which can be considered to be satisfactory for this type of analysis.

The concentration of fusarin C in sample M-84/F was found to be 0.28 mg/kg (Table II) while a low amount (0.02 mg/kg) could also be detected in sample M-84/C. This latter concentration was apparently too low to be detected in a mutagenicity assay (Figure 2).

It can be concluded that fusarin C is a highly mutagenic metabolite with a mutagenic potency comparable to the potent mutagens aflatoxin B_1 and sterigmatocystin. Fusarin C was shown to be a secondary metabolite of several strains of both *F. moniliforme* and *F. graminearum* but not of those strains of *F. moniliforme* var. *subglutinans* tested. It was proved to occur naturally in both *F. moniliforme* infected and "healthy" corn samples as the "fusarin C" peak coincided precisely with that of authentic fusarin C. Even after exposure to long-wave UV light, the HPLC chromatograms of the breakdown products of fusarin C and the isolated compound corresponded perfectly. In practice, the cooccurrence of fusarin C with the other *Fusarium* mycotoxins, moniliformin, deoxynivalenol, and zearalenone, in corn merits further investigation to determine any potential danger to human health.

Registry No. Fusarin C, 79748-81-5; aflatoxin B_1, 1162-65-8; sterigmatocystin, 10048-13-2.

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Identification of *p*-Hydrazinobenzoic Acid in the Commercial Mushroom *Agaricus bisporus*

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p-Hydrazinobenzoic acid (HBA) was identified in the cultivated mushrooms of the western hemisphere *Agaricus bisporus*. The commercial mushroom purchased locally was found to contain 10.7 ± 2.0 μg of HBA/g of mushroom (wet weight). HBA was quantitated in the fungus by high-performance liquid chromatography. The structure of HBA was confirmed by mass spectrometry.

The commercial cultivated mushroom, *Agaricus bisporus*, contains relatively large amounts of the hydrazide N^{β}-[γ-L-(+)-glutamyl]-4-(hydroxymethyl)phenylhydrazine (agarithine) (Levenberg, 1960, 1961). Kelly et al. (1962) found 0.04% agaritine in these mushrooms (wet weight), while we have found levels up to 0.07% (Ross et al., 1982).

Although agaritine did not induce tumors in mice (Toth et al., 1981a,b), over 50 hydrazine derivatives have induced tumors in laboratory animals (Toth, 1975, 1980, 1984; Toth et al., 1978, 1981a,b). The aim of this present study is to identify biosynthetic hydrazine precursors to agaritine that are present in this commercial mushroom. Schutte et al. (1972) have examined the incorporation of isotopically labeled compounds into agaritine by *A. bisporus*. There was no significant incorporation from 3-^{14}C-labeled tyrosine or phenylalanine. However, incorporation of 0.14%, 0.68%, and 4.1% was obtained with [U-^{14}C]shikimic acid, [2-^{14}C]glutamic acid, and *p*-aminobenzoic acid, respectively. This finding indicates that *p*-aminobenzoic acid is a precursor, with agaritine being one of the few natural products known to be derived from it. The postulated intermediates between *p*-aminobenzoic acid and agaritine have not yet been isolated (LaRue, 1977).

LaRue (1977) has postulated *p*-hydrazinobenzoic acid (HBA) and N^{β}-[γ-L-(+)-glutamyl]-4-carboxyphenylhydrazine as possible biosynthetic precursors of agaritine. We report herein the successful isolation, identification, and quantitation of HBA in *A. bisporus*.

EXPERIMENTAL SECTION

Materials. HBA was purchased from Eastman Kodak Co. (Rochester, NY) and purified by recrystallization. All solvents used for solvent extraction and thin-layer chromatography (TLC) were glass distilled, and solvents used for high-performance liquid chromatography (HPLC) were UV spectroscopic grade.

High-Performance Liquid Chromatography (HPL-

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C). Analysis of HBA was carried out on a Waters Associates (Milford, MA) HPLC system, which consisted of 6000A pumps, a 6 UK injector, and a 660 solvent programmer attached to a Holochrome HM UV–vis detector (Gilson, Middleton, WI) and a 3390A computing integrator (Hewlett-Packard, Avondale, PA). The column was 25 × 0.9 cm Whatman, Inc. (Clifton, NH) partisil C8, 10 μm. The mobile phase was a mixture of methanol and water (45:55 v/v) and ran at a flow rate of 4.0 mL/min. The detector wavelength was 322 nm. The peak areas were calculated by the computer integrator.

Thin-layer chromatography (TLC) was performed using precoated polyamide 6-F plates (J. T. Baker, Phillipsburg, NJ) and a solvent system of (1) 1-propanol–water–acetic acid (7:3:2 v/v) or (2) ether–methanol–water (5:4:2 v/v).

Mass Spectrometry (MS). Confirmation of the HBA structure was done using a MS system that consisted of an MS-9 analyzer (A.E.I., Ltd, Manchester, U.K.) and a MSS Series 200 electronics console (Mass Spectrometry Services, Ltd., Manchester, U.K.). Spectra were processed on a V.G. Series 2000 data system (V.G., Ltd., Manchester, U.K.). Samples were introduced by direct probe. The scan speed was 10 s/decade and resolving power was 10 000.

Mushrooms. The cultivated mushroom *A. bisporus* was purchased through an Omaha distributor between Nov 1983 and Feb 1984 from Camsco Mushroom Co., Inc. (West Chicago, IL). The estimated time from harvest to experimental use was 24–48 h.

Analysis of *A. bisporus* for HBA. A 55-g sample of the commercial mushrooms was homogenized in a Waring blender in 110 mL of methanol for 15 min. After centrifugation the supernatant was stored overnight at −20 °C and recentrifuged. The clear supernatant was passed through a 5 × 30 mm silica gel column. The sample was filtered through a Millex-GS 0.22-μm filter (Millipore Corp., Bedford, MA) and 20-μL samples were analyzed by HPLC as described above. Synthetic HBA had a retention time of 4.05 min.

Recovery of HBA from *A. bisporus*. Four aliquots of the above homogenate, each corresponding to 1.0 g of mushroom, were spiked with 20, 200, and 2000 μg of authentic HBA. Each sample was centrifuged, stored, recentrifuged and analyzed by HPLC as described above.

Confirmation of HBA Identity. The *Agaricus bisporus* mushrooms were extracted and processed as above. Six 200-μL injections were made on the HPLC, and the fraction eluting between 3.9 and 4.2 min was collected. Analytical HPLC of the combined fractions indicated the desired component was contaminated with a second component that eluted at 3.75 min. The combined fractions were concentrated and streaked on two polyamide TLC plates. Analytical standards of HBA were spotted alongside the extracts. The plates were developed consecutively with solvent systems 1 and 2. The plates were air-dried between the developments. The bands corresponding to HBA (R_f 0.46) were removed and extracted with ethanol. The concentrated extract was again purified by HPLC using the conditions previously described. The fraction eluting at 4.05 min was collected. Reinjection of a concentrated aliquot of this eluant indicated it was homogeneous by UV detection. The fractions were concentrated and analyzed by MS as described.

RESULTS AND DISCUSSION

HBA was isolated from a methanolic extract of *A. bisporus* by reverse-phase HPLC. Further purification was necessary since the fractions collected were contaminated with a compound that eluted slightly ahead of the desired peak. The mass spectra of an authentic standard of HBA and the compound isolated from the mushroom appear in Figure 1. The spectra correspond very well with the exception of enhanced intensities for ions at m/z 120 and 137 in the isolated sample, which are the major peaks in the mass spectra of the peak eluting at 3.75 min. The spectra of the authentic and isolated compounds exhibit a base peak at m/z 152, which corresponds to the molecular ion of HBA. The high-resolution mass spectral data confirms the elemental composition of the base peak in the isolated sample as $C_7H_8N_2O_2$ (deviation 0.3 mmu). Ions at m/z 135 and 107 correspond to loss of hydroxyl and carboxyl radicals, respectively, from HBA.

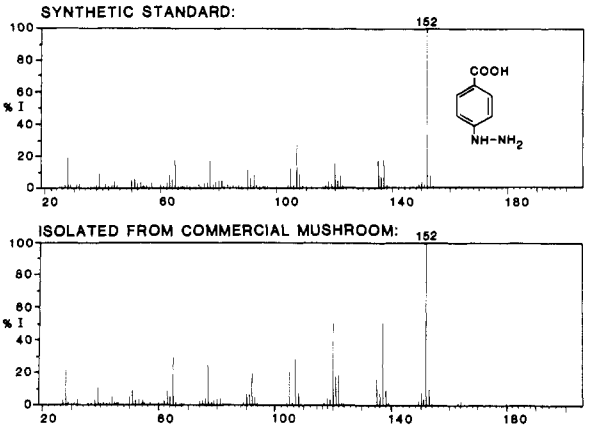

Figure 1. Mass spectra of *p*-hydrazinobenzoic acid.

Table I. Recovery of HBA from Mushroom Extracts

μg of HBA added/g of mushroom	% recovery[a]
2000	71 ± 3
200	53 ± 3
20	38 ± 4

[a] These values are from triplicate analyses; the variability is ± standard deviation.

Table II. Quantities of HBA in Commercial Samples of *A. bisporus*

batches	μg of HBA/g of mushroom[a]
batch 1	9.6
batch 2	10.2
batch 3	13.6
batch 4	8.3
batch 5	11.6

[a] Based on wet tissue weight and adjusted for recovery. HPLC analyses were performed in duplicate.

Table I lists the recovery of HBA from spiked samples of mushroom extracts. The recovery is very dependent upon the amount of HBA added and very low in the range of the HBA found in the mushroom samples analyzed. There appear to be at least two reasons for the variability in the recovery of HBA in the mushroom samples. In separate experiments we determined the recovery of HBA from a 5 × 30 mm silica gel column. When 2000 μg was added to the column, the recovery was 93%, but when 20 μg was added, the recovery dropped to 47%. Further the HPLC calibration curve deviated from linearity at low levels. Equal volumes were injected during these experiments. A small amount of irreversible absorption of HBA on the silica gel (which is also the backbone of the reverse-phase HPLC column) could be responsible for these observations. Table II lists the quantities of HBA found in commercial mushrooms purchased in Omaha. These values have been adjusted for recovery. We estimate our minimum detection limit for HBA to be 1.6 μg/g of

mushroom. The average value of HBA found in the mushroom samples analyzed was 10.7 ± 2.0 μg of HBA/g of mushroom. This value is surprisingly low if the sole or principal biosynthetic pathway leading to agaritine involved HBA. As mentioned earlier, agaritine has been found in these mushrooms in levels up to 0.07%. The level of agaritine in these mushrooms therefore is approximately 70-fold higher than that of HBA. It appears that either the biosynthesis of agaritine proceeds through alternate pathways not involving HBA or HBA is rapidly converted to other compounds involved in agaritine biosynthesis. HBA is presently under study for carcinogenic action in mice.

Registry No. HBA, 619-67-0.

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Paspalitrem C, a New Metabolite from Sclerotia of *Claviceps paspali*

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A new metabolite was purified from chloroform extracts of *Claviceps paspali* sclerotia by using column chromatography and preparative centrifugally accelerated, radial, thin-layer chromatography. The chemical structure of the metabolite was determined by ^1H and ^{13}C NMR spectroscopy to be 4-(3-methyl-2-butenyl)paspalinine (paspalitrem C). Paspalitrem C differed from the previously identified tremorgen, paspalitrem A, only by the position of attachment of the 3-methyl-2-butenyl unit to the indole ring.

Several tremorgenic as well as nontremorgenic, chemically related metabolites have been isolated from sclerotia of *Claviceps paspali* Stevens et Hall (Figure 1). This fungus infects *Paspalum* spp. and produces a staggers syndrome in cattle that graze the infected grass. The nontremorgens, paspaline (**1**) and paspalicine (**2**), were first reported from the dried mycelium of *C. paspali* (Fehr and Acklin, 1966), and their absolute stereochemical structures were subsequently determined by Springer and Clardy (1980). The tremorgens include paspalinine (**3**), 5-(3-methyl-2-butenyl)paspalinine (paspalitrem A) (**4**), and 5-(3-hydroxy-3-methyl-1-butenyl)paspalinine (paspalitrem B) (**5**) (Cole et al., 1977; Gallagher et al., 1980b). Another chemically related tremorgen, aflatrem (**6**), a metabolite of *Aspergillus flavus* (Wilson and Wilson, 1964; Gallagher et al., 1980a) and a logical distal product of *C. paspali* metabolism, has not been shown to be produced by *C. paspali*. In separate analyses of the mycelium-culture medium and sclerotia of several sclerotium-producing strains of *A. flavus*, Wicklow and Cole (1982) reported that aflatrem was detectable only in the sclerotia of *A. flavus*.

In the process of developing a high-performance liquid chromatographic system for the quantitation of these metabolites, a major unidentified component consistently appeared in *C. paspali* sclerotial extracts. We now report the purification and chemical identification of this previously unknown member of the paspalitrem group.

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EXPERIMENTAL SECTION

Extraction and Purification of Metabolites. Sclerotia of *C. paspali* were hand collected from infected *Paspalum* spp. in Georgia. Seven hundred grams of sclerotia was extracted 3 times with 2 L of chloroform per extraction by grinding with an Ultra Turrax homogenizer. The combined extracts were reduced in volume to an oily residue that was chromatographed on a silica gel column (4.5 cm i.d. × 40 cm) packed in benzene and eluted with a linear gradient of benzene to ethyl ether. One hundred eighty 17-mL fractions were collected, and every tenth fraction was subjected to thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Paspalitrem A (**4**), the unidentified metabolite, and related compounds (**1–3** and **5**) were contained in fractions 38–63. These fractions were combined, concentrated, and partitioned twice between 100 mL of hexane and 100 mL of 80% acetonitrile in water. The aqueous phase was reduced in volume and applied to a C_{18} reverse-phase column (3.5 cm i.d. × 27 cm) and eluted with a gradient of 50–90% acetonitrile in water. One hundred sixty fractions were collected and the following fractions were combined on the basis of TLC and HPLC analyses: 58–67, **5**; 79–90, **2**; 112–116, unknown metabolite; 117–120, unknown plus **4**; 121–124, **4**; 128–134, **1**.

High-performance liquid chromatographic analysis of the combined fractions 112–116 indicated the actual presence of two compounds eluting as a negatively skewed peak. A Model 7924 Chromatotron (Harrison Research,

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