wilkins, I. P. G.: Pudney, P.; Clifford, M. N. *Phytochemistry* **1997**, 46, 1397–1402.
- 7. Recently, Qi obtained **5** by non-enzymatic oxidation of **1** using potassium hexacyanoferrate (III): Qi, X. *Fitoterapia* **2010**, *81*, 205–209.
- 8. Whiting, D. A. In Comprehensive Organic Synthesis; Trost, B. M., Fleming, I., Eds.; Pergamon: Oxford, 1991; Vol. 3, pp 803-821.
- (a) Matsuo, Y.; Tanaka, T.; Kouno, I. *Tetrahedron* **2006**, *62*, 4774–4783; (b) Matsuo, Y.; Tanaka, T.; Kouno, I. *Tetrahedron Lett.* **2009**, *50*, 1348–1351. 9
- (a) Valcic, S.; Muders, A.; Jacobsen, N. E.; Liebler, D. C.; Timmermann, B. N. *Chem. Res. Toxicol.* **1999**, *12*, 382–386; (b) Valcic, S.; Burr, J. A.; Timmermann, B. 10 N.; Liebler, D. C. Chem. Res. Toxicol. 2000, 13, 801-810.
- Yanase, E.; Sawaki, K.; Nakatsuka, S. *Synlett* **2005**, 2661–2663.
 (a) Foo, L. Y.; Lu, Y.; McNabb, W. C.; Waghorn, G.; Ulyatt, M. J. *Phytochemistry* 1997, 45, 1689–1696; (b) Mayer, W.; Bauni, G.; Stolp, F. Justus Liebigs Ann. Chem. **1960**, 630, 19-25.