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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),