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New black tea polyphenol having *N*-ethyl-2-pyrrolidinone moiety derived from tea amino acid theanine: isolation, characterization and partial synthesis

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Abstract

Ethylpyrrolidinonyl theasinensin A, a novel polyphenol having a *N*-ethyl-2-pyrrolidinone moiety, was isolated from commercial black tea, and the structure was determined on the basis of spectroscopic analysis and chemical synthesis, which was achieved by condensation of theasinensin A with *N*-ethyl-5-hydroxy-2-pyrrolidinone. The *N*-ethyl-5-hydroxy-2-pyrrolidinone is spontaneously produced from theanine Strecker aldehyde by intramolecular cyclization; therefore, the presence of ethylpyrrolidinonyl theasinensin A in black tea suggested that theanine, the most abundant amino acid in tea leaf, was degraded to a Strecker aldehyde and conjugated with polyphenol A-rings during black tea production.

Keywords: Black tea; Theanine; Theasinensin; Polyphenol; Strecker aldehyde

1. Introduction

Recently, plant polyphenols in foods and beverages were believed to have various health benefits. Black tea is the most important source of the polyphenols, because it accounts for almost 80% of the world's tea production and contains polyphenols in high concentration (Lakenbrink, Lapczynski, Maiwald, & Engelhardt, 2000; Rechner et al., 2002). The chemistry of black tea polyphenols was opened up by Roberts at the end of the 1950s to the early 1960s (Roberts, 1962), and it has been shown that the black tea polyphenols are produced by complex oxidation of tea leaf catechins (flavan-3-ols). Some catechin dimers, such as theaflavins (Takino, Imagawa, Horikawa, & Tanaka, 1964) and theasinensins (Hashimoto, Nonaka, & Nishioka, 1988), were regarded as typical black tea polyphenols (Hashimoto, Nonaka, & Nishioka, 1992). Various flavan-3-ols, proanthocyanidin dimers and catechin-flavonoid dimers were also reported (Nonaka, Hashimoto, & Nishioka, 1986). However, black tea contains many other unknown metabolites besides these compounds, and the unknown polyphenols totally account for a significant part of the total polyphenols of black tea. Especially, relatively polar polyphenols, distributed into the 1-BuOH-soluble fraction after sequential solvent partitioning of black tea extracts were believed to be a complex mixture of polyphenols of large molecular weights. In order to understand the chemistry of black tea polyphenols, we have been studying the oxidation mechanism of tea catechins by simple model oxidation experiments, in which purified catechins are oxidized by plant enzymes (Tanaka & Kouno, 2003; Tanaka, Watarumi, Matsuo, Kamei, & Kouno, 2003). However, during black tea production, it has been demonstrated that many other constituents, such as amino acids, are

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concomitantly metabolized; therefore, cross condensation products between reactive metabolites derived from polyphenols and other constituents are possibly produced. Theanine (**1**), a major tea leaf amino acid, was known to decrease during tea fermentation (Co & Sanderson, 1970; Roberts & Sanderson, 1966). In addition, it was reported that Strecker aldehydes were produced from corresponding amino acids in the presence of epicatechin quinone, produced by enzymatic oxidation of epicatechin (Saijo & Takeo, 1970). Since formaldehyde and acetaldehyde easily react with catechin A-rings to form dimers and oligomers (Matsuo & Itoo, 1982; Tanaka, Takahashi, Kouno, & Nonaka, 1994), similar condensation reactions possibly occur during tea fermentation. Actually, catechin dimers, produced by condensation with formaldehyde, were isolated from semifermented tea (Hashimoto, Nonaka, & Nishioka, 1989a). In the present study, we isolated a novel polyphenol, having a *N*-ethyl-2-pyrrolidinone moiety, from relatively polar fractions of commercial black tea, and thus, this paper describes the structure determination, chemical synthesis, and biogenesis of the metabolite.

2. Materials and methods

2.1. General

IR and UV spectra were obtained with JASCO FT/IR-410 and JASCO V-560 spectrophotometers. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. CD spectra were measured with a JASCO J-720w apparatus. ^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, NOESY, HSQC and HMBC spectra were recorded with a Unity *plus* 500 spectrometer (Varian Inc, USA) operating at 500 MHz for ^{1}H, and 125 MHz for ^{13}C, respectively. ^{1}H and ^{13}C NMR spectra were also measured with a JEOL JMN-AL400 (JEOL Ltd., Japan), operating at 400 MHz for ^{1}H, and 100 MHz for ^{13}C, respectively. FAB and EIMS were recorded on a JMS DX-303 spectrometer (JEOL Ltd., Japan), and *m*-nitrobenzyl alcohol or glycerol was used as a matrix for FABMS. Elemental analysis was obtained with a Perkin–Elmer 2400 II analyzer (Perkin–Elmer, Inc.). Column chromatography was done on MCI-gel CHP 20P (Mitsubishi Chemical Co.), Chromatorex ODS (Fuji Silysia Chemical Ltd., Japan), TSK gel Toyopearl HW-40F (TOSOH Co.) and Sephadex LH-20 (Pharmacia Fine Chemical Co.). Thin-layer chromatography (TLC) was performed on precoated Kieselgel 60 F_{254} plates, 0.2 mm thick (Merck), with benzene–ethyl formate–formic acid (1:7:1, v/v) or $CHCl_3$–MeOH–H_2O (14:6:1, v/v) and spots were detected by UV illumination, sprayed with 2% ethanolic $FeCl_3$ or 10% sulfuric acid reagent, and followed by heating. Analytical high pressure liquid chromotography (HPLC) was performed on a Cosmosil 5C_{18}-AR II, 250 × 4.6 mm id column (Nacalai Tesque Inc., Japan) with gradient elution from 10% to 30% (30 min) and 30% to 75% (15 min) of CH_3CN in 50 mM H_3PO_4 at a flow rate of 0.8 ml/min, and detected with a MD-910 photodiode array detector (JASCO Co., Japan)].

2.2. Extraction and isolation

Commercial black tea (600 g), a blended tea produced in India and Sri Lanka, purchased in a local market, was extracted with boiling water (5 l × 3). The extract was concentrated and decaffeinated by partitioning with $CHCl_3$. The aqueous layer was successively partitioned with ethyl acetate and 1-butanol. The 1-butanol-soluble fraction (63.4 g) was separated into 10 fractions by Sephadex LH-20 column chromatography (5.0 × 35 cm) with H_2O containing increasing proportions of MeOH. The fraction 8, which was obtained by elution of 70–80% MeOH, was shown to contain epigallocatechin-3-*O*-gallate, theasinensin A (**3**), epicatechin 3-*O*-gallate and **2** by TLC and HPLC analysis. This fraction was successively subjected to column chromatography on MCI-gel CHP20P (H_2O–MeOH), Chromatorex ODS (H_2O–MeOH) and TSK gel Toyopearl HW-40F (H_2O–MeOH) to yield **2** (10 mg), along with epigallocatechin-3-*O*-gallate (**5**) (386 mg), epicatechin-3-*O*-gallate (165 mg) and theasinensin A (**3**) (729 mg). Similar chromatographic separation gave fraction 9, which was obtained by further elution, with of 80–90% MeOH, of the initial Sephadex LH-20 column, to yield theasinensin D (**4**) (16.5 mg).

2.3. Spectral data of 2

Compound **2** was a tan amorphous powder, $[\alpha]_D = -284.3°$ (*c* 0.1, methanol); UV λ_{max} nm (log ε): 276 (4.43); ^{1}H NMR (500 MHz, d_6-acetone) δ 7.12 (2H, s, 3-galloyl H-2,6), 7.03 (2H, s, 3′-galloyl H-2, 6), 6.94 (1H, s, H-16), 6.91 (1H, s, H-16′), 6.11 (1H, s, H-6), 6.01 (1H, d, *J* = 2.3 Hz, H-6′), 5.861 (1H, d, *J* = 2.3 Hz, H-8′), 5.33 (1H, br d, *J* = 4.8 Hz, H-3′), 5.26 (1H, dd, *J* = 5.3, 9.8 Hz, H-5″), 5.19 (1H, br d, *J* = 4.8 Hz, H-3), 4.73 (1H, br s, H-2), 4.70 (1H, br s, H-2′), 3.62 (1H, dq, *J* = 7.1, 13.7 Hz, NCH_2), 3.00 (1H, br d, *J* = 17.6 Hz, H-4), 2.84 (1H, br d, *J* = 17.4 Hz, H-4′), 2.83 (1H, dq, *J* = 7.1, 13.7 Hz, NCH_2), 2.56 (1H, dd, *J* = 4.8, 17.6 Hz, H-4), 2.51 (1H, dd, *J* = 4.8, 17.4 Hz, H-4′), 2.01, 1.84 (each 1H, m, H-3″), 1.67 (2H, m, H-4″), 1.01 (3H, q, *J* = 7.1 Hz, CH_3); ^{13}C NMR (125 MHz, d_6-acetone) δ 176.8 (C-2″), 166.6 (3′-galloyl C-7), 166.2 (3-galloyl C-7), 157.4 (2C), 157.3 (C-5″, 7′, 9′), 156.8 (C-5), 156.5 (C-9), 155.3 (C-7), 145.9 (C-15′), 145.8, 145.7 (C-15, 3- and 3′-galloyl C-3, 5), 145.1 (C-13), 144.7 (C-13′), 138.8, 138.7 (3- and 3′-galloyl C-4), 133.9 (C-14), 133.1 (C-14′), 128.9 (C-11′), 128.0 (C-11),

121.6, 121.7 (3- and 3′-galloyl C-1), 112.8 (C-12), 112.6 (C-12′), 109.9, 109.8 (3- and 3′ galloyl C-2, 6), 108.3 (C-16), 107.6 (C-16′), 106.1 (C-8), 99.6 (C-10), 98.6 (C-10′), 96.3 (C-6′), 96.0 (C-6), 95.8 (C-8′), 76.34 (C-2′), 76.28 (C-2), 68.9 (C-3), 68.7 (C-3′), 52.8 (C-5″), 35.9 (NCH_2), 32.1 (C-3″), 27.3 (C-4′), 27.1 (C-4), 23.4 (C-4″), 12.8 (CH_3); CD (EtOH, *c* 2.8 × 10-5) $[\theta]_{221} = -335{,}579$, $[\theta]_{247} = -2166$, $[\theta]_{287} = -42{,}222$; FABMS *m/z* 1026 $[M + H]^+$, Anal. Calcd for $C_{50}H_{43}O_{23}N\ 4.5H_2O$: C, 54.25; H, 4.74, N, 1.27. Found: C, 54.13; H, 4.85, N, 1.22.

2.4. Preparation of 1-ethyl-5-hydroxy-2-pyrrolidinone (6)

1-Ethyl-5-hydroxy-2-pyrrolidinone (**6**), which was formed by spontaneous intramolecular cyclization of theanine Strecker aldehyde (**1a**), was prepared according to the procedure reported previously. (Barco et al., 1979). To a solution of *cis*-4-octen-1,8-dioic acid (**7**) (Barco, Benetti, Pollini, & Teddia, 1974) (6.6 g) in thionyl chloride (25 ml) was added a few drops of dimethylformamide. The solution was gently heated (60 °C) for 2 h, and then evaporated in vacuo. The remaining thionyl chloride was removed by coevaporation with toluene. The acid chlorides, so obtained, were dissolved in $CHCl_3$ (20 ml) and mixed with triethylamine (15 ml) and a $CHCl_3$ solution (20 ml) containing monoethylamine (5 g). The mixture was stirred for 6 h at room temperature, and then mixed with water (50 ml). The organic layer was successively washed with 2 N HCl and 5% $NaHCO_3$, dried over $Na_2\ SO_4$, concentrated, and crystallized from toluene to yield *cis*-4-octen-1,8-dioic acid bisethylamide (**8**) (5.2 g) as colourless needles, m.p. 101–102 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.76 (2H, br s, NH), 5.38 (2H, m, H-4, 5), 3.26 (4H, dq, $J = 4.9$, 7.3 Hz, N–CH_2), 2.38 (4H, br q, $J = 7.2$ Hz, H-3, 6), 2.18 (4H, t, $J = 7.1$ Hz, H-2, 7), 1.1 (6H, t, $J = 7.4$ Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3 (C-1, 8), 129.2 (C-4, 5), 36.7 (NCH_2), 34.4, 23.6 (C-2, 3, 6, 7), 14.9 (CH_3); EIMS *m/z* 226 (M^+, 40), 140 (100), 87 (38), 72 (38). To a stirred solution of the bisethylamide (0.9 g) in dioxane (40 ml) and water (13 ml) was added a small crystal (5 mg) of OsO_4. After colour of the solution became brown, an aqueous solution (about 10 ml) of $NaIO_4$ (2.1 g) was added dropwise at room temperature and stirred for 30 min. The white precipitates were removed by filtration and the filtrate was evaporated in vacuo. The remaining dioxane and water was removed by coevaporation with toluene. The resulting residue was dissolved in $CHCl_3$ (50 ml), dried over $Na_2\ SO_4$, and separated by silica gel column chromatography with $CHCl_3$–Et_2O–acetone (5:7:3, v/v) and then with hexane–acetone (2:3, v/v) to yield **6** (387 mg): a colourless syrup; IR (neat) ν_{max} 3366, 2978, 2939, 2878, 1698, 1645, 1473 cm^{-1} ; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.20 (1H, ddd, $J = 2.2$, 6.1, 8.1 Hz, H-5), 4.46 (1H, br s, OH), 3.49, 3.17 (each 1H, dq, $J = 7.1$, 14.6 Hz, NCH_2), 2.51, 2.28, 2.24, 1.87 (each 1H, m, H-3, 4), 1.11 (3H, t, $J = 7.1$ Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4 (C-2), 82.9 (C-5), 34.7 (NCH_2), 29.1, 28.4 (C-3, 4), 13.0 (CH_3); EIMS *m/z* 129 (M^+, 62), 111 (100), 96 (28), 68 (84); Anal. Calcd for $C_6H_{11}\ NO_2$: C, 55.80; H, 8.58; N, 10.85. Found: C, 55.45; H, 8.96; N, 10.86.

2.5. Preparation of DNP derivative of 6

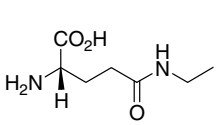
A mixture of **6** (54 mg) and 2,4-dinitrophenylhydrazine (83 mg) in EtOH (3 ml), containing 2 drops of conc. HCl, was heated at 80 °C for 1 h. The solution was evaporated and the residue separated by silica gel column chromatography with $CHCl_3$–MeOH (98:2 v/v) to give the DNP derivative **6a** (18.2 mg) as a red amorphous solid, ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.23 (1H, s, NH), 9.04 (1H, d, $J = 2.4$ Hz, H-3′), 8.27 (1H, dd, $J = 2.4$, 9.5 Hz, H-5″), 7.77 (1H, d, $J = 9.5$ Hz, H-6′), 4.80 (1H, br d, $J = 4.9$ Hz, H-5), 4.50 (1H, br s, NH), 3.65, 3.22 (each 1H, dq, $J = 7.3$, 14.6 Hz, NCH_2), 2.41, 2.33, 2.29, 1.85 (each 1H, m, H-3, 4), 1.21 (3H, t, $J = 7.3$ Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5 (C-2), 150.1 (C-1′), 137.5 (C-4′), 130.3 (C-5′), 129.8 (C-2′), 123.7 (C-3′), 115.7 (C-6′), 73.8 (C-5), 35.3 (NCH_2), 29.3(C-3), 22.9 (C-4), 13.1 (CH_3); FABMS *m/z* 310 $[M + H]^+$.

2.6. Synthesis of ethylpyrrolidinonyl theasinensin A (2)

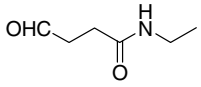
A solution of theasinensin A (3) (183 mg) and **6** (40 mg) in 1% aqueous trifluoroacetic acid (3 ml) was stirred at room temperature for 3 h. The reaction mixture was directly applied to a column of MCI gel CHP20P (0–40% MeOH in H_2O, gradient elution), and the product was purified by Sephadex LH-20 column chromatography (60–80% MeOH in H_2O, gradient elution) to give **2** (24.9 mg). The ^{1}H and ^{13}C NMR spectral data were completely identical to those of the sample isolated from black tea.

2.7. Preparation of ethylpyrrolidinonyl epigallocatechin-3-O-gallate (5a and 5b)

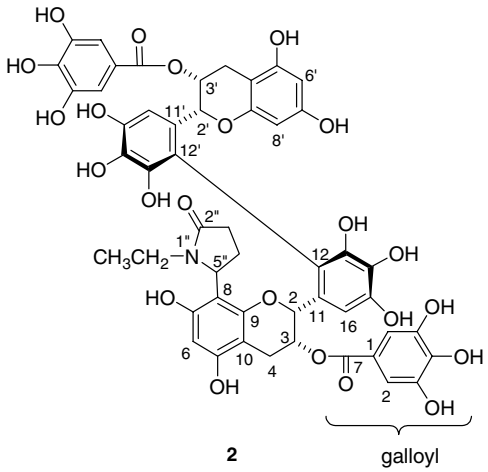
A solution of epigallocatechin-3-*O*-gallate (**5**) (100 mg) and **6** (14.9 mg) in 1% aqueous trifluoroacetic acid (5 ml) was stirred at room temperature for 3 h. The reaction mixture was separated by MCI gel CHP20P column chromatography (0–40% MeOH in H_2O, gradient elution) to give a mixture of **5a** and **5b** (20.7 mg) as a white amorphous powder, $[\alpha]_D = -361.2°$ (*c* 0.2, acetone); major isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (2H, s, galloyl C-2,6), 6.59 (2H, s, H-12, 16), 6.16 (1H, s, H-6), 5.46 (1H, dd, $J = 5.3$, 9.8 Hz, H-5″), 3.68 (1H, dq, $J = 7.1$, 13.7 Hz, NCH_2), 3.00 (2H, m, H-4), 2.83 (1H, dq, $J = 7.1$, 13.7 Hz, NCH_2), 2.60–2.18 (4H, m, H-3″,



1

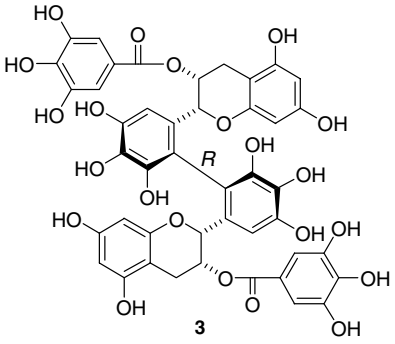


1a



2

galloyl



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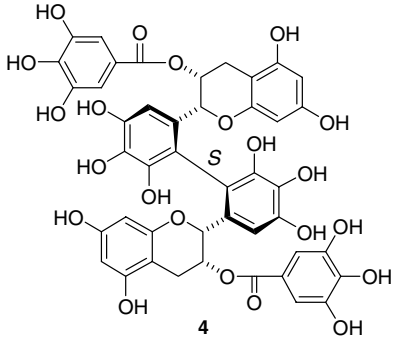


4

4″), 1.01 (3H, t, J = 7.1 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.9 (C-2″), 166.1 (galloyl C-7), 156.8, 156.2, 155.5 (C-5, 7, 9), 145.8, 145.6 (C-13, 15, galloyl C-3, 5), 138.8 (galloyl C-4), 133.1 (C-14), 129.8 (C-11), 121.3 (galloyl C-1), 109.8 (galloyl C-2, 6), 106.3 (C-12, 16), 104.9 (C-8), 99.7 (C-10), 95.8 (C-6), 78.6 (C-2), 70.1 (C-3), 53.2 (C-5″), 36.0 (NCH_2), 32.1 (C-3″), 26.9 (C-4), 23.5 (C-4″), 12.7 (CH_3). Minor isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00 (2H, s, galloyl H-2,6), 6.62 (2H, s, H-12, 16), 5.38 (1H, dd, J = 5.3, 9.8 Hz, H-5″); the signals due to H-2, H-3, H-4, H-6, H-2″, H-3″, H-5″, and H-6″ were overlapped with corresponding signals of the major isomer; ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.7 (C-13, 15), 132.9 (C-14), 130.1 (C-11), 109.7 (galloyl C-2, 6), 106.5 (C-12, 16), 77.8 (C-2), 69.4 (C-3), 35.6 (NCH_2), 31.8 (C-3″), 24.2 (C-2″); other signals were overlapped with corresponding signals of the major isomer; FABMS m/z 570 $[M + H]^+$, 592 $[M + Na]^+$.

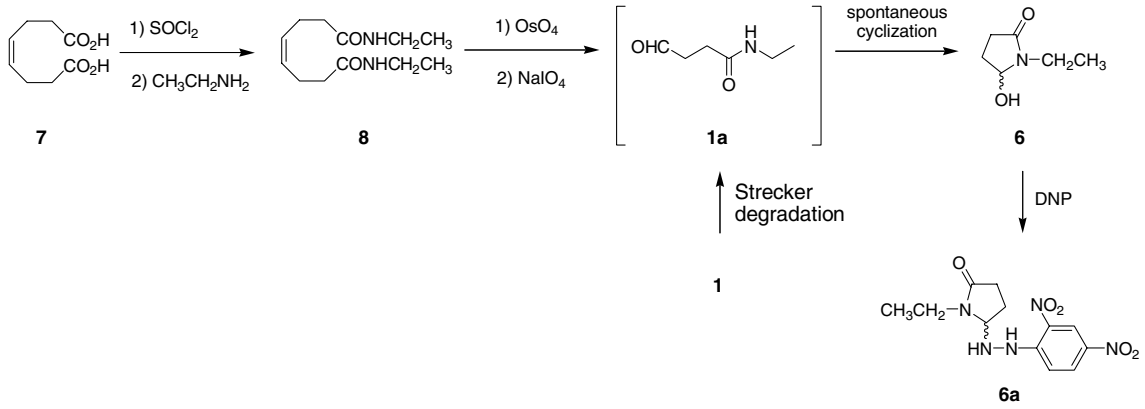
3. Results and discussion

3.1. Isolation

An aqueous extract of commercial black tea was successively partitioned with ethyl acetate and 1-BuOH, and the 1-BuOH-soluble fraction was fractionated by Sephadex LH-20 column chromatography. The ethylpyrrolidinonyl theasinensin A (**2**) was eluted with 70-80% MeOH, and purified by a combination of column chromatography with MCI-gel CHP20P, Chromatorex ODS, and TSK gel Toyopearl HW-40F.

3.2. Spectroscopic analysis

Compound **2** was obtained as a tan amorphous powder and gave a dark blue coloration with the $FeCl_3$ reagent, suggesting the presence of 3,4,5-trihydroxybenzene rings. The odd number of the molecular weight (1025) deduced from the FABMS (m/z 1026, $[M + H]^+$) suggested the presence of a nitrogen atom in the molecule. The ^{1}H and ^{13}C NMR spectra showed two sets of signals arising from flavan-3-ols and galloyl ester groups. The chemical shifts and coupling constants of the signals were closely related to theasinensins A (**3**) and D (**4**), which are symmetrical dimers of epigallocatechin 3-*O*-gallte. In their molecules of **3** and **4**, bulky groups at *ortho*-positions of the biphenyl bond restricted the free rotation at this position, and **3** and **4** differed in configuration at the biphenyl bond. The chemical shifts of proton signals of **2** were similar to those of **3** rather than **4** (Hashimoto et al., 1988). Despite the presence of two flavan-3-ol units, **2** showed only three A-ring proton signals, and one of the



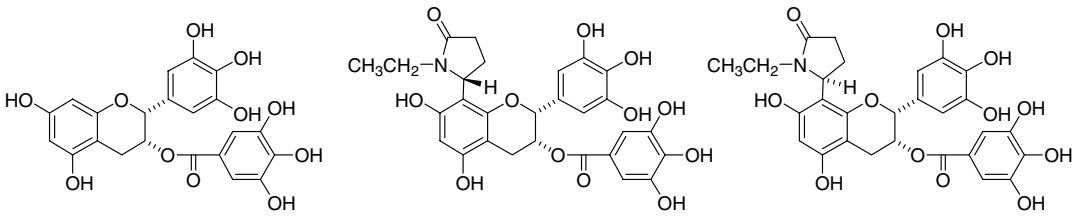
signals was observed at δ 6.11 as a singlet signal, suggesting substitution at C-6 or C-8 of the A-ring. Besides signals arising from two flavan and two galloyl units, the ^{1}H and ^{13}C NMR spectrum showed signals attributable to a methine (C-5″), two methylenes (C-3″ and C-4″), a carbonyl (C-2″) and an ethyl group. The ^{1}H–^{1}H COSY correlation of these methine and methylenes revealed the presence of a partial structure of –CH_2–CH_2–CH–. The ethyl group were shown to be attached to a nitrogen atom, based on the ^{1}H and ^{13}C NMR chemical shift (^{1}H: δ 3.62 and 2.83, each dq: ^{13}C: δ 35.9, NCH_2). In the HMBC spectrum, the methylene protons of the *N*-ethyl group were correlated with the carbonyl carbon (δ 175.8, C-2″) and the methine carbon (δ 53.8, C-5″). The carbonyl carbon was also correlated with one of the methylene protons (δ 2.01, 1.84, H-3″). These HMBC correlations indicated presence of an *N*-ethyl-2-pyrrolidinone ring. Furthermore, the methine proton (δ 5.26, H-5″) showed HMBC correlations with aromatic carbon signals at δ 106.1, 155.3 and 156.5, attributable to the A-ring carbons of one of the flavan-3-ol units. The position of the substitution was unambiguously determined to be the C-8 by observation of NOEs of the *N*-ethyl protons with the B-ring H-16′ (δ 6.91, s) and the galloyl H-2, 6 (δ 7.12) located at C-3. If the pyrrolidinone ring were attached to the C-6 position, a long distance between the *N*-ethyl group and the B-ring aromatic protons could not explain the NOE correlations. From these spectral data, the planar structure of **2** was deduced.

The atrop isomerism of the biphenyl bond between two B-rings was presumed to be *R* by comparison of the CD spectra: the spectra of **2** showed a large negative Cotton effect at 221 nm ($[\theta] = -335579$), which was similar to that of **3** (219 nm, $[\theta] = -258125$). The atrop isomer **4** also showed a negative Cotton effect at similar wavelength (219 nm); however, its molar ellipticity ($[\theta] = -96091$) was much smaller than those of **2** and **3**.

3.3. Partial synthesis of 2

The structure of **1** was determined by partial synthesis from **2**. First, we prepared 1-ethyl-5-hydroxy-2-pyrrolidinone (**6**), which is spontaneously produced from the Strecker aldehyde (**1a**) of theanine (**1**) by intramolecular cyclization. *cis*-4-Octen-1,8-dioic acid (**7**) was converted to a bisdiethylamide (**8**), and then the double bond of **8** was oxidatively cleaved with osmium tetraoxide to give **6**. To the best of our knowledge this is the first preparation of the theanine Strecker aldehyde equivalent.

When **6** and **3** was mixed in acidic aqueous solution, retention time of the major product coincided with that of **2**, and retention times of the products obtained by condensation between **6** and **4** were different from that of **1**. Finally, the condensation product of **6** and **3** was purified by column chromatography, and the spectrum of the product was completely identical to that of **1**. It is known that electrophilic substitutions on flavan-3-ol A-rings preferentially occur at the C-8 position rather than C-6 position (Charlton et al., 2000; Tanaka et al., 1994). In this experiment, the substitution reaction consistently occurred at the C-8 position of **3**. The configuration at the methine carbon of **2** couldnot be determined. HPLC analysis of the reaction mixture showed production of other minor products with similar UV absorption. Although we isolated only one diastereomer and the minor products were not purified in this experiments, an epimer at the C-5″ of **2** was probably produced in the reaction mixture. This was supported by following model experiment: reaction of **6** with epigallocatechin-3-*O*-gallate (**5**) yielded a mixture of two isomers of condensation products (**5a** and **5b**, 4:1). The ^{1}H and ^{13}C NMR chemical shift of the B-ring, galloyl and pyrrolidinonyl moieties of these isomers were slightly different each other; however, the chemical shifts of the A-ring carbons of these isomers were the same. When ^{1}H and ^{13}C NMR chemical shifts of some C-6 and C-8 positional isomers of related flavan-3-ol derivatives were compared, chemical shifts of the A-rings were different (Hashimoto,



5 5a , 5b

Nonaka, & Nishioka, 1989b; Kashiwada, Nonaka, & Nishioka, 1986); therefore, the results indicated that **5a** and **5b** were mixtures of epimers at the benzylic methine carbon of the pyrrolidinonyl unit.

4. Conclusion

Ethylpyrrolidinonyl theasinensin A (**2**) represents the first nitrogen-containing polyphenol isolated from tea. Since amino-carbonyl reactions, including Strecker degradation, usually usually occur on heating, the Strecker aldehydes of tea amino acids may be produced at the late stage of black tea production, where heat inactivates the enzymes and dries the tea leaf. In addition, Saijo & Takeo (1970) suggested that Strecker aldehydes of tea amino acids are produced by amino-carbonyl reaction with epicatechin quinone, produced during tea fermentation. In either case, the resulting aldehydes, which are reactive electropiles, probably attack A-rings of coexisting tea catechins. In persimmon fruits, acetaldehyde, which was produced under anaerobic conditions, reacts with proanthocyanidin A-rings and polymerizes the molecules (Tanaka et al., 1994). A similar reaction between Strecker aldehydes and tea polyphenols may occur in fermented tea leaf. Actually, oolonghomobisflavans, catechin dimers connected through a formaldehyde molecule, were isolated from semifermented tea (Hashimoto et al., 1989a). In order to examine whether **6** is present or not in the black tea and related tea products, a preliminary experiment was attempted. First, **6** was treated with 2,4-dinitrophenylhydrazine (DNP), to give a DNP derivative **6a**. Then, black tea, oolong tea, green tea, roasted green tea and crashed fresh tea leaf were treated with DNP under similar conditions and analyzed by HPLC. However, **6a** was not detected in the tea extracts. The aldehyde may have evaporated during manufacturing or may have reacted with polyphenols to form polymeric substances.

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References

Barco, A., Benetti, S., Pollini, G. P., & Teddia, R. (1974). Synthesis of *cis*-4-octene-1,8-dioic acid and its esters. *Organic Preparations and Procedures International, 6*, 217–220.

Barco, A., Benetti, S., Pollini, G. P., Baraldi, P. G., Simoni, D., & Vicentini, C. B. (1979). Synthesis of 5-hydroxy-2-oxotetrahydropyrroles. *Synthesis*, 68–70.

Charlton, A. J., Davis, A. L., Jones, D. P., Lewis, J. R., Davies, A. P., Haslam, E., & Williamson, M. P. (2000). The self-association of the black tea polyphenol theaflavin and its complexation with caffeine. *Journal of the Chemical Society, Perkin Transactions, 2*, 317–322.

Co, H., & Sanderson, G. W. (1970). Biochemistry of tea fermentation: conversion of amino acids to black tea aroma constituents. *Journal of Food Science, 35*, 160–164.

Hashimoto, F., Nonaka, G., & Nishioka, I. (1988). Tannins and related compounds. LXIX. Isolation and structure elucidation of B,B′-linked bisflavanoids, theasinensins D–G and oolongtheanin from oolong tea. *Chemical and Pharmaceutical Bulletin, 36*(2), 1676–1684.

Hashimoto, F., Nonaka, G., & Nishioka, I. (1989a). Tannins and related compounds. XC. 8-C-ascorbyl (−)-epigallocatechin 3-*O*-gallate and novel dimeric flavan-3-ols, oolonghomobisflavans A and B, from oolong tea., (3). *Chemical and Pharmaceutical Bulletin, 37*, 3255–3263.

Hashimoto, F., Nonaka, G., & Nishioka, I. (1989b). Tannins and related compounds. LXXVII. Novel clalcan-flavan dimers, assamicains A, B and C, and a new flavan-3-ol and proanthocyanidins from the leaves of *Camellia sinensis* L.var. *assamica* Kitamura. *Chemical and Pharmaceutical Bulletin, 37*, 77–85.

Hashimoto, F., Nonaka, G., & Nishioka, I. (1992). Tannins and related compounds. CXIV. Structures of novel fermentation products, theogallinin, theaflavonin and desgalloyl theaflavonin from black tea, and changes of tea leaf polyphenols during fermentation. *Chemical and Pharmaceutical Bulletin, 40*, 1383–1389.

Kashiwada, Y., Nonaka, G., & Nishioka, I. (1986). Tannins and related compounds. XLV. Rhubarb. (5). Isolation and characterization of flavan-3-ol and procyanidin glucosides. *Chemical and Pharmaceutical Bulletin, 34*, 3208–3222.

Lakenbrink, C., Lapczynski, S., Maiwald, B., & Engelhardt, U. H. (2000). Flavonoids and other polyphenols in consumer brews of tea and other caffeinated beverages. *Journal of Agricultural and Food Chemistry, 48*, 2848–2852.

Matsuo, T., & Itoo, S. (1982). A model experiment for de-astringency of persimmon fruit with high carbon dioxide treatment: in vitro gelation of kaki-tannin by reacting with acetaldehyde. *Agricultural and Biological Chemistry, 46*, 683–689.

Nonaka, G., Hashimoto, F., & Nishioka, I. (1986). Tannins and related compounds. XXXVI. Isolation and structures of theaflagallins, new red pigments from black tea. *Chemical and Pharmaceutical Bulletin, 34*, 61–65.

Rechner, A. R., Wagner, E., Van Buren, L., Van De Put, F., Wiseman, S., & Rice-Evans, C. A. (2002). Black tea represents a major source of dietary phenolics among regular tea drinkers. *Free Radical Research, 36*, 1127–1135.

Roberts, E. A. H. (1962). Economic importance of flavonoid substances: tea fermentation. In *The chemistry of flavonoid compounds* (pp. 468–512). Oxford: Pergamon Press.

Roberts, G. R., & Sanderson, G. W. (1966). Changes undergone by free amino acids during the manufacture of black tea. *Journal of the Science of Food and Agriculture, 17*, 182–188.

Saijo, R., & Takeo, T. (1970). Formation of aldehydes from amino acids by tea leaves extracts. *Agricultural and Biological Chemistry, 34*, 227–233.

Takino, Y., Imagawa, H., Horikawa, H., & Tanaka, A. (1964). Studies on the mechanism of the oxidation of tea leaf catechins. Part III. Formation of a reddish orange pigment and its spectral relationship to some benzotropolone derivatives. *Agricultural and Biological Chemistry, 28*, 64–71.

Tanaka, T., Takahashi, R., Kouno, I., & Nonaka, G. (1994). Chemical evidence for the de-astringency (insolubilization of tannins) of persimmon fruit. *Journal of the Chemical Society-Perkin Transactions, 1*, 3013–3022.

Tanaka, T., & Kouno, I. (2003). Oxidation of tea catechins: chemical structures and reaction mechanism. *Food Science and Technical Research, 9*, 128–133.

Tanaka, T., Watarumi, S., Matsuo, Y., Kamei, M., & Kouno, I. (2003). Production of theasinensins A and D, epigallocatechin gallate dimers of black tea, by oxidation–reduction dismutation of dehydrotheasinensin A. *Tetrahedron, 59*, 7939–7947.