A New Lignan Amide, Grossamide, from Bell Pepper (*Capsicum annuum* var. *grossum*)*

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Two new phenolic amides, grossamide (7) and *N*-*cis*-feruloyl tyramine (2), have been isolated from the roots of bell pepper (*Capsicum annuum* var. *grossum*) together with *p*-aminobenzaldehyde (1), *N*-*trans*-feruloyl tyramine (3), *N*-*trans*-*p*-coumaroyl tyramine (4), *N*-*trans*-feruloyl octopamine (5), and *N*-*trans*-*p*-coumaroyl octopamine (6).

Eggplant (*Solanum melongena* L.) roots have been reported¹⁾ to contain the four phenolic amides during a survey of the phenolic compounds in *Solanaceae*. Continued extension of the phytochemical survey led us to find two new amides in addition to the four amides from bell pepper (*Capsicum annuum* var. *grossum*). This deals with the isolation and the structure determination of these new compounds with identification of the several known compounds.

The roots of bell pepper were extracted with aqueous ethanol and the concentrated extract was partitioned between water-ether. The ether fraction, after the chromatographic separation, yielded an aromatic amine (P₃) and six phenolic compounds (P_{4a}, P_{4b}, P_{4c}, P₅, P_{6a}, and P_{6b}). Five of them were identified as *p*-aminobenzaldehyde (P₃, 1), *N*-*trans*-feruloyl tyramine (P_{4b}, 3), *N*-*trans*-*N*-coumaroyl tyramine (P₅, 4), *N*-*trans*-feruloyl octopamine (P_{6a}, 5) and *N*-*trans*-*p*-coumaroyl octopamine (P_{6b}, 6) by direct comparison with the authentic samples from eggplant.

The P_{4a} compound showed the mass spectrum similar to that of *N*-*trans*-feruloyl tyramine (3). The base peak at *m/z* 137 was characteristic of tyramine moiety. The frag-

ment ion at *m/z* 177 would be expected from ferulic acid moiety. The PMR spectrum of P_{4a} was similar to that of 3 except signals assigned to vinylic protons at δ 5.90 ($J=13$ Hz) and 6.65 ($J=13$ Hz). The smaller coupling constants of the vinylic protons than those of the *trans*-isomer (3, $J=16$ Hz) indicated the presence of *cis*-geometry together with lack of IR absorption around 970 cm^{-1} in P_{4a}. The P_{4a} compound was, therefore, assigned as *N*-*cis*-feruloyl tyramine (2). The structure was confirmed by synthesis of diacetyl *N*-*cis*-feruloyl tyramine (2a), in which *N*-*trans*-feruloyl tyramine (3) was isomerized by UV irradiation²⁾ and then acetylated. The higher chemical shift of β -vinylic proton at δ 5.90 than that of the *trans*-isomer (3) is considered to be owing to no deshield effect by the carbonyl group in 2.

The PMR spectrum of the P_{4c} compound, now named grossamide (7), showed a little similarity to the resonance pattern of *N*-*trans*-feruloyl tyramine (3), but differed from 3 in additional one methoxyl, two methine coupling each other ($J=8$ Hz) and six aromatic protons. The integral value of 7 in a region of $-\text{CH}_2-\text{CH}_2-$ (δ 2.6~2.9 and 3.4~3.7) showed two times that of 3. The presence of

and $-\text{CONH}-$ group in 7 was supported by IR absorption at 970 and

* Phenolic Compounds from the Roots of *Solanaceae* Plants. Part II. For Part I. see ref. 1.

TABLE I. SEPARATION OF THE POLYPHENOL MIXTURE FROM BELL PEPPER

Fraction No.	Eluent (MeOH:CHCl ₃)	Test tube No.	Weight (g)	Compound	Yield (mg)
P ₁	0:100	1~16	2.0	—	
P ₂	"	17~107	15.2	—	
P ₃	"	108~150	1.9	<i>p</i> -aminobenzaldehyde (1)	130
P ₄	5:95	151~211	3.3	<i>N</i> - <i>cis</i> -feruloyl tyramine (P _{4a} , 2) <i>N</i> - <i>trans</i> -feruloyl tyramine (P _{4b} , 3) grossamide (P _{4c} , 7)	35 260 68
P ₅	"	212~272	1.9	<i>N</i> - <i>trans</i> - <i>p</i> -coumaroyl tyramine (4)	50
P ₆	10:90	273~340	1.9	<i>N</i> - <i>trans</i> -feruloyl octopamine (P _{6a} , 5) <i>N</i> - <i>trans</i> - <i>p</i> -coumaroyl octopamine (P _{6b} , 6)	32 20

SCHEME 1.

SCHEME 2.

1655 cm⁻¹, respectively. The *trans*-arrangement about the double bond was confirmed by the coupling constant of 16 Hz between the vinyl protons. The mass spectrum exhibited fragment ions at *m/z* 137 and 120 indicating to contain tyramine moiety in the molecule. The PMR spectrum of an acetylated compound (7a) displayed two signals at δ 2.26 (3H) and 2.30 (6H) for three acetyl groups. There was no signal shifted to lower field. This indicated that 7a possessed three Ar-OH but no primary and secondary alcohol groups. The presence of three Ar-OH was confirmed

by PMR spectrum of a methylated compound (7b) which had three signals at δ 3.75, 3.86 and 3.89 in addition to those of 7. A molecular ion at *m/z* 666 appeared in the FD-MS of 7b.

Hydrogenation of 7 followed by hydrolysis with 6N HCl (Scheme 1) led to liberation of tyramine in aqueous fraction, which was identified by TLC and MS. The acid extracted with ethyl acetate from the aqueous phase was methylated with HCl-MeOH. The methyl ester (9), C₂₂H₂₄O₈, gave a positive reaction with Folin-Ciocalteu reagent. Its PMR spectrum showed the following structural features:

FIG. 1. Polyphenol Amides and an Aromatic Amine from Bell Pepper.

$-\text{CH}_2-\text{CH}_2-$ (δ 2.56 and 2.87, $J=7.5$ Hz), two $-\text{COOCH}_3$ (3.50 and 3.61), two $-\text{OCH}_3$ (3.78 and 3.90), two $-\text{OH}$ (5.60 and 5.70), five aromatic protons (6.5~6.62 and 6.7~6.9) and one vinylic proton (7.80). Finally, the structure of the ester was proposed as methyl 2-[2-hydroxy-3-methoxy-5-(2-methoxycarbonyl)ethyl]phenyl]-3-(4-hydroxy-3-methoxyphenyl)acrylate (9).

The structure of dihydrogrossamide (8) was elucidated on the basis of the formation of 9, in which the mechanism is shown in Scheme 1. Thus the whole structure of grossamide can be depicted as 7, an amide consisting of two tyramines and dehydrodiferulic acid (3-carboxy-5-*trans*-carboxyvinyl-7-methoxy-2-(4-hydroxy-3-methoxy)phenyl)-2,3-dihydrobenzofuran). Further confirmation of the proposed structure was obtained by comparing the natural product directly with a synthetic sample, which was prepared from *N-trans*-feruloyl tyramine (3) by an oxidative coupling (Scheme 2).

McCredie *et al.*³⁾ proposed that the term lignan is extended to cover all natural prod-

FIG. 2. Hordatine A (10), B (11) and M (12 and 13).

ucts of low molecular weight that arise primarily from the oxidative coupling of *p*-hydroxyphenylpropene unit. Grossamide is therefore classified into a group of lignan. Very few studies have been reported on oxidative coupling products possessing amide group. Hordatine A (10), B (11) and M (12 and 13) found as antifungal factors in barley⁴⁾ are the cases for them. In contrast with hordatines, grossamide is optically inactive. This fact implied that grossamide can occur in an optically inactive form in nature, because the conditions used for isolation were not so drastic as to alter the optical property. The relative configuration of grossamide (7) will be reported in a separate paper.

EXPERIMENTAL

Melting points were determined on Yanaco Micro Melting Point Apparatus MP-30 and MP-3S). UV spectra were recorded using HITACHI PERKIN-ELMER 139 UV-VIS spectrophotometer and Hitachi EPS-3T. IR spectra were measured with HITACHI 285. PMR spectra were run on HITACHI R-22 (90 Mc/sec) and JNM-FX 200 FT NMR Spectrometer (200 Mc/sec). FD-mass spectra were run on JEOL JMS-01SG-2 and EI-MS run JEOL JMS D-300. ORD curve was measured with a JASCO ORD/UV-5. UV irradiation was carried out with Halos PIH-300.

Isolation and separation. Chopped root materials of bell pepper (21 kg) were extracted with 70% EtOH (160 liter)

for 2 weeks. The extracts were evaporated to remove the solvent under reduced pressure. The aqueous layer was extracted with ether (1 liter $\times$ 8). The ether extracts were concentrated under reduced pressure to a syrup (40 g), which was chromatographed over a silica gel column (600 g). The elution was carried out with (1) CHCl_3 , (2) $\text{MeOH}-\text{CHCl}_3$ (5:95) and (3) $\text{MeOH}-\text{CHCl}_3$ (10:90), collecting each 15 g fraction which was monitored by UV absorption at 280 nm and TLC. Thus 6 major fractions as listed in Table I were obtained.

The fraction P_4 was further separated into three fractions (P_{4a} , P_{4b} and P_{4c} in their order of elution) by silica gel column chromatography (25 g) using $\text{MeOH}-\text{benzene}$ (7:93). Similarly the fraction P_6 was rechromatographed to yield P_{6a} and P_{6b} on a silica gel-celite (75 g:15 g) column using acetone- CHCl_3 (20:80).

P_3 compound (*p*-aminobenzaldehyde, 1). The P_3 fraction was evaporated to dryness under reduced pressure and rechromatographed on a silica gel column (50 g) with $\text{MeOH}-\text{CHCl}_3$ (2:98) and resulted in a syrup, which was crystallized from CHCl_3 to give *p*-aminobenzaldehyde (1) as colourless needles, mp 98~100°C. It gives a positive 2,4-dinitrophenyl hydrazine test and negative Folin-Ciocalteu and ninhydrin tests. PMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 6.15~6.30 (2H, m, $-\text{NH}_2$), 7.0 (2H, d, $J=9\text{ Hz}$, $2 \times \text{Ar-H}$), 7.85 (2H, d, $J=9\text{ Hz}$, $2 \times \text{Ar-H}$), 9.85 (1H, s, $-\text{CHO}$).

P_{4a} compound (*N*-cis-feruloyl tyramine, 2). The P_{4a} fraction (67 mg) was rechromatographed on a silica gel column (15 g) with $\text{MeOH}-\text{CHCl}_3$ (2:98) and resulted in a syrup of *N*-cis-feruloyl tyramine (2). IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 1640, 1590, 1500, 1020. EI-MS m/z : No M^+ (313), 177, 137 (base peak). PMR $\delta_{\text{TMS}}^{\text{acetone-d}_6}$: 2.6~3.0 (2H, m, $-\text{CH}_2-$), 3.3~3.7 (2H, m, $-\text{CH}_2-$), 3.89 (3H, s, $-\text{OCH}_3$), 5.90 (1H, d, $J=13\text{ Hz}$, $\text{H}_\text{C}=\text{C}^\text{H}$), 6.65 (1H, d, $J=13\text{ Hz}$, $\text{H}_\text{C}=\text{C}^\text{H}$), 6.8~7.3 (7H, m, $7 \times \text{Ar-H}$).

Acetylation of the amide (2, 70 mg) with Ac_2O (2 ml) and pyridine (2 ml) gave a syrup, which was chromatographed over silica gel (2 g) with $\text{MeOH}-\text{CHCl}_3$ (2:98) and yielded diacetyl *N*-cis-feruloyl tyramine (2a, 22 mg) as a syrup. IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 1765, 1660, 1605, 1590, 1505, 1365, 1205, 1195, 905, 830, 750. EI-MS m/z : 397 (M^+), 335 ($\text{M}^+ - \text{Ac}$), 192, 177 (base peak), 120, 43. PMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 2.28 (3H, s, $-\text{Ac}$), 2.29 (3H, s, $-\text{Ac}$), 2.65~2.90 (2H, m, $-\text{CH}_2-$), 3.4~3.8 (2H, m, $-\text{CH}_2-$), 3.82 (3H, s, $-\text{OCH}_3$), 5.96 (1H, d, $J=12\text{ Hz}$, $\text{H}_\text{C}=\text{C}^\text{H}$), 6.71 (1H, d, $J=12\text{ Hz}$, $\text{H}_\text{C}=\text{C}^\text{H}$), 6.9~7.3 (7H, m, $7 \times \text{Ar-H}$).

Isomerization of *N*-trans-feruloyl tyramine (3) to *N*-cis-feruloyl tyramine (2). The amide (3, 50 mg) was dissolved in MeOH (50 ml) and the solution was irradiated for 2 hr at room temperature at a distance of 5 cm with UV lamp.

The reaction mixture was chromatographed over silica gel-celite (5 g:1 g) with acetone- CHCl_3 (20:80). The eluate was concentrated to a syrup of *N*-cis-feruloyl tyramine (2, 20 mg) which was identical with the natural product in all spectral data.

Acetylation of the synthetic *cis*-amide (20 mg) with Ac_2O (1.0 ml) and pyridine (1.0 ml) gave a syrup of diacetyl *N*-cis-feruloyl tyramine (22 mg). The product was ascertained to be identical with natural diacetyl *N*-cis-feruloyl tyramine (2a) in spectral data.

P_{4b} compound (*N*-trans-feruloyl tyramine, 3). The fraction P_{4b} (514 mg) was concentrated under reduced pressure and the residues were crystallized from acetone- CHCl_3 to give *N*-trans-feruloyl tyramine (3), mp 144.5~145°C. PMR $\delta_{\text{TMS}}^{\text{acetone-d}_3}$: 2.76 (2H, t, $J=7\text{ Hz}$, $-\text{CH}_2-$), 3.40 (1H, t, $J=7\text{ Hz}$, $-\text{CH}_2-$), 3.56 (1H, t, $J=7\text{ Hz}$, $-\text{CH}_2-$), 3.87 (3H, s, $-\text{OCH}_3$), 6.51 (1H, d, $J=16\text{ Hz}$, $\text{H}_\text{C}=\text{C}^\text{H}$), 6.65~7.15 (7H, m, $7 \times \text{Ar-H}$), 7.47 (1H, d, $J=16\text{ Hz}$, $\text{H}_\text{C}=\text{C}^\text{H}$).

Acetylation of P_{4b} compound (3, 32 mg) with Ac_2O (1.0 ml) and pyridine (1.0 ml) followed by chromatography with silica gel (1.0 g) using $\text{MeOH}-\text{CHCl}_3$ (1:99) gave diacetyl *N*-trans-feruloyl tyramine (3a, 17 mg), mp 153~155°C on crystallization from CHCl_3 . PMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 2.22 (6H, s, $2 \times -\text{Ac}$), 2.75 (2H, t, $J=6\text{ Hz}$, $-\text{CH}_2-$), 3.45 (1H, t, $J=6\text{ Hz}$, $-\text{CH}_2-$), 3.53 (1H, t, $J=6\text{ Hz}$, $-\text{CH}_2-$), 3.72 (3H, s, $-\text{OCH}_3$), 5.6~5.85 (1H, m, $>\text{NH}$), 6.12 (1H, d, $J=16\text{ Hz}$, $\text{H}_\text{C}=\text{C}^\text{H}$), 6.8~7.15 (7H, m, $7 \times \text{Ar-H}$), 7.38 (1H, d, $J=16\text{ Hz}$, $\text{H}_\text{C}=\text{C}^\text{H}$).

P_{4c} compound (grossamide, 7). The P_{4c} fraction (250 mg) was concentrated and the residue was crystallized from $\text{MeOH}-\text{CHCl}_3$ -cyclohexane to give grossamide (7) as colorless crystals, mp 174~175°C. UV $\lambda_{\text{EtOH}}^{\text{max}}$ nm (ϵ): 289 (20,400), 305 (20,000), 322 (21,200). IR $\nu_{\text{KBr}}^{\text{max}}$ cm^{-1} : 1655, 1600, 1505, 1270, 1145, 970, 820. FD-MS m/z : 625 (2.8%), 624 (M^+ , 1.7%), 487 (100%). EI-MS m/z : No M^+ (624), 487 ($\text{M}^+ - 137$), 367, 350 ($\text{M}^+ - 2 \times 137$), 234, 217, 137 (tyramine moiety), 120 (base peak). PMR $\delta_{\text{TMS}}^{\text{acetone-d}_6}$: 2.6~2.9 (4H, m, $2 \times -\text{CH}_2-$), 3.4~3.7 (4H, m, $2 \times -\text{CH}_2-$), 3.80 (3H, s, $-\text{OCH}_3$), 3.82 (3H, s, $-\text{OCH}_3$), 4.22 (1H, d, $J=8\text{ Hz}$, $-\text{CH}$), 6.02 (1H, d, $J=8\text{ Hz}$, $-\text{CH}$), 6.50 (1H, d, $J=16\text{ Hz}$, $\text{H}_\text{C}=\text{C}^\text{H}$), 6.5~7.7 (16H, m, $13 \times \text{Ar-H}$, $\text{H}_\text{C}=\text{C}^\text{H}$ and $2 \times -\text{NH}-$), 7.73 (1H, bs, $-\text{OH}$), 8.24 (1H, bs, $-\text{OH}$), 8.78 (1H, bs, $-\text{OH}$). ORD ($c=1.0$, MeOH) $[\alpha]$ (nm): 0° (700~435).

Acetylation of P_{4c} compound (7, 60 mg) with Ac_2O (2 ml) and pyridine (2 ml) and crystallization from CHCl_3 -cyclohexane gave triacetyl grossamide (7a, 40 mg), mp

203.5~204.5°C. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (ϵ): 305 (16,200), 322 (17,400). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1760, 1655, 1650, 1605, 1600, 1500, 970, 905, 840. MS m/z : No M^+ (750, $\text{C}_{42}\text{H}_{42}\text{N}_2\text{O}_{11}$), 571.1828 ($\text{C}_{32}\text{H}_{29}\text{NO}_9$, Calcd. 571.1841, M^+ - acetyl tyramine), 529.1737 ($\text{C}_{30}\text{H}_{27}\text{NO}_8$, 529.1737, $\text{C}_{32}\text{H}_{29}\text{NO}_9$ -Ac), 499.1602 ($\text{C}_{29}\text{H}_{25}\text{NO}_7$, 499.1630), 487.1609 ($\text{C}_{28}\text{H}_{25}\text{NO}_7$, 487.1630, $\text{C}_{30}\text{H}_{27}\text{NO}_8$ -Ac), 393.0979 ($\text{C}_{22}\text{H}_{17}\text{O}_7$, 393.0974, M^+ - 2 $\times$ acetyl tyramine). EI-MS m/z : No M^+ (750), 571, 529, 487, 393, 380, 367, 351, 150, 137, 120 (base peak), 107. FT-PMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 2.26 (3H, s, -Ac), 2.30 (6H, s, 2 $\times$ -Ac), 2.65~2.95 (4H, m, 2 $\times$ -CH₂-), 3.4~3.7 (4H, m, 2 $\times$ -CH₂-), 3.79 (3H, s, -OCH₃), 3.91 (3H, s, -OCH₃), 4.05 (1H, d, $J=6.4$ Hz, $\text{H}-\text{C}(\text{H})=\text{C}(\text{H})$), 5.8~6.0 (2H, m, 2 $\times$ -CONH-), 6.03 (1H, d, $J=6.9$ Hz, $\text{H}-\text{C}(\text{H})=\text{C}(\text{H})$), 6.14 (1H, d, $J=15.6$ Hz, $\text{H}-\text{C}(\text{H})=\text{C}(\text{H})$), 6.6~7.3 (13H, m, 13 $\times$ Ar-H), 7.49 (1H, d, $J=15.6$ Hz, $\text{H}-\text{C}(\text{H})=\text{C}(\text{H})$).

Methylation of grossamide (7). Grossamide (7, 25 mg) was methylated under reflux in anhydrous acetone (10 ml) containing K_2CO_3 (0.5 g) and $(\text{CH}_3)_2\text{SO}_4$ (0.4 ml) for 20 hr. The solid in the reaction mixture was removed by filtration and the filtrate was concentrated under reduced pressure to a syrup, which was partitioned between dil. NH_4OH -ethyl acetate. The ethyl acetate fraction was evaporated to dryness. The residue was passed over a silica gel column (1.5 g) with $\text{MeOH}-\text{CHCl}_3$ (3:97) yielded a powder of trimethyl grossamide (7b), 18 mg (68% yield). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1655, 1650, 1605, 1505, 1240, 1140, 1020, 965. FD-MS m/z : 668 (21%), 667 (49%), 666 (M^+ , 100%). EI-MS m/z : No M^+ (666), 548, 516, 381, 337, 151, 134, 121 (base peak). PMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 2.7~2.95 (4H, m, 2 $\times$ -CH₂-), 3.4~3.7 (4H, m, 2 $\times$ -CH₂-), 3.75 (3H, s, -OCH₃), 3.79 (3H, s, -OCH₃), 3.85 (3H, s, -OCH₃), 3.86 (3H, s, -OCH₃), 3.89 (3H, s, -OCH₃), 4.08 (1H, d, $J=7$ Hz, $\text{H}-\text{C}(\text{H})=\text{C}(\text{H})$), 5.7~6.0 (2H, m, 2 $\times$ -NH), 5.94 (1H, d, $J=7$ Hz, $\text{H}-\text{C}(\text{H})=\text{C}(\text{H})$), 6.07 (1H, d, $J=16$ Hz, $\text{H}-\text{C}(\text{H})=\text{C}(\text{H})$), 6.7~7.2 (13H, m, 13 $\times$ Ar-H), 7.49 (1H, d, $J=16$ Hz, $\text{H}-\text{C}(\text{H})=\text{C}(\text{H})$).

Hydrogenation of grossamide (7). Grossamide (7, 20 mg) was stirred in ethanol (1 ml) with 10% Pd-C (2 mg) under hydrogen at atmospheric pressure for 2 hr. The reaction mixture was monitored by UV absorption at 250 to 360 nm. On removing the catalyst and solvent, dihydrogrossamide (8) resulted in 15 mg yield.

IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 1640, 1600, 1505, 1260, 1210, 1130, 1090, 1020, 810, 650. FD-MS m/z : No M^+ (626), 489 (M^+ - 137). EI-MS m/z : No M^+ (626), 489, 335, 235, 164, 137, 120, 108 (base peak). PMR $\delta_{\text{TMS}}^{\text{acetone-d}_3}$: 2.3~2.5 (2H, m, -CH₂-), 2.5~2.9 (6H, m, 3 $\times$ -CH₂-), 3.2~3.6 (4H, m, 2 $\times$ -CH₂-), 3.78 (6H, s, 2 $\times$ -OCH₃), 4.12 (1H, d,

$J=7$ Hz, $\text{H}-\text{C}(\text{H})=\text{C}(\text{H})$), 5.94 (1H, d, $J=7$ Hz, $\text{H}-\text{C}(\text{H})=\text{C}(\text{H})$), 6.6~7.4 (13H, m, 13 $\times$ Ar-H).

Hydrolysis and methylation of dihydrogrossamide (8). The dihydrogrossamide (8, 15 mg) was refluxed with a solution of 6N HCl (5 ml) and MeOH (4 ml) for 8.5 hr. To the concentrated solution was added H_2O (20 ml) and the solution was extracted with ethyl acetate (20 ml $\times$ 3).

a) The aqueous layer after adjusted to pH 10 with dilute aq. KOH was extracted with ethyl acetate (20 ml $\times$ 3). The ethyl acetate layer was dried on Na_2SO_4 and concentrated to give tyramine. TLC with microcrystalline cellulose (avicel) in $n\text{-BuOH}-\text{AcOH}-\text{H}_2\text{O}$ (4:1:2): R_f 0.70, R_f of tyramine: 0.70. The MS pattern was completely identical with that of tyramine; m/z 137 (M^+), 120, 108 (base peak), 107, 77.

b) The ethyl acetate layer was concentrated *in vacuo* and the residue (10 mg) was methylated under reflux in 3% HCl-MeOH (10 ml) for 2 hr. The reaction mixture was concentrated under reduced pressure to a syrup, which was chromatographed over silica gel (1 g) using CHCl_3 to give a syrup of 9 (1 mg). IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 1730, 1705, 1595, 855, 815, 790, 750. EI-MS m/z : 416 (M^+ , 416.1489 $\text{C}_{22}\text{H}_{24}\text{O}_8$, requires 416.1472), 384 (base peak), 311, 283. FT-PMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 2.56 (2H, t, $J=7.5$ Hz, -CH₂-), 2.87 (2H, t, $J=7.5$ Hz, -CH₂-), 3.50 (3H, s, -COOCH₃), 3.61 (3H, s, -COOCH₃), 3.78 (3H, s, Ar-OCH₃), 3.90 (3H, s, Ar-OCH₃), 5.60 (1H, s, -OH), 5.70 (1H, s, -OH), 6.5~6.62 (2H, m, 2 $\times$ Ar-H), 6.7~6.9 (3H, m, 3 $\times$ Ar-H), 7.80 (1H, s, $\text{H}-\text{C}(\text{H})=\text{C}(\text{H})$).

Synthesis of grossamide (7). To a mixture of *N-trans*-feruloyl tyramine (2, 150 mg), peroxidase (Boehringer Mannheim GmbH, horseradish, 2 mg) and M/15 phosphate buffer solution (pH 7.38, 80 ml), 0.022% H_2O_2 (130 ml) was added dropwise by stirring for 18 hr at 38°C. Being allowed to stir for 13 hr, NaCl was added to prevent the reaction. Ethyl acetate fraction (150 ml $\times$ 3) was concentrated to a syrup (148 mg), which was passed over a silica gel column (15 g) with $\text{MeOH}-\text{CHCl}_3$ (5:95), yielded a powder of synthetic grossamide (30 mg, 20% yield). Its spectral data of PMR and IR were identical with those of the natural product (7).

P_5 compounds (*N-trans-p*-coumaroyl tyramine, 4). Rechromatography of the fraction P_5 using silica gel (25 g) with $\text{MeOH}-\text{CHCl}_3$ (10:90) afforded a powder of *N-trans-p*-coumaroyl tyramine (4). PMR $\delta_{\text{TMS}}^{\text{acetone-d}_6}$: 2.6~2.9 (2H, m, -CH₂-), 3.3~3.6 (2H, m, -CH₂-), 6.45 (1H, d, $J=16$ Hz, $\text{H}-\text{C}(\text{H})=\text{C}(\text{H})$), 6.65~7.20 (6H, m, 6 $\times$ Ar-H), 7.40 (2H, d, $J=8$ Hz, 2 $\times$ Ar-H), 7.45 (1H, d, $J=16$ Hz, $\text{H}-\text{C}(\text{H})=\text{C}(\text{H})$).

Acetylation of *N-trans-p*-coumaroyl tyramine (4, 73 mg)

with Ac_2O (0.5 ml) and pyridine (0.5 ml) and chromatography with silica gel (4 g) using CHCl_3 gave diacetyl *N-trans-p-coumaroyl* tyramine (**4a**), 27 mg. EI-MS m/z : 367 (M^+), 283, 164, 147, (base peak), 120. PMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 2.30 (6H, s, $2 \times -\text{Ac}$), 2.88 (2H, t, $J=7\text{ Hz}$, $-\text{CH}_2-$), 3.55~3.80 (2H, m, $-\text{CH}_2-$), 5.60~5.80 (1H, m, $-\text{NH}$), 6.27 (1H, d, $J=16\text{ Hz}$, $\text{H}-\text{C}=\text{C}-\text{H}$), 6.95~7.25 (6H, m, $6 \times \text{Ar-H}$), 7.50 (2H, d, $J=8\text{ Hz}$, $2 \times \text{Ar-H}$), 7.60 (1H, d, $J=16\text{ Hz}$, $\text{H}-\text{C}=\text{C}-\text{H}$).

P_{6a} compound (*N-trans-feruloyl* octopamine, **5**). The **P_{6a}** fraction (300 mg) was chromatographed on silica gel (15 g) using $\text{MeOH}-\text{CHCl}_3$ (3:97) to give a powder of *N-trans-feruloyl* octopamine (**5**). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1650, 1590, 1500, 970, 830, 750. EI-MS m/z : No M^+ (329), 311 ($\text{M}^+ - \text{H}_2\text{O}$, base peak), 254, 239, 177, 135, 107. PMR $\delta_{\text{TMS}}^{\text{acetone-d}_6}$: 3.35~3.80 (2H, m, $-\text{CH}_2-$), 3.92 (3H, s, $-\text{OCH}_3$), 4.75~5.05 (2H, m, $-\text{CH}$ and $-\text{OH}$), 6.05~6.30 (1H, m, $-\text{NH}$), 6.75 (1H, d, $J=16\text{ Hz}$, $\text{H}-\text{C}=\text{C}-\text{H}$), 6.90~7.50 (7H, m, $7 \times \text{Ar-H}$), 7.69 (1H, d, $J=16\text{ Hz}$, $\text{H}-\text{C}=\text{C}-\text{H}$), 8.20 (1H, bs, OH), 8.55 (1H, bs, OH).

Acetylation of *N-trans-feruloyl* octopamine (**5**, 45 mg) with Ac_2O (1.0 ml) and pyridine (1.0 ml) and chromatography with silica gel (2 g) using CHCl_3 gave triacetyl *N-trans-feruloyl* octopamine (**5a**), 13 mg, mp 164~165°C (from benzene-cyclohexane). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1760, 1730, 1660, 1500, 960, 840, 820. EI-MS m/z : 455 (M^+), 413, 395, 353, 177 (base peak), 135. PMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 2.11 (3H, s, $-\text{Ac}$), 2.31 (6H, s, $2 \times -\text{Ac}$), 3.7~3.95 (2H, m, $-\text{CH}_2-$), 3.89 (3H, s, $-\text{OCH}_3$), 5.8~6.1 (2H, m, $-\text{NH}$ and $-\text{CH}$), 6.36 (1H, d, $J=16\text{ Hz}$, $\text{H}-\text{C}=\text{C}-\text{H}$), 7.1~7.55 (7H, m, $7 \times \text{Ar-H}$), 7.66 (1H, d, $J=16\text{ Hz}$, $\text{H}-\text{C}=\text{C}-\text{H}$).

P_{6b} compound (*N-trans-p-coumaroyl* octopamine, **6**). The **P_{6b}** fraction (230 mg) was crystallized to give *N-trans-p-coumaroyl* octopamine (**6**), mp 214~215°C. EI-MS m/z : No M^+ (299), 281, 224 (base peak), 163, 147, 135, 107. PMR $\delta_{\text{TMS}}^{\text{acetone-d}_6}$: 3.2~3.6 (2H, m, $-\text{CH}_2-$), 4.65~4.85 (2H, m, $-\text{CH}$ and $-\text{OH}$), 6.53 (1H, d, $J=16\text{ Hz}$, $\text{H}-\text{C}=\text{C}-\text{H}$), 6.70~6.95 (4H, m, $4 \times \text{Ar-H}$), 7.15~7.55 (4H, m, $4 \times \text{Ar-H}$), 7.50 (1H, d, $J=16\text{ Hz}$, $\text{H}-\text{C}=\text{C}-\text{H}$), 8.20 (1H, bs, $-\text{OH}$), 8.75 (1H, bs, $-\text{OH}$).

Acetylation of crude **P_{6b}** compound (**6**, 310 mg) with Ac_2O (2.0 ml) and pyridine (2.0 ml) and chromatography with silica gel (10 g) using CHCl_3 gave triacetyl *N-trans-p-coumaroyl* octopamine (**6a**, 6 mg), mp 156~159°C. PMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 2.12 (3H, s, $-\text{Ac}$), 2.37 (6H, s, $2 \times -\text{Ac}$), 3.7~4.1 (2H, m, $-\text{CH}_2-$), 5.9~6.2 (1H, m, $-\text{CH}$), 6.41 (1H, d, $J=16\text{ Hz}$, $\text{H}-\text{C}=\text{C}-\text{H}$), 7.1~7.7 (8H, m, $8 \times \text{Ar-H}$), 7.70 (1H, d, $J=16\text{ Hz}$, $\text{H}-\text{C}=\text{C}-\text{H}$).

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REFERENCES

- 1) T. Yoshihara, S. Takamatsu and S. Sakamura, *Agric. Biol. Chem.*, **42**, 623 (1978).
- 2) F. R. Van Heerden, E. V. Brandt and D. G. Roux, *Phytochem.*, **19**, 2125 (1980).
- 3) R. S. McCredie, E. Ritchie and W. C. Taylor, *Aust. J. Chem.*, **22**, 1011 (1969).
- 4) A. Stoessl, *Can. J. Chem.*, **45**, 1745 (1967).