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TRYPTOPHAN 4-AMINOCINNAMAMIDES FROM A MYXOMYCETE *FULIGO AUREA*

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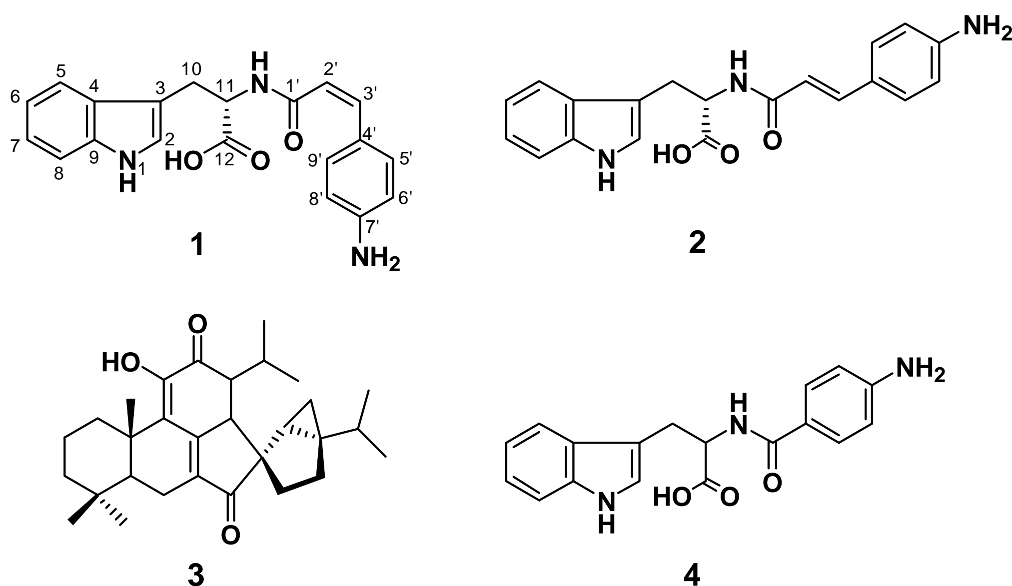
Abstract – Two new tryptophan derivatives, *cis*- and *trans*- tryptophan 4-aminocinnamamides (**1** and **2**), were isolated from field-collected fruit bodies of a myxomycete *Fuligo aurea*, along with a hexacyclic triterpenoid, chamaecydin (**3**), previously known as a plant constitutent. The structures of these compounds were elucidated on the basis of spectroscopic data.

The myxomycetes (true slime molds) are an unusual group of primitive organisms that may be assigned to one of the lowest classes of eukaryote. The chemical investigation of the secondary metabolites has been limited so far.[1] During our studies on search for bioactive natural products from myxomycetes,[2,3] we recently investigated a field-collected sample of fruit bodies of *Fuligo aurea* (Penz.). There has been no previous report before on the chemical constituents of *F. aurea*. From the fruit bodies of a myxomycete of the same genus *Fuligo candida*,[4] we previously isolated cycloanthranilylproline derivatives as well as 4-aminobenzoyltryptophan. Here we describe isolation and structure elucidation of two new tryptophan derivatives, *cis*- and *trans*-tryptophan 4-aminocinnamamides (**1** and **2**), from field-collected fruit bodies of a myxomycete *Fuligo aurea*, along with a hexacyclic triterpenoid, chamaecydin (**3**), previously known as a plant constitutent.

The fruit bodies of *F. aurea*, collected in Kochi Prefecture, were extracted with 90% MeOH and 90% acetone, and the combined crude extract was partitioned with between hexane, EtOAc, *n*-BuOH and H_2O. The *n*-BuOH-soluble portion (0.5 g) was submitted to an ODS column chromatography, followed by purification by reversed-phase HPLC to give compounds (**1** and **2**). On the other hand, the combined residue of hexane and EtOAc fractions (2.3 g) was subjected to a silica gel column chromatography and then a Sephadex LH-20 column chromatography to give compound (**3**).



Compound (**1**), obtained as amorphous solid, $[\alpha]_D^{23}$ +97° (c 0.8, MeOH), had the molecular formula $C_{20}H_{19}O_3N_3$ as determined by its HRESIMS (*m/z* 350.1505, $[M+H]^+$, Δ +0.2 mmu). The IR spectum of **1** suggested the presence of amino (3337 cm^{-1}) and amide (1645 cm^{-1}) groups. The UV absorption maxima were observed at 325, 291 and 222 nm. The ^{1}H NMR spectrum in CD_3OD (Table 1) showed the signals for eleven aromatic/olefinic protons (δ_H 7.57, 7.29, 7.23×2, 7.04, 7.00, 6.94, 6.49×2, 6.47, and 5.67), an sp^3 methine proton (δ_H 4.68) and methylene protons (δ_H 3.37 and 3.19). The ^{13}C NMR spectrum showed signals due to two carbonyl carbons (δ_C 178.7 and 169.6), sixteen aromatic or olefinic carbons (δ_C 111 – 149), an sp^3 methine carbon (δ_C 56.9), and an sp^3 methylene carbon (δ_C 29.1). Analysis of the ^{1}H-^{1}H COSY [H-5/H-6, H-6/H-7 and H-7/H-8; H_2-10/H-11; H-2'/H-3'; H-5'(9')/H-6'(8')] and the HMBC spectra (Figure 1) suggested the presence of a tryptophan residue [H-2/C-4, C-9 and C-10; H-5/C-3, C-7 and C-9; H-6/C-4 and C-8; H-7/C-5 and C-9; H-8/C-4 and C-6; H_2-10/C-2, C-4 and C-12; H-11/C-3] and *p*-substituted cinnamic acid residue [H-2'/C-3' and C-4'; H-3'/C-1', C-2', C-5'(9'); H-5'(9')/C-3', C-7', and C-9'(5'); H-6'(8')/C-4' and C-8'(6')]. The *p*-substituted cinnamoyl group was shown to be attached at C-11 amino group through an amide bond from the HMBC correlation observed between the carbonyl carbon at δ_C 169.6 (C-1') and the methine proton δ_H 4.68 (H-11).

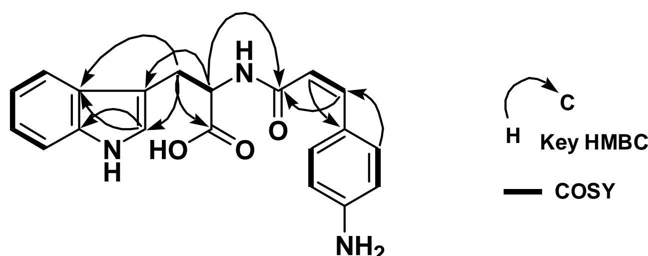


Figure 1. Key ^{1}H-^{1}H COSY and HMBC Data of **1**

In the ^{1}H NMR spectrum on a cinnamoyl residue, the configuration of the C-2'/C-3' double bond was suggested from the coupling constant ($J_{2',3'}$: *ca.* 13 Hz for *Z*, *ca.* 15 Hz for *E*)[5] as well as the chemical shifts data (H-2': δ_H *ca.* 5.6 for *Z*, δ_H *ca.* 6.5 for *E*; H-3': δ_H *ca.* 6.3 for *Z*, δ_H *ca.* 7.3 for *E*).[5] The C-2'/C-3' double bond of compound (**1**) was indicated to have *Z*-configuration from the coupling constant and chemical shift values [H-2': δ_H 5.67 (1H, d, *J*=13.0 Hz); H-3': δ_H 6.47 (1H, d, *J*=13.0 Hz)]. The presence of a tryptophan and a cinnamoyl group accounted for the molecular formula of **1** ($C_{20}H_{20}O_3N_3$) except for an amino group (NH_2), which remained to be elucidated. The ^{13}C NMR chemical shift of the C-4' position (δ_C 149.7) in the cinnamoyl group was indicative that an amino group was attached to C-4' position. From all of these findings, the structure of compound (**1**) was determined to be tryptophan *cis*-4-aminocinnamamide. Compound (**1**) was new on the basis of SciFinder search, and we have isolated a related tryptophan derivative, tryptophan 4-aminobenzamide (**4**), from the same genus of the myxomycete, *Fuligo candida*.[4]

Table 1. ^{1}H and ^{13}C NMR Spectral Data for compounds (**1** and **2**) in methanol-d_4

position	1		2	
	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C
2	7.00 (1H,s)	124.3	7.09 (1H, s)	124.3
3		111.9		112.0
4		129.4		129.4
5	7.57 (1H, d, 8.0)	119.7	7.60 (1H, d, 8.0)	119.7
6	6.94 (1H, t, 8.0)	119.5	6.94 (1H, t, 8.0)	119.5
7	7.04 (1H, t, 8.0)	122.0	7.03 (1H, t, 8.0)	122.0
8	7.29 (1H, d, 8.0)	111.9	7.28 (1H, d, 8.0)	111.9
9		137.9		137.8
10	3.19 (1H, dd, 15.0, 6.5)	29.1	3.24 (1H, dd, 15.0, 6.5)	29.2
	3.37 (1H, dd, 15.0, 5.0)		3.42 (1H, dd, 15.0, 5.0)	
11	4.68 (1H, dd, 6.5, 5.0)	56.9	4.72 (1H, dd, 6.5, 5.0)	57.1
12		178.7		178.6
1'		169.6		168.7
2'	5.67 (1H, d, 13.0)	120.0	6.32 (1H, d, 15.5)	125.4
3'	6.47 (1H, d, 13.0)	138.4	7.33 (1H, d, 15.5)	142.1
4'		125.8		124.3
5', 9'	7.23 (2H, d, 8.0)	132.1	7.26 (2H, d, 8.5)	130.4
6', 8'	6.49 (2H, d, 8.0)	115.4	6.63 (2H, d, 8.5)	115.7
7'		149.7		151.3

The molecular formula of compound (**2**), $[\alpha]_D^{23}$ +49° (c 0.7, MeOH), was determined to be $C_{20}H_{19}O_3N_3$ by the HRESIMS (*m/z* 350.1506, $[M+H]^+$, Δ +0.3 mmu). The UV and IR spectra of **2** were similar to those of **1**. Comparison of the ^{1}H and ^{13}C NMR spectra of **2** (Table 1) with those of **1** suggested that compound **2** was also a tryptophan 4-aminocinnamamide, which was further supported by the analysis of its ^{1}H-^{1}H COSY and HMBC correlation data. The major differences of ^{1}H NMR spectral data between **1** and **2** were the chemical shifts and their coupling constant of the C-2'/C-3' double-bond portion of the cinnamoyl group [H-2': δ_H 6.32 (1H, d, *J*=15.5 Hz); H-3': δ_H 7.33 (1H, d, *J*=15.5 Hz)], implying that this

double bond of **2** had *trans*-configuration.[5] Thus, the structure of compound (**2**) was concluded to be tryptophan *trans*-4-aminocinnamamide.[6]

The absolute stereochemistry of the chiral center at C-11 position of compounds (**1** and **2**) was determined by chiral TLC analysis after acid hydrolysis of each compound (6N HCl, 110°C, 8 h), revealing that the tryptophan residue contained in each compound (**1** and **2**) was L (11*S*-configuration).[7]

Compound (**3**) was isolated from hexane- and EtOAc-soluble fractions of the extract of this myxomycete. The ^{1}H and ^{13}C NMR spectral data as well as the EIMS data [*m/z* 448 (M^{+})] suggested that compound (**3**) was identical with chamaecydin, a hexacyclic triterpenoid previously isolated from the seed of *Chamaecyparis obtuse*[8] and the cones of *Cryptomeria japonica.*[9] Here, chamaecydin (**3**) was first isolated from a mycomycete.

The cytotoxic activity[10] of compounds (**1**), (**2**), and (**3**) against Jurkat cells were examined, but all three compounds proved to be inactive at 25 μg/mL.

EXPERIMENTAL

Optical rotations were measured on a JASCO P-1020 polarimeter. UV spectra were obtained on a SHIMADZU UV mini 1240 spectrophotometer, and IR spectra were recorded on a JASCO FT/IR-230 spectrophotometer. The NMR spectra were recorded on JEOL JNM ecp600 and A500 spectrometers. The chemical shift values are reported in ppm (δ) units and the coupling constants (J) are in Hz. High-resolution ESI-MS was obtained on a Micromass LCT spectrometer. EI-MS was acquired on JMS GC-Mate mass spectrometer. HPLC was carried out on Shimadzu LC-10ADvp pump equipped with a SPD-M10vp detector (λ 254 nm), and preparative HPLC on a JASCO PU-2080 Plus equipped with a UV-2075 Plus detector (λ 254 nm).

Organism: The fruit bodies of *Fuligo aurea* were collected and identified by Y.Y. at Ohtsu, Kochi-shi in Kochi Prefecture, Japan, in 2005. Voucher specimens (#28680-28683) are maintained by Y.Y. (Ohtsu-ko, Kochi).

Extraction and isolation: The field-collected fruit bodies of *Fuligo aurea* (47.7 g) were extracted with 90% MeOH (600 mL × 6), 90% acetone (600 mL × 1) and H_2O (600 mL × 6) at rt. The combined MeOH, acetone and H_2O extracts (11.0 g) were partitioned between hexane (500 mL × 3), EtOAc (500 mL × 4), *n*-BuOH (500 mL × 2), and H_2O (500 mL). The hexane- and EtOAc-extracts were combined to give a residue (2.3 g), which was applied to a silica gel column (column A: 30 × 400 mm) and eluted with hexane/$CHCl_3$ mixture (1:1), $CHCl_3$, $CHCl_3$/MeOH (10:1, 9:1, 4:1, 7:3 and 1:1) and MeOH. A fraction (20 mg) of column A eluted with hexane/$CHCl_3$ (1:1) was subjected to a Sephadex LH-20 column (10 × 600 mm) with MeOH to give **3** (1.3 mg).

The *n*-BuOH-soluble fraction (0.5 g) was subjected to an ODS column chromatography (column B: 30 × 150 mm) with a H_2O/MeOH gradient system (9:1, 7:3, 1:1, 3:1 and 0:1) to afford 7 fractions (fractions A-G). Fr. D (51 mg) of column B eluted with H_2O/MeOH (7:3) was further purified with ODS HPLC (Develosil ODS-HG-5, 10 × 250 mm; eluent, 20% MeOH; flow rate, 2.0 mL/min) to afford **1** (16.3 mg, t_R 21 min) and **2** (12.0 mg, t_R 30 min).[6]

Tryptophan *cis*-4-aminocinnamamide (1): Amorphous solid; $[\alpha]_D^{23}$ +97° (c 0.8, MeOH); UV (MeOH) λ_{max} nm (log ε) 325 (3.9), 291 (3.9), 222 (4.3); IR (film) ν_{max} 3337, 1645, 1597, 1517 and 1399 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; HRESIMS *m/z* 350.1505 $[M+H]^+$ (calcd for $C_{20}H_{20}O_3N_3$, 350.1505).

Tryptophan *trans*-4-aminocinnamamide (2): Amorphous solid; $[\alpha]_D^{23}$ +49° (c 0.7, MeOH); UV (MeOH) λ_{max} nm (log ε) 325 (3.8), 291 (3.8), 222 (4.2); IR (film) ν_{max} 3338, 1647, 1590, 1517 and 1397 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; HRESIMS *m/z* 350.1506 $[M+H]^+$ (calcd for $C_{20}H_{20}O_3N_3$, 350.1505).

Acid Hydrolysis and chiral TLC analysis of 1 and 2: Compound (**1**) (1.2 mg) was treated with 6N HCl (2 mL) and heated at 110°C for 8 h. After cooling, the mixture was passed through a short Amberlite IRA96SB AG column and then the eluate was evaporated to dryness. The hydrolyzate was subjected to the chiral TLC analysis (Merck HPTLC plate CHIR Art. 1410; MeOH/H_2O/CH_3CN 1:1:4; visualization, ninhydrorin) to give a spot corresponding to L-Trp (R_f-values: D-Trp 0.50, L-Trp 0.58). Compound (**2**) (2.5 mg) was treated under the same conditions as above to show the same results.

Cytotoxic activity: Jurkat cells (1.0×10^4 cells/mL) were seeded in 195 μL of culture medium per well in 96-well mictoritre plates, and were treated with 5 μL of graded concentrations of samples and were then incubated for 72 h at 37°C in a 5% CO_2-95% air atmosphere. Cell viability was determined by the colorimetric assay using alamer blue.[10]

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6. HPLC separation (Develosil C30-UG-5, 10 × 250 mm; eluent, 30% MeOH; flow rate, 2.0 mL/min) of a fraction (fraction E, 108 mg) of column B (see, Experimental section) afforded compounds (**1**) (3.5 mg, t_R 15 min), (**2**) (3.6 mg, t_R 22 min), and (**2a**) (30.2 mg, t_R 40 min). Compound (**2a**): amorphous solid; $[\alpha]_D^{23}$ –2.4° (c 0.7, MeOH); UV (MeOH) λ_{max} nm (log ε) 325 (3.8), 291 (3.8), 223 (4.2); IR (film) ν_{max} 3384, 1645, 1586, 1519 and 1417 cm^{-1}; ^1H NMR (CD_3OD) 7.08 (1H, s, H-2), 7.57 (1H, d, *J*=6.6 Hz, H-5), 6.93 (1H, t, *J*=6.6 Hz, H-6), 7.03 (1H, t, *J*=6.6 Hz, H-7), 7.27 (1H, d, *J*=6.6 Hz, H-8), 3.19 (1H, br d, *J*=13.5 Hz, H-10), 3.23 (1H, dd, *J*=13.5 and 5.8 Hz, H-10), 4.71 (1H, m, H-11), 6.29 (1H, d, *J*=15.5 Hz, H-2'), 7.34 (1H, d, *J*=15.5 Hz, H-3'), 7.22 (2H, d, *J*=6.5 Hz, H-5' and H-9'), and 6.59 (2H, d, *J*=6.5 Hz, H-6' and H-8'); ^{13}C NMR (CD_3OD) δ_C 122.9 (C-2), 111.9 (C-3), 127.9 (C-4), 118.2 (C-5), 118.2 (C-6), 120.8 (C-7), 110.7 (C-8), 137.9 (C-9), 27.5 (C-10), 55.9 (C-11), 178.7 (C-12), 168.1 (C-1'), 115.1 (C-2'), 141.4 (C-3'), 123.9 (C-4'), 129.1 (C-5' and C-9'), 114.4 (C-6' and C-8'), and 150.1 (C-7'); HRESIMS *m/z* 350.1514 $[M+H]^+$ (calcd for $C_{20}H_{20}O_3N_3$, 350.1505). Although compound (**2a**) had a different HPLC retention time and different ^1H NMR spectral data from those of **2**, compound (**2a**) was identified as tryptophan *trans*-4-aminocinnamamide with a different ionic state from **2**, since under a different HPLC condition (Develosil C30-UG-5, 10 × 250 mm; eluent, 40% MeOH with 0.1% TFA), **2** and **2a** afforded an identical retention time ($t_{R=}$22 min), and methylation of **2** and **2a** with $TMSCHN_2$ afforded an identical methyl ester: ^1H NMR (CD_3OD) δ_H 7.08 (1H, s, H-2), 7.53 (1H, d, *J*=7.0 Hz, H-5), 7.00 (1H, t, *J*=7.0 Hz, H-6), 7.08 (1H, t, *J*=7.0 Hz, H-7), 7.32 (1H, d, *J*=7.0 Hz, H-8), 3.23 (1H, dd, *J*=14.4, 6.4 Hz, H-10), 4.84 (1H, dd, *J*=9.2, 6.4 Hz, H-11), 6.38 (1H, d, *J*=15.6 Hz, H-2'), 7.39 (1H, d, *J*=15.6 Hz, H-3'), 7.29 (2H, d, *J*=8.4 Hz, H-5' and H-9'), 6.65 (2H, d, *J*=8.4 Hz, H-6' and H-8'), and 3.66 (3H, s, -COOMe); HRESIMS *m/z* 364.1673 $[M+H]^+$ (calcd for $C_{21}H_{22}O_3N_3$, 364.1661). Detailed ionic states for **2** and **2a** remained uncharacterized.
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