Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 64 (2003) 965–969
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Phenylpropanoid amides of serotonin accumulate in witches' broom diseased bamboo

Eiji Tanaka[a], Chihiro Tanaka[a,*], Naoki Mori[b], Yasumasa Kuwahara[b], Mitsuya Tsuda[a]

[a]*Division of Environmental Science and Technology, Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-Cho, Sakyo-Ku, Kyoto, 606-8502, Japan*
[b]*Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-Cho, Sakyo-Ku, Kyoto, 606-8502, Japan*

Received 29 October 2002; received in revised form 4 June 2003

Abstract

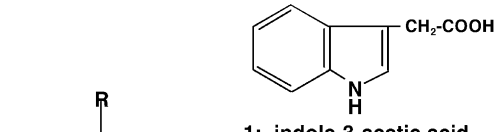
Aciculosporium take (Ascomycota; Clavicipitaceae), causes the witches' broom disease in bamboo, particularly *Phyllostachys bambusoides*. Since it was observed that endogenous indole-3-acetic acid is reduced in the twigs of the diseased bamboo, the symptoms (bushy appearance) may be induced by reduction in auxin levels. Furthermore, two indolic compounds accumulated in diseased twigs, these being identified as *N*-*p*-coumaroylserotonin and *N*-feruloylserotonin by LC-MS, ^{1}H NMR and ^{13}C NMR spectroscopic analyses. *N*-*p*-Coumaroylserotonin possesses antifungal activity against *A. take*.

Keywords: *Phyllostachys bambusoides*; Poaceae; Indole-3-acetic acid; *N*-*p*-Coumaroylserotonin; *N*-Feruloylserotonin; Antifungal activity; *Aciculosporium take*; Witches' broom of bamboo

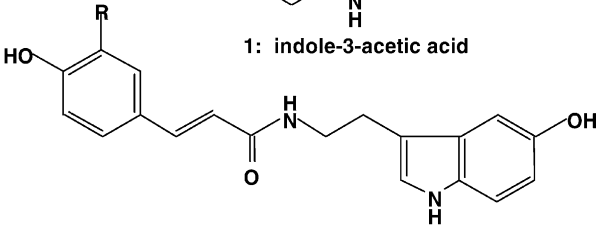
1. Introduction

Witches' broom is the disease most destructive to bamboo forests in Japan, China and Taiwan (Tsuda et al., 1997; Zhu and Huang, 1988; Chen, 1970). The symptoms of this disease include a bushy appearance with dwarfed leaves numerous twigs growing successively. The symptoms in *P. bambusoides* Sieb. et Zucc. are especially severe, although the causal agent *Aciculosporium take* Miyake (Ascomycota, Clavicipitaceae; Anamorph: *Albomyces take* Miyake) can infect many other bamboo species (Tsuda et al., 1997). In order to investigate the cause of development of the symptoms, it is necessary to examine the physiological changes in diseased bamboo. Auxin (indole-3-acetic acid, **1**) deficit led to a bushy phenotype in Arabidopsis mutants (Lincoln et al., 1990; Bak et al., 2001). Thus, a reduction in auxin **1** may be implicated in the witches' broom appearance of infected bamboo plants.

In this article, we analyzed indolic compounds in diseased and healthy bamboo twigs. The bamboo with witches' broom contained significantly less IAA (indole-3-acetic acid) (**1**) than healthy twigs. Furthermore, we confirmed that diseased twigs accumulated two indolic compounds, *N*-*p*-coumaroylserotonin (**2**) and *N*-feruloylserotonin (**3**). We studied the antifungal activity of these two compounds.



1: indole-3-acetic acid



2: *N*-*p*-coumaroylserotonin (R = H)
3: *N*-feruloylserotonin (R = OCH_3)

* Corresponding author. Tel.: +81-75-753-6466; fax: +81-75-753-6312.
E-mail address: chihiro@remach.kais.kyoto-u.ac.jp (C. Tanaka).

0031-9422/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0031-9422(03)00429-1

2. Results and discussion

2.1. IAA (1) amount related to the symptoms of witches' broom

The amount of free IAA (**1**) in diseased and healthy bamboo twigs were 5.4±1.8 μg/g ($N=5$) and 31.2±21.2 μg/g ($N=4$) dry wt of twigs, respectively (Table 1). Features of witches' broom resemble the phenotype of some bushy mutants of Arabidopsis, e.g. a reduced auxin **1** sensing mutant (Lincoln et al., 1990) and a CYP83B1 over-expression mutant, which may consequently reduce free auxin **1** (Bak et al., 2001). The low concentration of free IAA (**1**) in the twigs possibly explains the bushy appearance.

2.2. Diseased bamboo accumulated N-p-coumaroyl- and N-feruloylserotonin (2 and 3)

Two indole-related compounds accumulated in diseased bamboo twigs. These compounds were isolated and identified as *N*-*p*-coumaroyl- and *N*-feruloylserotonin (**2** and **3**) on the basis of LC-MS, ^{1}H NMR and ^{13}C NMR spectroscopic analyses. The identity of each amide was established by chemical synthesis from the corresponding hydroxycinnamic acids and serotonin using dicyclohexylcarbodiimide. Next, the amounts of *N*-*p*-coumaroyl- and *N*-feruloylserotonin (**2** and **3**) were measured in diseased and healthy bamboo twigs (Table 1). Both compounds (**2** and **3**) accumulated in diseased twigs, compared to healthy twigs. The detection of the compounds (**2** and **3**) in bamboo has not been reported before, although the occurrence of cinnamic acid derivatives in bamboo has been carefully examined (Tachibana et al., 1992). *N*-*p*-Coumaroylserotonin (**2**) was detected in significant amount in the diseased twigs but only at trace levels in healthy twigs, wherease *N*-feruloylserotonin (**3**) was detected in trace amounts in both diseased and healthy bamboo twigs. These observations imply that *N*-*p*-coumaroyl- and *N*-feruloylserotonin (**2** and **3**) are produced by the bamboo plant and not by the pathogenic fungi.

We then searched for *N*-*p*-coumaroyl- and *N*-feruloylserotonin (**2** and **3**) in bamboo species other than *Phyllostachys bambusoides*. Both amides were detected in all bamboo species we studied, including *P. bambusoides* var. *marliacea* Makino, *P. pubescens* Maz.ex Houz.de Le. and *Shibataea kumasaca* (Zoll.) Nakai (Table 2). *N*-*p*-Coumaroylserotonin (**2**) was detected even in *P. pubescens* and *S. kumasaca*, which showed no symptoms in response to the witches' broom fungus.

Previously, both phenylpropanoid amides of serotonin (**2** and **3**) had been identified from safflower seeds and oil cake (Sakamura et al., 1978; Sakamura et al., 1980; Zhang et al., 1997). *N*-*p*-Coumaroylserotonin (**2**) was isolated from Japanese barnyard millet grains (Watanabe, 1999) and a powder of konnyaku-imo (*Amorphophallus konjac*) (Niwa et al., 2000). *N*-Feruloylserotonin (**3**) was reported in Sweet Sultan (*Centaurea moschata*) (Sarker et al., 1997) and Cornflower (*Centaurea cyanus*) as moschamine (Sarker et al., 2001). *N*-*p*-Coumaroylserotonin (**2**) has been reported as having strong antioxiditant activity (Zhang et al., 1997; Watanabe et al., 1999) and inhibiting the production of proinflammatory cytokines by human monocytes (Takii et al., 1999; Kawashima et al., 1998). It is, however, not known whether *N*-*p*-coumaroyl- and *N*-feruloylserotonin (**2** and **3**) are important for the plant. Some amides of hydroxycinnamic acids possess antifungal activity, e.g. avenanthramides (Miyagawa et al., 1995) and feruloylagmatine (Stoessl and Unwin, 1970). We therefore investigated the antifungal activity of *N*-*p*-coumaroyl- and *N*-feruloylserotonin (**2** and **3**).

2.3. N-p-coumaroylserotonin (2) possesses antifungal activity against Aciculosporium take

The antifungal activities of *N*-*p*-coumaroyl- and *N*-feruloylserotonin (**2** and **3**) were tested against three fungi pathogenic to bamboo, *A. take*, *Heteroepichloë bambusae* and *Shiraia bambusicola* (Fig. 1). The concentrations of these amides were set at 100 μg/ml or less as in most examinations of antifungal activity. *Aciculosporium take* was unable to grow at concentrations higher than 100 μg/ml of *N*-*p*-coumaroylserotonin (**2**) (MIC = 100 μg/ml), and the EC_{50} value for mycelial grow was 84 μg/ml. However, *A. take* grew at 100 μg/ml of *N*-feruloylserotonin (**3**) (MIC > 100 μg/ml, EC_{50} > 100 μg/ml). *Heteroepichloë bambusae* and *Shiraia bambusicola* grew on complete medium amended with

Table 1
The amounts of IAA (indole-3-acetic acid) (**1**), *N*-*p*-coumaroylserotonin (**2**) and *N*-feruloylserotonin (**3**) in bamboo twigs[a]

Sample bamboo twigs	Amount (μg/g)		
	IAA (1)	*N*-*p*-coumaroylserotonin (2)	*N*-feruloylserotonin (3)
Healthy bamboo ($N=4$)	31.2±21.2	Trace	2.8±1.0
Witches' broom diseased bamboo ($N=5$)	5.4±1.8	3.4±1.4	9.6±6.4

[a] *Phyllostachys bambusoides* was used. Statistically significant differences were confirmed with the Mann–Whitney *U* test.

Table 2
Relative amounts of *N*-*p*-coumaroylserotonin (**2**) and *N*-feruloylserotonin (**3**) in selected bamboo species

Bamboo species	Witches' broom symptoms	Amount (μg/g)	
		N-*p*-coumaroylserotonin (**2**)	*N*-feruloylserotonin (**3**)
Phyllostachys bambusoides	Healthy	Trace	2.8±1.0
	Diseased	3.4±1.4	9.6±6.4
P. bambusoides var. *marliacea*	Healthy	Trace	1.6±1.0
	Diseased	0.8±0.4	4.2±2.4
P. pubescens	Healthy	1.6±0.8	6.0±2.2
Shibataea kumasaca	Healthy	11.0±5.0	12.8±7.4

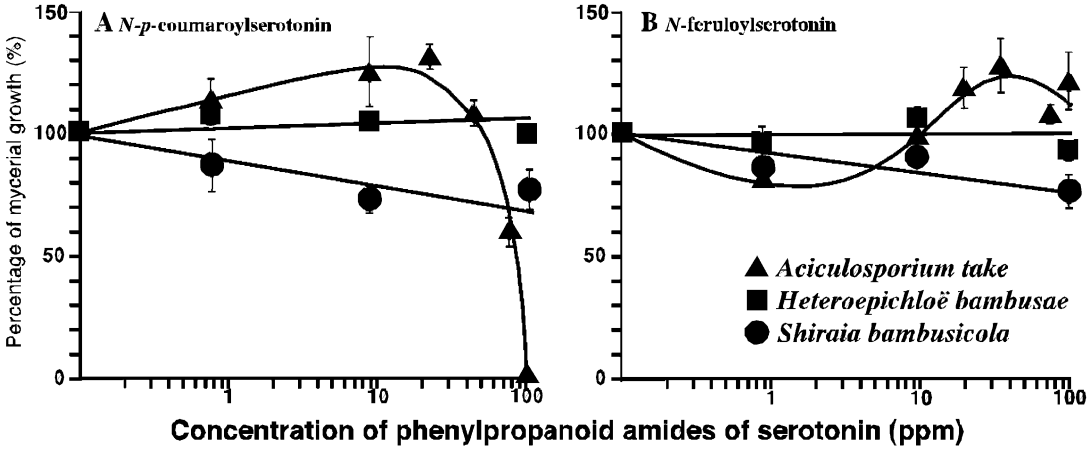


Fig. 1. Inhibition of mycelial growth by *N*-*p*-coumaroylserotonin (**2**) and *N*-feruloylserotonin (**3**). The mycelial growth is the diameter of the colony from which the diameter of the inoculum disk (4 mm) was subtracted. The colony diameter 7-days after incubation was measured and its growth compared to that of the corresponding control, *Aciculosporium take* (5.3 mm), *Heteroepichloë bambusae* (20.7 mm) and *Shiraia bambusicola* (12.1 mm). Results are the mean of 3 replicates.

100 μg/ml of *N*-*p*-coumaroyl- and *N*-feruloylserotonin (**2** and **3**) (MIC > 100 μg/ml, EC_{50} > 100 μg/ml), respectively.

This is the first report of *N*-*p*-coumaroylserotonin (**2**) possessing antifungal activity. This activity is much stronger than the activity of several indole derivatives or serotonin (Yue et al., 2000; Cornelia et al., 2002).

Hydroxycinnamic acid amides have been implicated in the inducible defense of plants. Avenanthramides, *N*-cinnamoylanthramides, were characterized as oat phytoalexins (Mayama et al., 1981; Miyagawa et al., 1995). *N*-Hydroxycinnamoyltyramine in solanaceous plants was produced in response to various environmental stimuli (Clarke, 1982; Negrel and Martin, 1984; Pearce et al., 1988). *N*-*p*-Coumaroylserotonin (**2**) accumulates in diseased bamboo plants, and possesses antifungal activity. Thus *N*-*p*-coumaroylserotonin (**2**) can be considered as a defense compound of bamboo plants.

The witches' broom disease frequently occurs in *P. bambusoides* and related species, and symptoms are especially severe among the host species of *A. take* (Tsuda et al., 1997). In our research, the amount of *N*-*p*-coumaroylserotonin (**2**) in diseased *P. bambusoides* was less than the level effective against *A. take*. Thus, *P. bambusoides* produces insufficient amounts of antifungal compounds. On the other hand, the witches' broom disease rarely occurred in *P. pubescens* and *S. kumasaca*, although these two species were reported as a host of *A. take* (Tsuda et al., 1997). Our studies showed that healthy *P. pubescens* and *S. kumasaca* also produced *N*-*p*-coumaroylserotonin (**2**) (Table 2). From this, it is expected that these species have a greater capacity to produce *N*-*p*-coumaroylserotonin (**2**) than *P. bambusoides*. When these bamboo species are attacked by *A. take*, they may produce sufficient amounts of *N*-*p*-coumaroylserotonin (**2**) to prevent invasion. Thus, the development of witches' broom symptoms may be affected by the accumulation of *N*-*p*-coumaroylserotonin (**2**).

3. Experimental

3.1. Plant materials

Diseased and healthy bamboo twigs were obtained in Kyoto, Japan. One-year-old-twigs were sampled, and the leaves and sheaths were removed. The plant materials were freeze-dried and ground in liquid nitrogen using a mortar and pestle. The powdered material was then

extracted three times with MeOH containing 1% HOAc. All voucher specimens (PRI-Cla-087; PRI-Cla-088) are deposited at the herbarium of the Kyoto University Museum (KYO).

3.2. Quantification of IAA (1), N-p-coumaroyl- and N-feruloylserotonin (2 and 3) in host plants

The MeOH-extract from bamboo twigs (1 g dry weight) was evaporated in vacuo at 40 °C, and dissolved in distilled water (10 ml). The aqueous phase was extracted with EtOAc (10 ml). The EtOAc layer was concentrated in vacuo and dissolved in distilled water (10 ml). The solution was loaded on a Sep-pak C18 cartridge (Waters) and eluted with MeOH–H_2O. The MeOH–H_2O (6:4) fraction, containing IAA (**1**) and the other indole compounds was analyzed by HPLC (column, Wakosil II 5C18-HG 250×4.6 mm; solvent, MeCN–H_2O (18:88); flow rate, 1.2 ml/min), with monitoring of UV (280 nm) and fluorescence intensity (excitation wavelength of 280 nm and emission wavelength of 355 nm). The calibration curve was linear in the concentration range from 0 to 10 mg/l. The recovery yield of IAA (**1**) and serotonin conjugates (**2** and **3**) was determined by the following recovery experiments. The concentrations of these compounds in the powdered plant materials were determined by the method described above. Then each compound (10 μg) was mixed with the powdered plant materials (1 g), and the concentrations were again determined. The recoveries of the compounds were calculated from differences of the concentrations in the samples with or without additions of exogenous IAA (**1**) and serotonin conjugates (**2** and **3**). The numeric values in Tables 1 and 2 were corrected using recovery yield. Non parametric statistical methods (Mann–Whitney *U* test) were used for analysis. Each study shows a significant difference between diseased and healthy twigs.

3.3. Purification and identification of N-p-coumaroyl- and N-feruloylserotonin (2 and 3)

The MeOH-extracts were developed by silica gel TLC (Merck) with $CHCl_3$–MeOH (1:9). The developed plate was sprayed with Ehrlich's reagent (4-dimethylaminobenzaldehyde 1 g, MeOH 75 ml, 36% HCl 25 ml). From diseased bamboo extracts, two major violet spots appeared at R_f 0.3 (A) and R_f 0.35 (B). From healthy bamboo extracts, the spot corresponding to compound B was faint. These two compounds were purified by the following procedures, while the fraction that contains the compounds was checked by TLC. The extract corresponding to 250 g dry weight of bamboo twigs was evaporated in vacuo, and the residue was dissolved in distilled water (250 ml) and partitioned with EtOAc (250 ml). The EtOAc extract was applied to a silica gel (10 g) column, and eluted with $CHCl_3$–MeOH. The fraction eluted with 10% MeOH was concentrated in vacuo. The solution was loaded on a Sep-pak C18 cartridge (Waters) and eluted with MeOH–H_2O. The MeOH–H_2O (40:60) fraction, containing the compounds of interest, was concentrated in vacuo and subjected to HPLC (column, Wakosil II 5C18-HG 250×4.6 mm; solvent, MeCN–H_2O (23:77); flow rate, 1.2 ml / min; detection, UV 280 nm; 50 °C). Each compound gave a single peak on reversed phase HPLC, at R_t 9.8 min (A) and R_t 11.5 min (B). The purified compounds were subjected to LC-MS (LCMS-2010A, SHIMADZU) analyses. The following HPLC conditions were used: linear gradient: 30–60% AcCN for 15 min (column, Cadenza CD-C18 75×30 mm Imatakt; flow rate, 0.2 ml/min; detection, UV 280 nm; 50 °C). LC-MS analysis of each compound revealed a molecular ion at m/z 323 (A) and m/z 353 (B) $[M+H]^+$. ^1H- and ^{13}C NMR spectra were recorded on a Bruker AC-300 spectrometer in MeOH-d_4 solution using TMS as an internal standard. The ^1H NMR spectral data and other results, including the ^{13}C NMR spectrum, compound A and compound B were identified as *N*-*p*-coumaroylserotonin (**2**) and *N*-feruloylserotonin (**3**), respectively. Comparisons were made with synthetic compounds and data from the literature (Zhang et al., 1997).

3.4. Preparation of synthetic N-p-coumaroyl- and N-feruloylserotonin (2 and 3)

Synthesis of *N*-*p*-coumaroylserotonin (**2**) was carried out according to the method of caffeoyl DOPA synthesis (Tebayashi et al., 2000) with a slight modification. *p*-Coumaric acid (3.0 mmol), serotonin (1.5 mmol) and dicyclohexylcarbodiimide (3.3 mmol) were dissolved in dry pyridine (10 ml) and the mixture was stirred at room temperature for 24 h. After evaporation of pyridine in vacuo, the residue was dissolved in MeOH (20 ml). The solution was cooled in an ice-bath and then 2 M KOH (200 ml) was added. The mixture was stirred at room temperature for 4 h and then neutralized with HOAc. The solvent was extracted with EtOAc (200 ml). The extract was evaporated in vacuo and the residue was dissolved in MeOH (50 ml). Dicyclohexylurea and salts were removed by filtration. After evaporation of solvent, the filtrate was fractionated using a silica-gel column by eluting with EtOAc–hexane (3:1, v/v) to give *N*-*P*-coumaroylserotonin (0.14 mmol). *N*-Feruloylserotonin (**3**) was also synthesized using the same procedure with ferulic acid and serotonin to give *N*-*p*-coumaroyl- and *N*-feruloylserotonin (**2** and **3**) (0.30 mmol).

3.5. Antifungal activities of N-p-coumaroyl- and N-feruloylserotonin (2 and 3)

The antifungal activities of *N*-*p*-coumaroyl- and *N*-feruloylserotonin (**2** and **3**) were tested against three bamboo

pathogenic fungi, *A. take*, *Heteroepichloë bambusae* and *Shiraia bambusicola*. Sensitivity to the compounds was evaluated as the minimum inhibitory concentration (MIC) and effective concentration to inhibit 50% of mycelial growth (EC_{50}), using the plate dilution method. Each synthetic compound was dissolved in MeOH and incorporated into a series of complete media [0.15% $Ca(NO_3)_2$–$4H_2O$, 0.05% KCl, 0.05% $MgSO_4$–$7H_2O$, 0.04% KH_2PO_4, 0.003% K_2HPO_4, 0.1% yeast extract, 0.1% tryptone, 1% glucose (w/v)] (Nakada et al., 1994) to yield final concentrations of 1–100 μg/ml. Each medium was poured into petri dishes. Mycelial discs (4 mm in diameter) were cut with a sterilized cork borer from the margin of 1-week-old colonies. The EC_{50} value was determined 7 days after incubation at 25 °C by plotting the percentage decrease in colony diameter against the log concentration of the chemicals. Each experiment was done in triplicate.

Acknowledgements

We thank Dr. A. Ishihara, Dr. N. Shimidzu, Dr. S. Kugimiya and Dr. M. Takemura, Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, for suggestions on the chemical synthesis and analyses. We also thank the two anonymous reviewers for their kind suggestions on the revision of the manuscript.

References

Bak, S., Tax, F.E., Feldmann, K.A., Galbraith, D.W., Feyereisen, R., 2001. CYP83B1, a cytochrome P450 at the metabolic branch point in auxin and indole glucosinolate biosynthesis in Arabidopsis. Plant Cell 13, 101–111.

Chen, C.C., 1970. Witches' broom, a new disease of bamboo in Taiwan. Mem. Coll. Agric. Natl.Taiwan Univ. 11, 101–111. (in Chinese).

Clarke, D.D., 1982. The accumulation of cinnamic acid amides in the cell walls of potato tissue as an early response to fungal attack. In: Wood, R.K.S. (Ed.), Active Defence Mechanisms in Plants. Plenum Press, New York, pp. 321–322.

Cornelia, L.F., Wiedauer, B., Mayr, A., Kirchmair, M., Jenewein, I., Ledochowski, M., Dierich, M.P., 2002. Antifungal properties of 5-hydroxytryptamine (serotonin) against *Aspergillus* spp. in vitro. Int. J. Med. Microbiol. 291, 655–657.

Kawashima, S., Hayashi, M., Takii, T., Kimura, H., Zhang, H.L., Nagatsu, A., Sakakibara, J., Murata, K., Oomoto, Y., Onozaki, K., 1998. Serotonin derivative *N*-(*p*-coumaroyl) serotonin, inhibits the production of TNF-α, IL-1α, IL-1β and IL-6 by endotoxin-stimulated human blood monocytes. J. Interferon Cytokine Res. 18, 423–428.

Lincoln, C., Britton, J.H., Estelle, M., 1990. Growth and development of the *axr1* mutants of Arabidopsis. Plant Cell 2, 1071–1080.

Mayama, S., Tani, T., Matsuura, Y., Ueno, T., Fukami, H., 1981. The production of phytoalexins by oat *Avena sativa* in response to crown rust *Puccinia coronata* f. sp. *avenae*. Physiol. Plant Pathol. 19, 217–226.

Miyagawa, H., Ishihara, A., Nishimoto, T., Ueno, T., Mayama, S., 1995. Induction of avenanthramides in oat leaves inoculated with crown rust fungus, *Puccinia coronata* f. sp. *avenae*. Biosci. Biotechnol. Biochem. 59, 2305–2306.

Nakada, M., Tanaka, C., Tsunewaki, K., Tsuda, M., 1994. RFLP analysis for species separation in genera *Bipolaris* and *Curvularia*. Mycoscience 35, 271–278.

Negrel, J., Martin, C., 1984. The biosynthesis of feruloyltyramine in *Nicotiana tabacum*. Phytochemistry 23, 2797–2801.

Niwa, T., Etoh, H., Shimizu, A., Shimizu, Y., 2000. *cis*-*N*-(*p*-coumaroyl)serotonin from Konnyaku, *Amorphophallus konjak* K. Koch. Biosci. Biotechnol. Biochem. 64, 2269–2271.

Pearce, G., Marchand, P.A., Griswold, J., Lewis, N.G., Ryan, C.A., 1998. Accumulation of feruloyltyramine and *p*-coumaroyltyramine in tomato leaves in response to wounding. Phytochemistry 47, 659–664.

Sakamura, S., Terayama, Y., Kawakatsu, S., Ichihara, A., Saito, H., 1978. Conjugated serotonins related to cathartic activity in safflower seeds (*Carthamus tincrotorius* L.). Agric. Biol. Chem. 42, 1805–1806.

Sakamura, S., Terayama, Y., Kawakatsu, S., Ichihara, A., Saito, H., 1980. Conjugated serotonins and phenolic constituents in safflower seeds (*Carthamus tincrotorius* L.). Agric. Biol. Chem. 44, 2951–2954.

Sarker, S.D., Savchenko, T., Whiting, P., Sik, V., Dinan, L.N., 1997. Moschamine, *cis*-moschamine, moschamindole and moschamindolol: four novel indole alkaloids from *Centaurea moschata*. Nat. Prod. Lett. 9, 189.

Sarker, S.D., Laird, A., Nahar, L., Kumarasamy, Y., Jaspars, M., 2001. Indole alkaloids from the seeds of *Centaurea cyanus* (Asteraceae). Phytochemistry 57, 1273–1276.

Stoessl, A., Unwin, C.H., 1970. The antifungal factors in barley. V. Antifungal activity of the hordatines. Can. J. Botany 48, 465–470.

Tachibana, S., Ohkubo, K., Towers, G.H.N., 1992. Cinnamic acid derivatives in cell walls of bamboo and bamboo grass. Phytochemistry 31, 3207–3209.

Takii, T., Hayashi, M., Hiroma, H., Chiba, T., Kawashima, S., Zhang, H.L., Nagatsu, A., Sakakibara, J., Onozaki, K., 1999. Serotonin derivative, *N*-(*p*-coumaroyl)serotonin, isolated from safflower (*Carthamus tinctorius* L.) oil cake augments the proliferation of normal human and mouse fibroblasts in synergy with basic fibroblast growth factor (bFGF) or epidermal growth factor (EGF). J. Biochem. 125, 910–915.

Tebayashi, S., Ishihara, A., Tsuda, M., Iwamura, H., 2000. Induction of clovamide by jasmonic acid in red clover. Phytochemistry 54, 387–392.

Tsuda, M., Shimizu, K., Matsumura, K., Tanaka, E., Tanaka, C., Doi, Y., 1997. Host range of *Aciculosporium take*, the causal agent of witches' broom of bamboo plants. Bull. Nat. Sci. Mus., Tokyo Ser. B 23, 25–34.

Watanabe, M., 1999. Antioxidative phenolic compounds from Japanese barnyard millet (*Echinochloa utilis*) grains. J. Agric. Food Chem. 47, 4500–4505.

Yue, Q., Miller, C.J., White Jr., J.F., Richardson, M.D., 2000. Isolation and characterization of fungal inhibitors from *Epichloë festucae*. J. Agric. Food Chem. 48, 4687–4692.

Zhang, H., Nagatsu, A., Watanabe, T., Sakakibara, J., Okuyama, H., 1997. Antioxidative compounds isolated from safflower (*Carthamus tinctorius* L.) oil cake. Chem. Pharm. Bull. 45, 1910–1914.

Zhu, X., Huang, H., 1988. Studies on witches' broom of bamboo I. Symptoms, isolation of pathogen and inoculation test. Scientica Silvae Sinicae 24, 483–487.