

(S)-3-Amino-1-ethylglutarimide from green tea (*Camellia sinensis*)

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Abstract

The sub-critical water extraction of green tea (*Camellia sinensis*), composing of free amino acid, was investigated to produce a glutarimide derivative, (S)-3-amino-1-ethylglutarimide (1) along with a known compound, pyroglutamic acid. The structure of 1 was decided on the basis of spectroscopic evidence, including NMR (^1H , ^{13}C , DEPT, HSQC and HMBC) and FAB-MS studies. The taste assessment of 1 showed higher *umami* (good-tasting), bitterness, and astringency than those of the theanine standard. A large quantity preparation of 1 was achieved by sub-critical water extraction of aq. theanine at 180 °C and 3 min reaction time. The first isolation of 1 from the plant source is described.

Key words

sub-critical water extraction, green tea, theanine, (S)-3-amino-1-ethylglutarimide, pyroglutamic acid

1. Introduction

Green tea, utilized as a healthy beverage in Japan, is produced from fresh leaves of *Camellia sinensis* O. Kuntze (Theaceae) by steaming or panning just after harvest, followed by a systematic processes of kneading, crumpling, and drying. The first heat treatment inactivated enzymes that catalyze degradation of chemical constituents. The good taste of green tea has been originated from theanine and glutamic acid for *umami* (good-tasting), caffeine for bitterness, and catechins for astringency (Nakagawa, 1975; Yoneda et al., 1994; Ujihara et al., 2013). Theanine is one of the major amino acid components in green tea (Figure 1) and derived from ethylamine and glutamine. Also, theanine was reported to refresh the mental condition through the generation of alpha brain waves and the regulation of dopamine and serotonin levels in the brain (Cooper et al., 2005; Matsuo et al., 1997; Okuda et al., 1984; Taniguchi et al., 1992; Yokogoshi et al., 1995). This paper describes the isolation of amino acids,

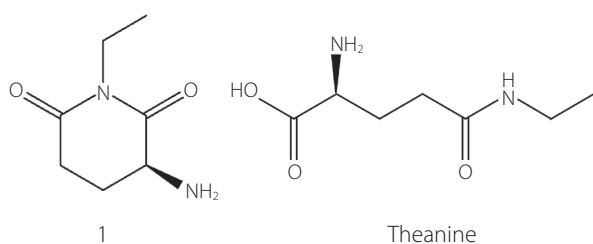


Figure 1: Chemical structures of compound 1 and theanine

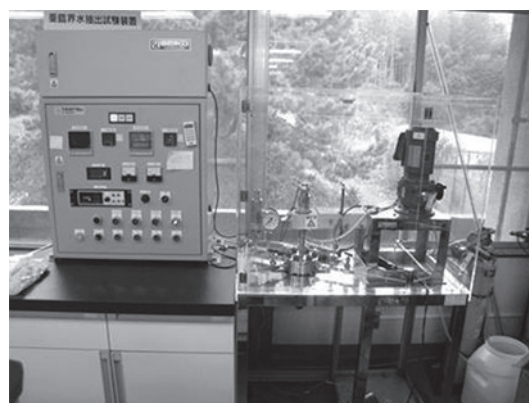


Figure 2: Sub-critical water extraction system

(S)-3-amino-1-ethylglutarimide (1), from green tea under sub-critical water extraction (Figures 2 and 3) and report the taste evaluation of compound 1. Furthermore, the sub-critical water extraction of aq. theanine to find a large-scale synthesis process of 1 was carried out.

2. Experimental procedure

2.1 General experimental procedures

Optical rotation was measured on a Jasco DIP-1000 digital polarimeter at room temperature (22 °C). IR spectrum was obtained on a JASCO FT/IR-400 spectrophotometer. The ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were measured using a Varian UNITY INOVA 500 spectrometer in D_2O with TMS as an external standard. The high resolution FAB-MS spectrum was recorded using a JEOL JMS-T100LP mass spectrometer with *m*-nitrobenzyl alcohol as a matrix. Spots on TLC plates (Merck silica gel 60 F_{254}) were detected by visualizing with vanillin sulfate. The taste sensor analysis was measured

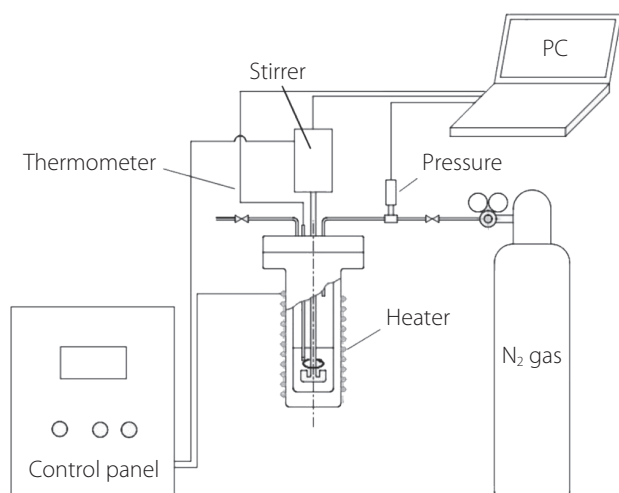


Figure 3: Model diagram of sub-critical water extraction

using SA402B, Interlligent Sensor Technology.

2.2 Materials

Green tea leaves (*C. sinensis*), collected in Shizuoka Prefecture, Japan, in May, 2013, were purchased at the speciality tea store in Shizuoka. A voucher specimen (No. 201305) was identified by one of the authors (Prof. H. Etoh) and was deposited at the Faculty of Agriculture, Shizuoka University. L-Theanine (320-93461) was purchased at Wako pure chemical industries, Japan.

2.3 Extraction and isolation of compound 1

The powdered leaves of green tea (100 g) were macerated with hot water for 10 min. The filtrate was evaporated *in vacuo* to give a residue (91 g). The residual material was subjected to repeated silica gel column chromatography (EtOAc/EtOH/H₂O/AcOH=4/1/1/3), followed by preparative TLC (EtOAc/EtOH/H₂O/AcOH=4/1/1/3) to give compound 1 (5.2 mg) and pyroglutamic acid (2.0 mg).

2.4 (S)-3-Amino-1-ethylglutarimide (1)

Amorphous powder; $[\alpha]_D^{25} -32^\circ$ (c 1.0, H₂O); IR (KBr) ν_{\max} : 1660 and 1575 cm^{-1} ; $^1\text{H NMR}$ (D₂O): δ 4.09 (1H, dd, $J = 9.0, 6.0$ Hz, H-3), 2.95 (2H, q, $J = 7.3$ Hz, H-1'), 2.41 (1H, m, H-4a), 2.30 (2H, m, H-5), 1.95 (1H, m, H-4b), 1.18 (3H, t, $J = 7.3$ Hz, H-2'). $^{13}\text{C NMR}$ (D₂O): δ 184.6 (C-6, C), 182.8 (C-2, C), 61.1 (C-3, CH), 37.8 (C-1', CH₂), 32.5 (C-5, CH₂), 28.1 (C-4, CH₂), 14.7 (C-2', CH₃); HR-FAB-MS: m/z $[\text{M}+\text{H}]^+$ calcd for C₇H₁₃N₂O₂: 157.0982; found: 157.0977.

2.5 L-Theanine

Colourless oil; $^1\text{H NMR}$ (D₂O): δ 3.68 (1H, dd, $J = 9.0, 6.0$ Hz), 3.12 (2H, q, $J = 7.3$ Hz), 2.30 (2H, m), 2.04 (2H, m), 1.02 (3H, t, $J = 7.3$ Hz). $^{13}\text{C NMR}$ (D₂O): δ 177.1, 176.8, 57.0, 37.3, 34.4, 29.3, 16.2.

2.6 Preparation of (S)-3-amino-1-ethyl glutarimide (1)

A solution of theanine (300 mg) in water (300 mL) was subjected to sub-critical water extraction (3.0 MPa, 180 °C, 3 min), followed by removal of solvent to give compound 1 (255 mg, 94 % yield). The detailed procedure was described in our previous study (Kulkarni et al., 2008).

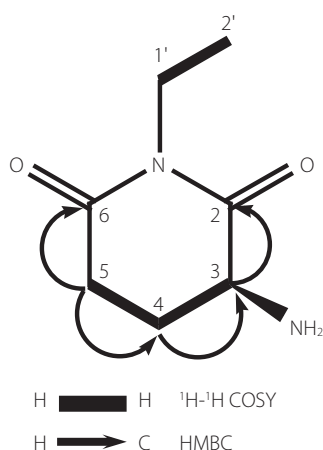
2.7 The taste sensor analysis

The taste sensor analysis compared compound 1 with theanine. Samples were placed in 50 mL special cups and analyzed using a taste sensor. Bitterness, astringency, and umami were evaluated using the sensor. Both "initial taste", perceiving at the moment the drink was placed in the mouth, and "last taste", perceiving after the drink was swallowed, were evaluated. The potential of the reference solution was regarded as zero, and the difference in potential of the samples was measured as the initial taste. The sensor was then rinsed gently, and the difference in the potential on re-measurement of the reference solution was determined as the last taste. The measurement was performed five times for each sample, and the means of the sensor outputs were calculated (Hayashi et al., 2006).

3. Results and discussion

Silica gel chromatography of the sub-critical water extraction of green tea gave (S)-3-amino-1-ethylglutarimide (1), together with pyroglutamic acid, which was identified by direct comparison with authentic sample.

The compound 1, $[\alpha]_D^{25} -32^\circ$ (c 1.0, H₂O), was obtained as an amorphous powder, whose molecular formula was determined as C₇H₁₂N₂O₂ from the HR-FAB-MS ($[\text{M}+\text{H}]^+$ m/z 157.0982). The IR spectrum showed carbonyl band (1660 cm^{-1}). The $^1\text{H NMR}$ spectrum revealed two adjacent methylene protons (δ_{H} 1.95, 2.41, and 2.30) and a methine proton (δ_{H} 4.09) on a glutarimide moiety, as well as a methylene protons (δ_{H} 2.95) and a methyl group (δ_{H} 1.18) on an ethyl side chain. The $^{13}\text{C NMR}$ spectrum of compound 1 showed seven carbon resonances, which composed of one CH₃ (δ_{C} 14.7), three CH₂ (δ_{C} 28.1, 32.5, and 37.8), one CH (δ_{C} 61.1), and two C (δ_{C} 182.8 and 184.6) from the DEPT experiment. The two carbons at δ_{C} 182.8 (C-2) and 184.6 (C-6) were observed for carbonyl groups, of which the C-6 signal assignment was determined from HMBC spectrum, indicating correlation between H-5 and C-6 (Figure 4). The assignment of a methine proton at the C-3 position (δ_{H} 4.09) was decided using the HMBC technique [correlations: H-3/C-2 and H-4/C-3]. A downfield shifts of δ_{C} 182.8 at C-2 and δ_{C} 184.6 at C-6 resonances of measurements in D₂O when compared with those of theanine (δ_{C} 176.8 and δ_{C} 177.1) indicated the presence of a cyclic ring as shown by Sandal et al (Sandal et al., 2011). The stereochemistry at the C-3 position was assigned as S configuration from negative

Figure 4: Selective ^1H - ^1H COSY and HMBC correlations for 1

optical rotation. Thus, compound 1 was characterized as (S)-3-amino-1-ethylglutarimide (CAS Registry Number: 672883-73-7). The isolation of compound 1 from plant source is reported for the first time.

Pyroglutamic acid was first discovered by Haitinger in 1882 (Haitinger, 1882), who found that when heated at 180 °C, glutamic acid is converted into 5-membered ring heterocyclic compound, pyroglutamic acid with losing a H_2O (Figure 5). On the other hand, theanine was subjected to the sub-critical water extraction to produce 6-membered ring heterocyclic compound 1.

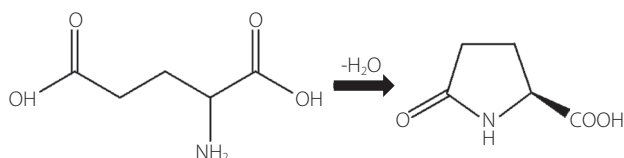


Figure 5: Formation of pyroglutamic acid from glutamic acid

A taste assessment of 1 was implemented using a taste sensor analyzer with a known standard, theanine (five times of measurements). Results are summarized in Figure 6. Compound 1 was estimated for bitterness (initial taste: $+5.96 \pm 0.46$), astringency (initial taste: $+3.33 \pm 0.23$), umami (initial taste: $+0.92 \pm 2.36$), bitterness (last taste: $+0.50 \pm 0.04$), astringency (last taste: $+0.14 \pm 0.01$) and umami (last taste: -0.87 ± 0.87) as compared to the theanine. The taste assessment of 1 revealed higher bitterness, astringency, and umami than those of theanine.

For multigram manufacture of 1, sub-critical water extraction of aq. theanine (3.0 MPa, 180 °C, 3 min) was carried out to provide compound 1 in high yield (94 % yield).

4. Conclusion

The isolation of compound 1 was the first finding from the sub-critical water extraction of green tea. The taste evaluation

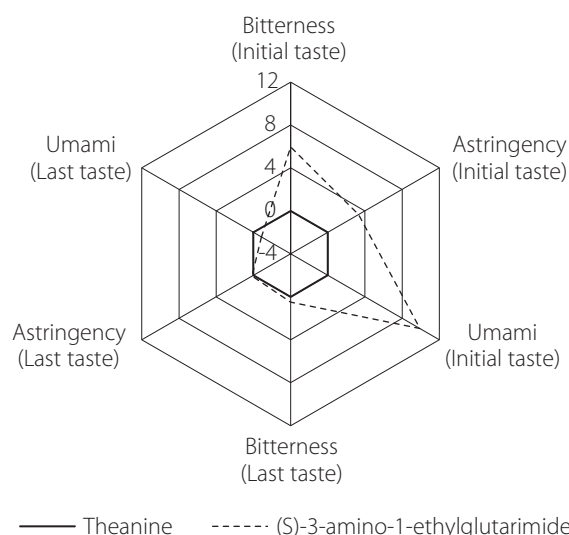


Figure 6: Taste evaluation of comparison between compound 1 and theanine using taste sensor analysis

Note: Data are expressed as mean \pm standard deviation ($n = 5$).

of 1 showed higher bitterness and astringency and umami than those of the theanine standard. A large quantity preparation of 1 was achieved by sub-critical water extraction of aq. theanine at 180 °C and 3 min reaction time. It is reported that 1 is useful for treating inflammatory conditions, diseases of the central nervous systems and insulin resistant diabetes (Thomas et al., 2004). Therefore, compound 1 might be expected to become a new multifunction medicine.

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