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Biosynthesis of Phenylpropanoid Amides by an Endophytic *Penicillium brasilianum* Found in Root Bark of *Melia azedarach*

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Biosynthetic studies on brasiliamides, potently convulsive and bacteriostatic compounds from an endophytic *Penicillium brasilianum* isolated from *Melia azedarach* (Meliaceae), confirms their phenylpropanoid origin, which is very uncommon in fungi. Feeding experiments with [2-^{13}C]-phenylalanine indicated the incorporation of two units of this amino acid on brasiliamide structures. The first step in the phenylpropanoid pathway to those compounds was evaluated through enzymatic bioassays and confirmed the phenylalanine ammonia-lyase (PAL) participation. The metabolism of phenylalanine in this fungus is discussed.

Keywords: Biosynthesis, *Penicillium*, endophytic, *bis*-phenylpropanoyl amides, phenylpropanoid

Cumarins and lignans are typical phenylpropanoid compounds, formed respectively of one and two phenylalanine units [20]. The enzyme phenylalanine ammonia-lyase (PAL) converts the amino acid phenylalanine to cinnamic acid, the basic C_6–C_3 carbon skeleton present in these compounds, in a key enzymatic process in the phenylpropanoid pathway [20, 44]. The cinnamic acid may incorporate a triketide (C_6) by the action of chalcone synthase (CHS), forming the flavonoids, one of the most abundant and diverse class of natural products [9, 37]. Phenylpropanoids are considered to be plant-specific secondary metabolites [41]. The expression of the enzyme PAL, the entry point of the phenylpropanoid pathway, has often been reported as a plant response against invading microorganisms, indicating that phenylpropanoid may act as phytoalexins [18, 27].

Although heterologous gene expressions lead some bacteria to produce flavonoids [40, 42], the phenylpropanoid biosynthetic pathway has not been found to be naturally expressed in microorganisms. Recently, the presence of genes encoding the core enzymes of the phenylpropanoid pathway in the fungus *Aspergillus oryzae* was detected [14, 34]. However, these genes are silenced and no phenylpropanoid compounds are produced by this *Aspergillus*. A bacterial route to benzoate *via* PAL was shown to occur in *Streptomyces*, but leading to flaviolin and its analogs [26] instead of phenylpropanoids. There are some reports of cinnamates in filamentous fungi, but their origin (whether they are truly fungi metabolites) appears to be doubtful. One of the first fungi *bis*-phenylpropanoid compounds reported was the *bis*-lactone of dihydrocaffeic acid isolated from *Inonotus* sp. [16]. Flavonoids were obtained from extracts of *Aspergillus* species [22, 23, 39], but these compounds may have originated from the soy-based cultivation medium used to grow the fungus. The biosynthetic route leading to *bis*-phenylbenzoquinone and related pigments and cyclopeptide compounds appears to be an important way for the accumulation of amino acids by fungi secondary metabolism [38], but apparently without the involvement of PAL producing cinnamates as intermediates.

The biosynthesis of phenylpropanoid compounds has received widespread interest, mainly because they have various important biological activities [17], are useful as pigments [35], and are involved in many ecological contexts since they are often related to biotic (*e.g.*, microorganism infection) and abiotic (*e.g.*, temperature, pH) stress induction in plants [6, 21]. Moreover, PAL is one of the few nonhydrolytic enzymes that have important commercial applications [21], being useful for the production of L-phenylalanine (L-Phe) from *trans*-cinnamic acid through the reverse physiological reaction [47]. In addition, PAL is effective in the treatment of certain mouse tumors [12] and useful in quantitative analysis of serum L-Phe in monitoring patients with phenylketonuria [43].

During our continued studies on the biochemistry of plant–microbe consortiums, we have investigated the compounds produced by an endophytic *Penicillium brasilianum* isolated from *Melia azedarach* (Meliaceae) [10, 11, 33]. The known

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brasiliamides A (**1**) and B (**2**), and a new brasiliamide *N*-oxide, named brasiliamide F (**6**) [11] (Fig. 1), were isolated and structurally characterized. Compounds **1** and **2** were also previously isolated along with brasiliamides C–E (**3–5**) by Fujita *et al.* [13, 36] from a soil-collected *P. brasilianum* strain. Analyses of the brasiliamides molecular structures indicated that they are apparently biosynthesized from two phenyalanine units, which is uncommon in fungi. In the present work, the biosyntheses of these brasiliamides were investigated through the addition of labeled L-phenylalanine in the culture medium. Extraction and bioassays with PAL were also conducted in order to investigate the possibility of phenylpropanoid pathway occurrence in the fungus metabolism.

MATERIALS AND METHODS

Materials

The fungus *Penicillium brasilianum* was isolated from root bark of *Melia azedarach* following the procedure described by Santos *et al.* [33], and is deposited at Laboratório de Bioquímica Micromolecular de Microorganismos in São Carlos, Brazil.

Instruments and Reagents

Low-resolution ESI/MS and ESI/MS/MS data were acquired in positive-ion mode, using a Micromass Quattro-LC instrument equipped with an ESI/APCI "Z-spray" ion. This mass spectrometer was coupled with a Waters HPLC (Alliance 2695) equipped with PDA detector (Alliance 2996) for the analysis. $_{1}H$ and $_{13}C$ NMR experiments were recorded on a Bruker DRX-400 spectrometer with deuteron chloroform ($CDCl_3$) as the solvent and TMS as the internal standard. [2-^{13}C]-phenylalanine and all other reagents were purchased from Sigma.

Penicillium brasilianum Cultivation and Feeding Experiments

The strain was cultivated on dextrose–potato agar (PDA) slants at 25°C. The inoculum was prepared by suspending spores from 7-day cultures in water to a density of 10^7 spores/ml. The inoculum of *Penicillium brasilianum* was transferred under sterile conditions to 5 of 8 Erlenmeyer flasks containing sterilized chemically defined medium (Czapek) developed in previous studies [12], which contained glucose, 80.0 g; NH_4NO_3, 0.48 g; K_2HPO_4, 5.0 g; $MgSO_4$, 1.0 g; $CuSO_4$, 0.015 g; $ZnSO_4$, 0.161 g; $MnSO_4$, 0.01 g; $FeSO_4 \cdot 7H_2O$, 0.1 g; and $(NH_4)_2MoO_4$, 0.1 g; per liter of distilled water. The other 3 flasks were kept for control purposes. The initial pH of the medium was adjusted to 6.5. Feeding experiments were performed with use of [2-^{13}C]-phenylalanine (99% ^{13}C). The precursor was added to the fungal culture before inoculation into the growth media. The final concentration of labeled phenylalanine was 1 mg/ml for each experiment. After fermentation of more than 15 days, the mycelium was separated from the culture medium and extracted with ethanol (150 ml). The ethanol extracts were dried to yield the crude isotopically labeled extract (321.0 mg). Purification of brasiliamide A (**1**) was performed by column chromatography in a column (diameter, 25 mm) packed with silica gel (70–230 Mesh). Brasiliamide A was eluted with 250 ml of a hexane:ethyl acetate (9:1) mixture. The yield of the vacuum-dried, high-purity brasiliamide A was 1.5 mg.

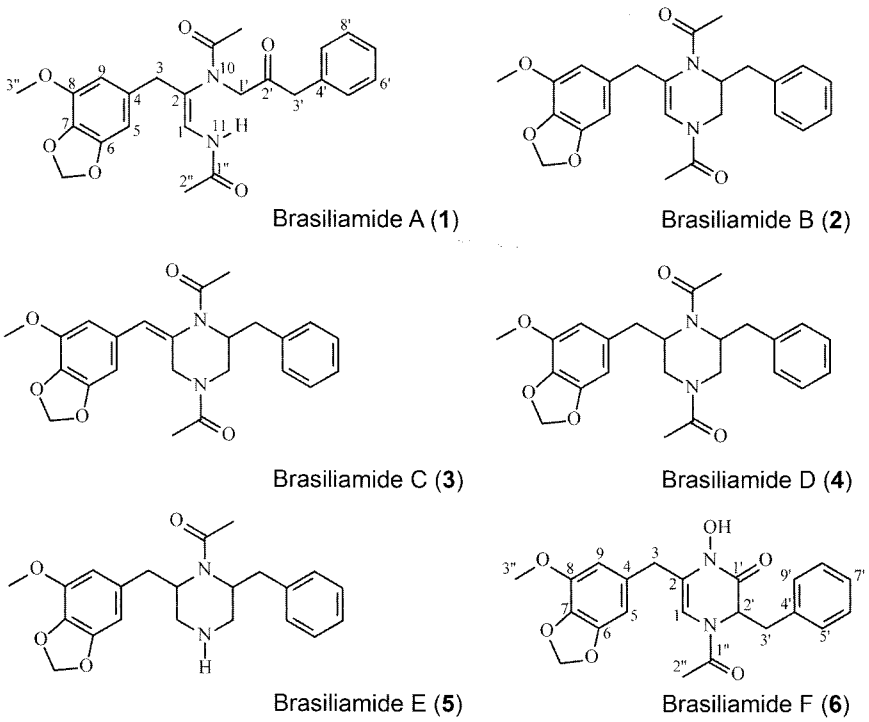


Fig. 1. Chemical structure of brasiliamides produced by the endophytic *P. brasilianum* isolate (**1**, **2**, and **6**), and those originated from the soil strain (**3**, **4**, and **5**) [13, 36].

Phenylalanine Ammonia-Lyase Activity

The fungus *Penicillium brasilianum* was cultivated for 3 days under static conditions on Czapek medium enriched with 2% of yeast extract, as described previously. The enzymatic activity was measured in two different environments, first using the filtrate (to check if it is a excreted enzyme) and then using the mycelium resuspended in a poor medium (buffer solution) containing Tris-HCl buffer, pH 8.8. The specific substrate for PAL (L-phenylalanine - 2.5 mM) was added to both assays. After 30 min of reaction, an aliquot of 5 ml of the filtrate mixture and the resuspended mycelium were collected and added to 5 ml of HCl (0.1 M) to stop the reaction. The desired product, cinnamic acid, was extracted with 5 ml of alcohol reagent and monitorated by HPLC/UV analyses.

LC/MS/MS Analyses

A LUNA C18 (Phenomenex) HPLC column (250×4.60 mm, 5 μ particle size) was used for sample separation. The HPLC mobile phase flow rate was set at 0.70 ml/min, using linear gradient elution with acetonitrile and water, for 45 min. In both eluents, 0.1% TFA (trifluoroacetic acid) was added. In the ESI/MS analyses, the capillary voltage was 3.88 kV and the cone voltage was 11 V. In the collision cell, argon was used as the collision gas at a collision energy of 10 eV and a CID pressure of 1.3×10^{-3} mbar. The ion source temperature was held at 50°C.

Results

The ^{13}C isotopically labeled phenylalanine (2-^{13}C-Phe) was added to the Czapek's medium used to grow the fungus *P. brasilianum* in order to investigate the incorporation of this amino acid into brasiliamides. After 15 days of inoculation, the fungus was harvested by filtration under reduced pressure and the amides were extracted from the mycelium using ethanol. Brasiliamide A, the major brasiliamide accumulated in the extract, was monitored for ^{13}C-Phe incorporation using LC/UV/MS/MS and ^{13}C NMR spectroscopy. Brasiliamide A was detected in both experiment (^{13}C-Phe added) and control (only fungus in the basic Czapek's medium) EtOH extracts as clear chromatographic peaks with identical retention time and UV spectra compared with the brasiliamide A standard.

The positive-ion ESI full-scan mass spectrum of brasiliamide A in the control cultivation contained a prominent peak at *m/z* 439 ($[M+H]^+$), which corresponds to the molecular formula $C_{24}H_{26}N_2O_6$. A sodium adduct ($[M+Na]^+$) was also detected at *m/z* 461 (Fig. 2). The MS spectrum obtained from the extract of the experiment (^{13}C-Phe added) contained peaks at *m/z* 441 and 463 in addition to 439 and 461, previously ascribed to $[M+H]^+$ and $[M+Na]^+$ of the standard brasiliamide A (Fig. 2). The mass shift of these two units is probably due to incorporation of two labeled phenylalanines into brasiliamide A. The product ion spectra of both precursor ions *m/z* 439 and 441 ($[M+H]^+$ and $[*M+H]^+$ respectively) were also very similar, with the most abundant product ions also showing a 2 Da shift

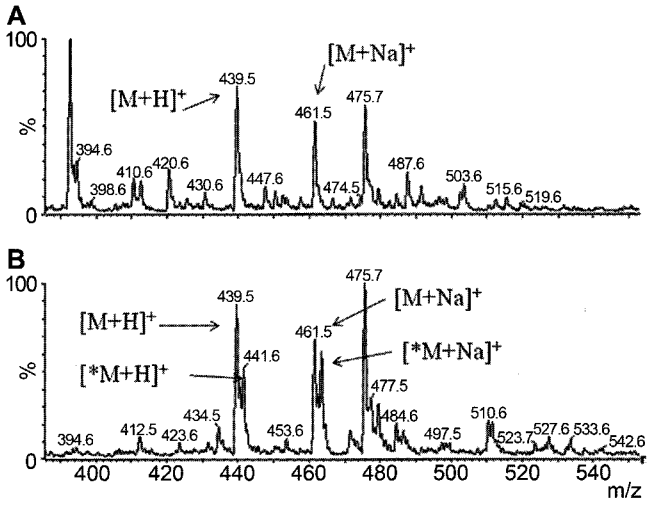


Fig. 2. Full-scan mass spectra of brasiliamide A for control extract (**A**) and for ^{13}C-labeled compounds in EtOH extract (**B**).

pattern (Fig. 3). These fragmentations correspond to loss of one acetamide (59 Da) and a ketene (42 Da), both from *N*-acetyl groups, to form the peaks at *m/z* 380/382 (**1d**) and 338/340 (**1e**), respectively (Scheme 2). Therefore, these two ions contain the two phenylalanine units, and the 2 Da mass shifts confirm the incorporation of two ^{13}C-Phe into brasiliamide A.

Labeled brasiliamide A was then purified using preparative scale atmospheric pressure chromatography and analyzed by ^{13}C NMR spectroscopy, in order to confirm the mass spectral analysis and to determine the ^{13}C-enriched position. An extensive analysis of the NMR data of brasiliamide A was previously performed using $^{1}H\{^{13}C\}$ and $^{1}H\{^{15}N\}$ HSQC and HMBC 2D spectra, to assure chemical shift ascriptions [11]. Although only a small sample of the

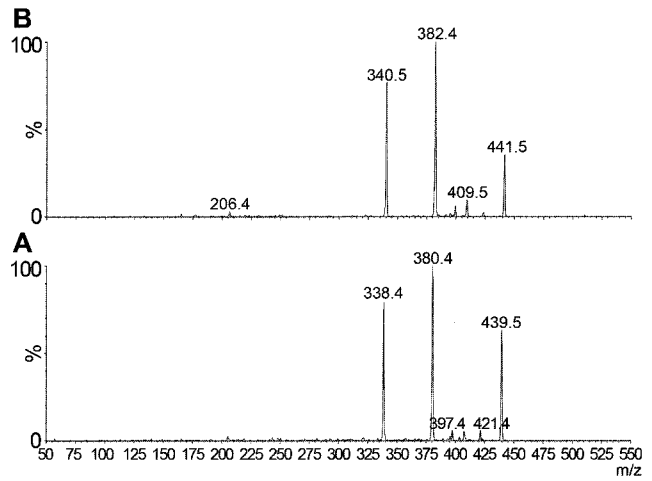


Fig. 3. Product ion mass spectra of $[M+H]^+$ precursor ions generated from unlabeled brasiliamide A (**A**) and brasiliamide A isolated from labeled extract (**B**).

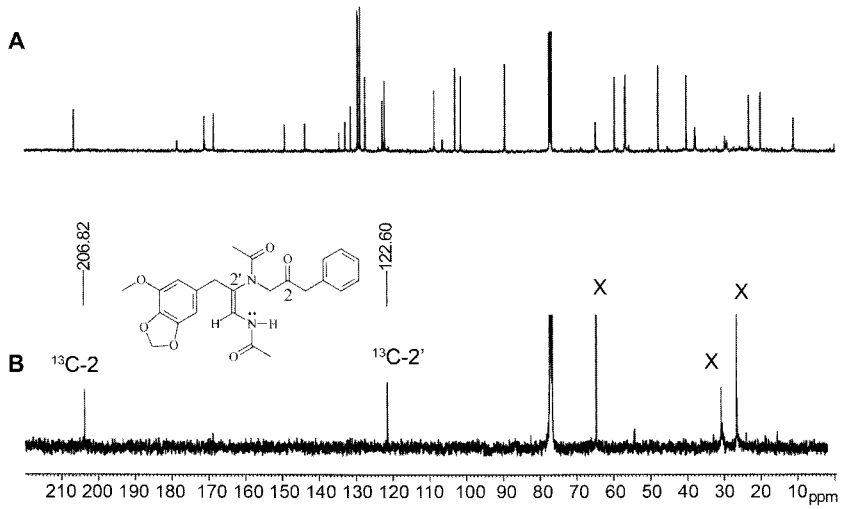


Fig. 4. ^{13}C NMR spectra obtained for brasiliamide A (**A**) and ^{13}C-enriched brasiliamide A (**B**). Signals labeled with "X" are due to impurities.

labeled compound was used to acquire ^{13}C NMR data, and two prominent signals were clearly detected at δ 122.6 and 206.8 (Fig. 4B), which corresponded respectively to C-2 and C-2' in brasiliamide A (Fig. 4A) [11]. This is in complete agreement with the interpretation of the MS data and confirms that two 2-^{13}C-Phe were incorporated into brasiliamide A, the major brasiliamide produced by this *Penicillium*. Minor brasiliamides B (**2**) and F (**6**) were also shown to incorporate 2-^{13}C-Phe, when analyzed by mass spectrometry (Fig. 5).

As stated before, *bis*-phenylpropanoid compounds are usually formed after phenylalanine molecules have been deaminated by PAL. Then, since the two C_6–C_3 units are present in brasiliamides, enzymatic experiments were performed in order to verify the possibility of PAL activity

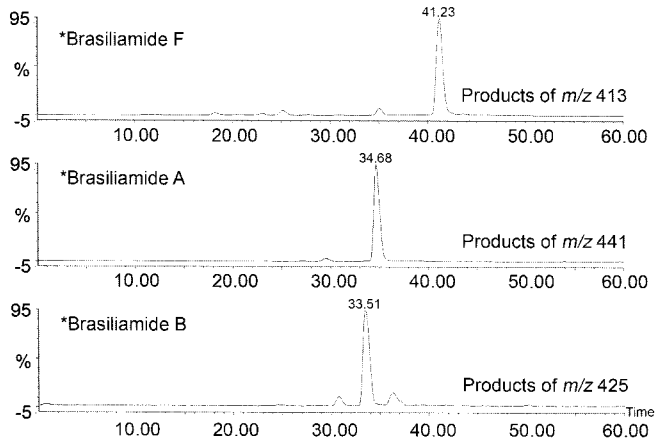


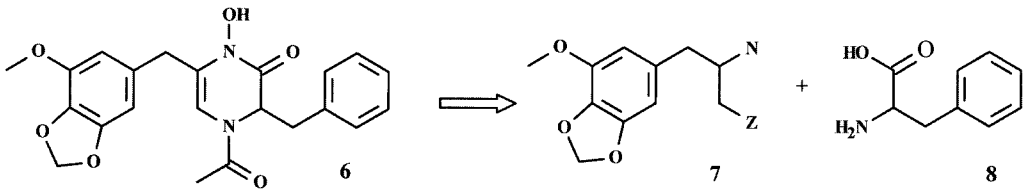
Fig. 5. Chromatograms of product ions generated from precursors of *m/z* 425 (^{13}C-labeled brasiliamide B), 441(^{13}C-labeled brasiliamide A), and 413 (^{13}C-labeled brasiliamide F) found in labeled extract.

in *P. brasilianum*. Enzyme extracts were obtained from growing cells after fungus cultivation in Czapek's medium. Thus, incubation of L-phenylalanine in these enzymatic extracts led to production of cinnamic acid, which was directly identified using LC/MS. It was observed that the enzyme was able to convert the substrate in a short time after incubation, which shows how active this enzyme is during the fungus development.

DISCUSSION

The brasiliamides A and B (**1** and **2**) produced by our endophytic isolate of *P. brasilianum* [11] are identical to those found by Fujita *et al.* in their soil isolate [13, 36]. What these compounds have in common are the presence of two aromatic rings, one of them with no substituent and the other being a methoxypiperonyl partial structure, and at least one *N*-acetyl group, which characterize them as amides. Although in all of these molecules the aromatic rings are bonded to a C_3 side chain, only in brasiliamide F (**6**) is an intact phenylalanine unit (**8**) promptly identified (Fig. 1). A retro-synthetic analysis on brasiliamide F (Scheme 1) indicates that the phenylalanine is connected to a more drastically modified C_6–C_3 (**7**), which can also originate from another phenylalanine unit, probably with the carboxyl group reduced to an aldehyde (Z corresponding to C=O).

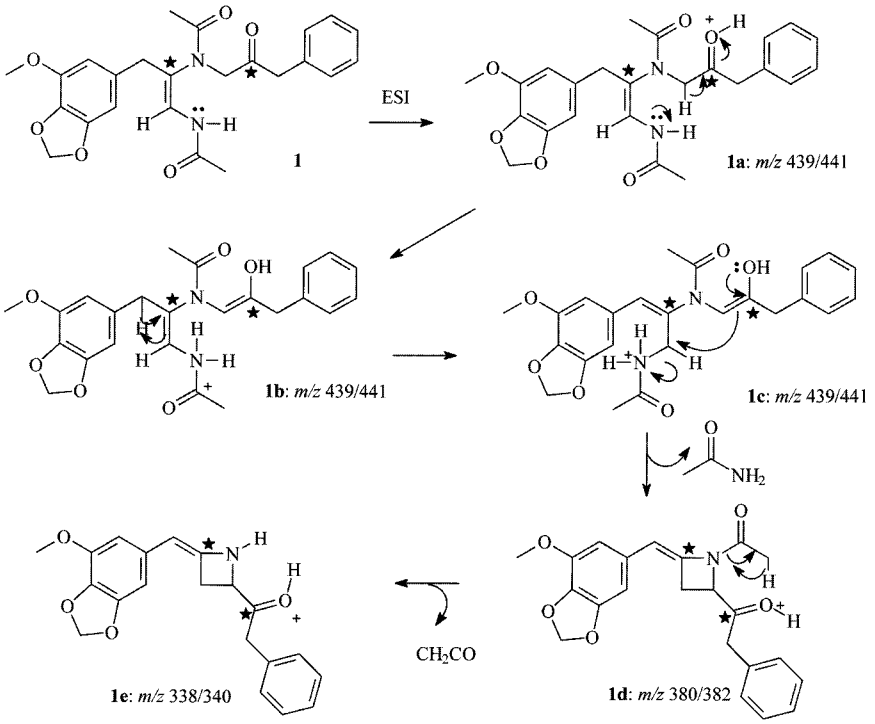
In the present work, it was demonstrated that brasiliamides are biosynthetized by *P. brasilianum* using two Phe units and that cinnamic acid is produced when Phe is incubated with an enzymatic extract obtained from this fungus, indicating that PAL is active in this extract and probably participates in brasiliamides biosynthesis. The six brasiliamides



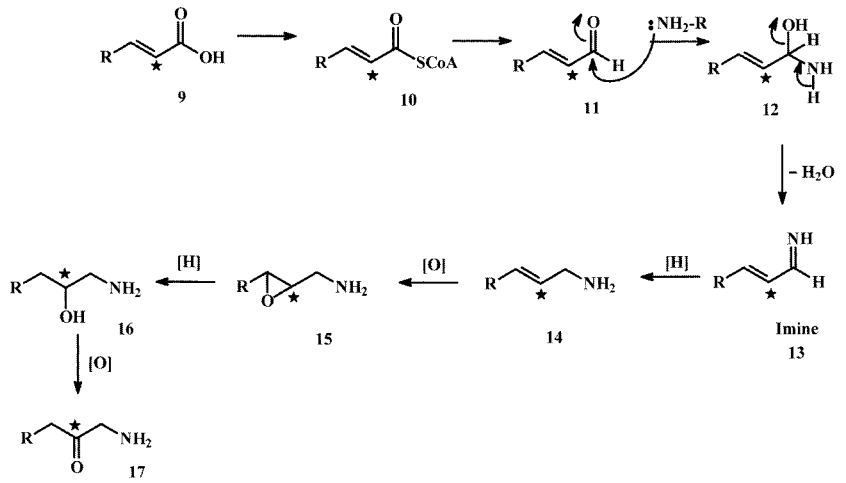
Scheme 1. Retro-synthetic analysis of brasiliamide F (**6**).

known so far (Fig. 1) have many structural features in common. With the exception of brasiliamide F (**6**), in all of the other congeners (**1–5**), the carboxyl carbon (C-1) of the Phe precursor unit is reduced and aminated. Therefore, a reductive amination in a cinnamaldehyde (**11**), followed by alternating oxidation and reduction steps, may occur to produce two 1-aryl-3-amino-2-propanone (**17**) units (Scheme 3), which are putative key intermediates to brasiliamides **1–5**. The coupling of these two aminopropanones is very much expected, in the organic chemistry point of view (Scheme 4), to form compounds **1–6**. The formation of the enamine function present in brasiliamides A, B, C, and F occurs during the dehydration of the unstable intermediate **20** in this scheme, and this is compatible with the presence of the C–C double bonds at $\Delta^{1(2)}$ in **1**, **2**, and **6**, and at $\Delta^{2(3)}$ in **3**. Only two further *N*-acetylation steps in **21** are necessary to form brasiliamide A (**1**). Thus, Schemes 3 and 4 are in complete agreement with the ^{13}C isotopic labeling experiment. Further cyclization of the aminoketone **21** in Scheme 4, followed by reductions and *N*-acetylation, can lead to the natural products **2–5**.

The presence of 1-aryl-3-amino-2-propanone partial structures (**18** and **19** in Scheme 4) is rare in natural secondary metabolites. An *Aspergillus* species has shown to accumulate 1-(4-hydroxyphenyl)-3-amino-2-propanone [1, 15], being one of the very few examples. Another expressive structural feature in these brasiliamides is the presence of a 3-methoxy-4,5-methylenedioxyphenyl (methoxypiperonyl) group. The classes of natural products in which the methoxypiperonyl group is most frequently found are the lignoids (lignans and neolignans) [4, 45] and some flavonoids [3, 5, 31, 32, 46], being typical plant phenylpropanoids, although a huge number of synthetic compounds contains this group. It appears that the only fungal metabolites containing the methoxypiperonyl as a partial structure are the brasiliamides (Fig. 1); the PF118 compounds produced by *Chrysosporium*, which are three complex antitumor amides [36]. CODEN: JKXXAF JP 2001139577 A 20010522



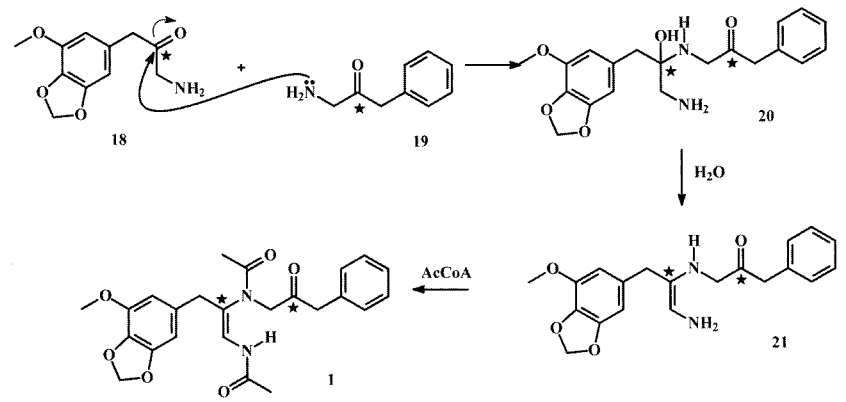
Scheme 2. Fragmentation mechanism suggested for brasiliamide A.



Scheme 3. Redutive amination and isomerization of phenylalanine producing 1-aryl-3-amino-2-propanone precursors of brasiliamides.

CAN 134:352364 AN 2001:366097 CAPLUS]; and the tremorgenic toxins territrems A and B produced by *A. terreus* [9], which seem to contain a cinnamic acid derivative, with the aromatic ring being a methoxypiperonyl, incorporated in a terpenoyl carbon chain. In plants, many typical enzymes of the phenylpropanoid pathway (*e.g.*, cinnamate 4-hydroxylase, 4-coumarate CoA ligase, caffeic acid/5-hydroxyferulic acid *O*-methyltransferase, *etc.*) act on phenylalanine to produce synapate [8], which is further oxidized to form the methoxypiperonyl. Thus, brasiliamides have many similarities with plant *bis*-phenylpropanoids biosynthesis and represent a special fungal metabolism of phenylalanine.

The most frequent ways followed by fungi to incorporate unmodified phenylalanine structures in its metabolites are the production of cyclopeptides and depsipeptides, besides construction of protein [3]. The deamination in phenylalanine/tyrosine leading to diphenylbenzoquinones and related pigments in fungi appears to occur *via* another enzymatic process producing 2-hydroxycinnamates as first intermediates [24, 25]. In an overall view, it appears that the phenylpropanoid pathway in plants is used to produce larger molecules from phenylalanine, resulting in secondary metabolites that may mediate their biotic (microbes, insects, *etc.*) and abiotic (mainly UV light) environmental interactions [8, 37, 41, 44]. On the other hand, in fungi, generally this metabolic pathway appears to be shorter and the initial biosynthetic step involves oxidizing enzymes that degrade phenylpropanoid precursors [28] back to smaller molecules such as benzoates and styrene, like some *Penicillium* species do [2, 29]. Thus, it seems that the phenylpropanoid pathway is an important biochemistry field of battle for survival. A very interesting theory was recently developed, which indicates that the land plants would have acquired the ability to produce phenylpropanoid compounds from microbes *via* horizontal gene transfer (HGT) encoding the PAL enzyme [7]. During their evolution, many microorganisms appear to have kept these metabolic abilities to defend themselves and be able to associate with plants controlling their chemical responses during colonization. Perhaps the biosynthesis of brasiliamides represents a new strategy developed by the fungus *P. brasilianum* to be accepted by



Scheme 4. Coupling of two 1-aryl-3-amino-2-propanones to produce brasiliamide A.

Melia azedarch as it host plant. Experiments to verify this hypothesis must be designed and tested.

Acknowledgments

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References

1. Benz, F., F. Kniisel, J. Nuesch, H. Treichler, W. Voser, R. Nyfeler, and W. Keller-Schierlein. 1974. Stoffwechselprodukte von mikroorganismen echinocandin B, ein neuartiges polypeptid - Antibioticum aus *Aspergillus nidulans* war. echinulatus: Isolierung und bausteine. *Helv. Chim. Acta* **57:** 2459–2477.
2. Campbell, I. M., M. A. Gallo, C. A. Jones, P. R. LaSitis, and L. M. Rosato. 1987. Role of cinnamates in benzoate production in *Penicillium brevicompacum*. *Phytochemistry* **26:** 1413–1415.
3. Cole, R. J., M. A. Schweikert, and B. B. Jarvis. 2003. *Handbook of Secondary Fungal Metabolites*, 3rd Ed. Academic Press, Amserdam.
4. Coy, E. D., L. E. Cuca, and M. Sefkow. 2009. Macrophyllin-type bicyclo[3.2.1]octanoid neolignans from the leaves of *Pleurothyrium cinereum*. *J. Nat. Prod.* **72:** 1245–1248.
5. De Tommasi, N., S. Piacente, F. DeSimone, and C. Pizza. 1996. Constituents of *Cydonia vulgaris*: Isolation and structure elucidation of four new flavonol glycosides and nine new a-ionol-derived glycosides. *J. Agric. Food Chem.* **44:** 1676–1681.
6. Dixon, R. A. and N. L. Paiva. 1995. Stress-Induced Phenylpropanoid metabolism. *Plant Cell* **7:** 1085–1097.
7. Emiliani, G., M. Fondi, R. Fani, and S. Gribaldo. 2009. A horizontal gene transfer at the origin of phenylpropanoid metabolism: A key adaptation of plants to land. *Biol. Direct* **4:** 1–12.
8. Ferrer, J. L., M. B. Austin, C. Stewart Jr., and J. P. Noel. 2008. Structure and function of enzymes involved in the biosynthesis of phenylpropanoids. *Plant Physiol. Biochem.* **46:** 356–370.
9. Ferrer, J. L., J. M. Jez, M. E. Bowman, R. A. Dixon, and J. P. Noel. 1999. Structure of chalcone synthase and the molecular basis of plant polyketide biosynthesis. *Nat. Struct. Biol.* **6:** 775–784.
10. Fill, T. P., G. K. Pereira, R. M. G. Santos, and E. Rodrigues-Filho. 2007. Four additional meroterpenes produced by *Penicillium* sp. found in association with *Melia azedarach*: Possible biosynthetic intermediates to austin. *Z. Naturforsch.* **62B:** 1035–1044.
11. Fill, T. P., R. M. G. Santos, A. Barisson, E. Rodrigues-Filho, and A. Q. L. Souza. 2009. Co-production of bisphenylpropanoid amides and meroterpenes by an endophytic *Penicillium brasilianum* found in the root bark of *Melia azedarach*. *Z. Naturforsch.* **64c:** 355–360.
12. Frits, R. R., D. S. Hodgins, and C. W. Abell. 1976. Phenylalanine ammonia-lyase. Induction and purification from yeast and cleareance in mammals. *J. Biol. Chem.* **251:** 4646–4650.
13. Fujita, T., D. Makishima, K. Akiyama, and H. Hayashi. 2002. New convulsive compounds, brasiliamides A and B, from *Penicillium brasilianum* Batista JV-379. *Biosci. Biotechnol. Biochem.* **66:** 1697–1705.
14. Juvvadi, P. R., Y. Seshime, and K. Kitamoto. 2005. Genomics reveals traces of fungal phenylpropanoid–flavonoid metabolic pathway in the filamentous fungus *Aspergillus oryzae*. *J. Microbiol.* **43:** 475–486.
15. Keller-Schierlein, W. and J. Widmer. 1976. Stoffwechselprodukte von mikroorganismen 159. Mitteilung. Uber die aromatishe aminosaure des echinocandins B, 3,4-dihihydroxyhomotyrosin. *Helv. Chim. Acta* **59:** 2021–2031.
16. Kumada, Y., H. Naganawa, H. Iinuma, M. Matsuzaki, T. Takeuchi, and H. Umezawa. 1976. Dehydrodicaffeic acid dilactone, an inhibitor of catechol-*O*-methyl transferase. *J. Antibiot.* **29:** 882–889.
17. Lee, E. R., G. H. Kang, and S. G. Cho. 2007. Effect of flavonoids on human health: Old subjects but new challenges. *Recent Pat. Biotechnol.* **1:** 139–150.
18. Liang, X. W., M. Dron, C. L. Cramer, R. A. Dixon, and C. J. Lamb. 1989. Differential regulation of phenylalanine ammonia-lyase genes during plant development and by environmental cue. *J. Biol. Chem.* **264:** 14486–14492.
19. Ling, K. H., C. K. Yang, and F. T. Peng. 1979. Territrems, tremorgenic mycotoxins of *Aspergillus terreus*. *Appl. Environ. Microbiol.* **37:** 355–357.
20. Mabry, T. J. and A. Ulubelen. 1980. Chemistry and utilization of phenylpropanoids including flavonoids, coumarins, and lignans. *J. Agric. Food Chem.* **28:** 189–196.
21. MacDonald, M. J. and G. B. D'Cunha. 2007. A modern view of phenylalanine ammonia lyase. *Biochem. Cell Biol.* **85:** 273–282.
22. Marchelli, R. and L. C. Vining. 1973. Biosynthesis of flavonoid and terphenyl metabolites by the fungus *Aspergillus candidus*. *J. Chem. Soc. Chem. Commun.* 555–556.
23. Marchelli, R. and L. C. Vining. 1973. The biosynthetic origin of chlorflavonin, a flavonoid antibiotic from *Aspergillus candidus*. *Can. J. Biochem.* **51:** 1624–1629.
24. Massow, F. V. 1977. Incorporation of phenylpropanes into xylerythrin-type pigments in *Peniophora sanguinea*. *Phytochemistry* **16:** 1695–1698.
25. Massow, F. V. and H. E. Noppel. 1977. Biosynthesis of the xylerythrin-type pigments in *Peniophora sanguinea*. *Phytochemistry* **16:** 1699–1700.
26. Moore, B. S., C. Hertweck, J. N. Hopke, M. Izumikawa, J. A. Kalaitzis, G. Nilsen, *et al.* 2002. Plant-like biosynthetic pathways in bacteria: from benzoic acid to chalcone. *J. Nat. Prod.* **65:** 1956–1962.
27. Naoumkina, M., M. A. Farag, L. W. Sumner, Y. Tang, C. Liu, and R. A. Dixon. 2007. Different mechanisms for phytoalexin induction by pathogen and wound signals in *Medicago truncatula*. *Proc. Natl. Acad. Sci. U.S.A.* **104:** 17909–17915.
28. Nuutinen, J. T. and S. Timonen. 2008. Identification of nitrogen mineralization enzymes, L-amino acid oxidases, from the

ectomycorrhizal fungi *Hebeloma* spp. and *Laccaria bicolor*. *Mycol. Res.* **112:** 1453–1464.
29. Pagot, Y., J. Belin, F. Husson, and H. Spinnler. 2007. Metabolism of phenylalanine and biosynthesis of styrene in *Penicillium camemberti*. *J. Dairy Res.* **74:** 180–185.
30. Parmar, V. S., H. N. Jha, A. K. Gupta, S. Prasad, and A. K. Agamanone. 1992. A flavanone from *Agave americana*. *Phytochemistry* **31:** 2567–2568.
31. Quijano, L., J. S. Calderon, G. F. Gomez, I. E. Soria, and T. Rios. 1980. Highly oxygenated flavanoids from *Ageratum corymbosum*. *Phytochemistry* **18:** 2439–2442.
32. Rani, M. and S. B. Kalidhar. 1996. Trioxygenations sites in the A-ring of naturally occurring flavanones and isoflavanones using 1H NMR spectroscopy. *J. Med. Aromatic Plant Sci.* **18:** 473–476.
33. Santos, R. M. G., E. Rodrigues-Filho, W. Caldas Rocha, and M. F. S. Teixeira. 2003. Endophytic fungi from *Melia azedarach*. *World J. Microbiol. Biotechnol.* **19:** 767–770.
34. Seshime, Y., P. R. Juvvadi, I. Fujii, and K. Kitamoto. 2005. Genomic evidences for the existence of a phenylpropanoid metabolic pathway in *Aspergillus oryzae*. *Biochem. Biophys. Res. Commun.* **337:** 747–751.
35. Tanaka, Y., N. Sasaki, and A. Ohmiya. 2008. Biosynthesis of plant pigments: Anthocyanins, betalains and carotenoids. *Plant J.* **54:** 733–749.
36. Tomoyuki, F. and H. Hideo. 2004. New brasiliamide congeners, brasiliamides C, D and E, from *Penicillium brasilianum* Batista JV-379. *Biosci. Biotechnol. Biochem.* **68:** 820–826.
37. Turnbull, J. J., J. Nakajima, R. W. D. Welford, M. Y. K. Saito, and C. J. Schofield. 2004. Mechanistic studies on three 2-oxoglutarate-dependent oxygenases of flavonoid biosynthesis: Anthocyanidin synthase, flavonol synthase, and flavanone 3-hydroxylase. *J. Biol. Chem.* **279:** 1206–1216.
38. Turner, W. B. and D. C. Aldridge. 1983. *Fungal Metabolites II.* pp. 594. 1st Ed. Academic Press, London.
39. Umezawa, H., H. Tobe, N. Shibamoto, F. Nakamura, K. Nakamura, M. Matsuzaki, and T. Takeuchi. 1975. Isolation of isoflavones inhibiting dopa decarboxylase from fungi and *Streptomyces*. *J. Antibiot.* **28:** 947–952.
40. Vannelli, T., W. W. Qi, J. Sweigard, A. A. Gatenby, and F. S. Sariaslani. 2007. Production of p-hydroxycinnamic acid from glucose in *Saccharomyces cerevisiae* and *Escherichia coli* by expression of heterologous genes from plants and fungi. *Metabol. Eng.* **9:** 142–151.
41. Ververidis, F., E. Trantas, C. Douglas, G. Vollmer, G. Kretzschmar, and N. Panopoulos. 2007. Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Part I: Chemical diversity, impacts on plant biology and human health. *Biotechnol. J.* **2:** 1214–1234.
42. Ververidis, F., E. Trantas, C. Douglas, G. Vollmer, G. Kretzschmar, and N. Panopoulos. 2007. Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Part II: Reconstruction of multienzyme pathways in plants and microbes. *Biotechnol. J.* **2:** 1235–1249.
43. Watanabe, S. K., G. Hernandez-Velazco, F. Iturbe-Chinas, and A. Lopez-Mungia. 1992. Phenylalanine ammonia-lyase from *Sporidiobolus pararoseus* and *Rhodosporidium toruloides*: Application for phenylalanine and tyrosine deamination. *World J. Microbiol. Biotechnol.* **8:** 406–410.
44. Weisshaar, B. and G. I. Jenkins. 1998. Phenylpropanoid biosynthesis and its regulation. *Curr. Opin. Plant Biol.* **1:** 251–257.
45. Wenhui, M., M. Xiaolin, Y. Lu, and D. Chen. 2009. Lignans and triterpenoids from the stems of *Kadsura induta*. *Helv. Chim. Acta* **92:** 709–715.
46. Yadav, R. N. and D. Brasainya. 1977. A novel 8,5'-methylenedioxy 3,7-dihydroxy flavone from seeds of *Centratherum anthelminticum* Kuntze. *J. Instit. Chem.* **69:** 60–62.
47. Yamada, S., K. Nabe, N. Izuo, K. Nakamichi, and I. Chibata. 1981. Production of L-phenylalanine from *trans*-cinnamic acid with *Rhodotorula glutinis* containing L-phenylalanine ammonia-lyase activity. *Appl. Environ. Microbiol.* **42:** 773–778.