Phytochemistry, Vol. 27, No. 3, pp. 929–930, 1988.
Printed in Great Britain.

0031–9422/88 $3.00 + 0.00
Pergamon Journals Ltd.

SHORT REPORTS

ISOLATION OF β-*N*-(γ-GLUTAMYL)-4-FORMYLPHENYLHYDRAZINE (AGARITINAL) FROM *AGARICUS CAMPESTRIS*

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(*Received* 21 *May* 1987)

Key Word Index—*Agaricus campestris*; mushroom; aminoacid; agaritinal; β-*N*-(γ-glutamyl)-4-formylphenylhydrazine.

Abstract—The new compound, agaritinal, β-*N*-(γ-glutamyl)-4-formylphenylhydrazine has been isolated from *Agaricus campestris*. It was purified by using combination of ion-exchange and reverse phase HPLC. The structure was established by UV, MS and ^{1}H NMR spectral data.

INTRODUCTION

In our research to identify metabolites associated with the reproduction of fungi, our attention was drawn to a compound isolated from *Agaricus campestris*, exhibiting a strong absorption at 313 nm, similar to that of mycosporines (ex P310) [1, 2]. However, Agaricales species are known to synthesize compounds in which the glutamic acid is linked either to phenylhydrazine [agaritine (**4**) and related compounds (**1**, **2**)] [3–6] or to aniline [7–10]. We now report the isolation and characterization, from this fungus, of β-*N*-(γ-glutamyl)-4-formylphenylhydrazine (**3**), a new compound which has been earlier considered as a possible intermediate in agaritine biosynthesis [11].

RESULTS AND DISCUSSION

After successive purifications on ion exchange resins and semi-preparative HPLC, we obtained the hydrophilic compound **3**, which gave a positive reaction with ninhydrin and diazo-benzidine. In dilute sodium hydroxide, the UV spectrum of **3** shifted from 313 to 385 nm with a hyperchromic effect, suggesting, in comparison with compounds **4** [3], **2** [12] and **1** [6], a distinct chromophore with an aldehydic group. This was confirmed both in MS and ^{1}H NMR. Indeed, we observed in FAB$^+$MS the quasi molecular ion MH^+ at *m/z* 266 for **3**, and in EIMS the ions at *m/z* 649 M^+ ($C_{20}H_{11}F_{12}N_3O_3$) and *m/z* 331 $[M-H_2O]^+$, for the trifluoroacetate and the acetate derivatives respectively. In ^{1}H NMR, we characterized an aldehydic proton, a *p*-substituted aromatic ring and a glutamic residue –CO–CH_2–CH_2–CH(NH_2)–COOH. The deshielding of two aromatic protons (δ 7.91 ppm), the UV absorption both in neutral (313 nm) and alkaline (385 nm) solution and the occurrence of an important ion at *m/z* 136 ($C_7H_8N_2O$), were consistent with a *p*-formylphenylhydrazine unit. This moiety was linked to the glutamic residue by an amide bond to form **3**. Final proof for the aminoacid unit was obtained by acid hydrolysis of **3**, which yielded glutamine and glutamic acid, characterized by TLC and HPLC (OPA derivatives). Lastly, after treatment of **3** with KBH_4, we isolated the reduction product indistinguishable by TLC and HPLC from agaritine (**4**).

Compound **3** appears to be transitorily synthesized in all parts of *Agaricus campestris* fruit bodies: gills, stipes, and caps. From the biosynthetic point of view, it has been demonstrated that the aromatic part of the phenylhydrazine and aniline derivatives derived from the shikimate-chorismate pathway with the *p*-aminobenzoate as a common intermediate [11, 13]. In the proposed biosynthetic scheme of agaritine (**4**) [11], some of the postulated intermediates between *p*-aminobenzoate and agaritine have been recently characterized in *Agaricus bisporus*: *p*-hydrazinobenzoic acid (**5**) [14] and β-*N*-(γ-glutamyl)-4-carboxyphenylhydrazine (**2**) [12].

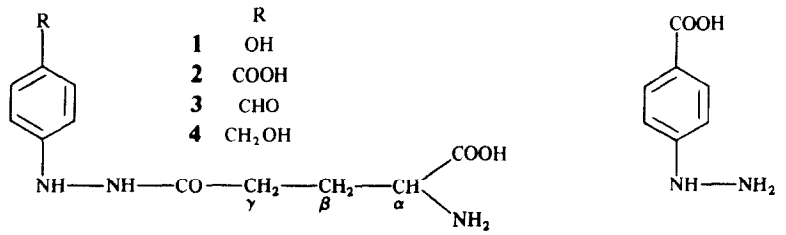
In the same way, occurrence in *A. campestris* of agaritinal (**3**) together with agaritine (**4**), is a convincing confirmation of the earlier postulated biosynthetic scheme of agaritine [11].

EXPERIMENTAL

Plant material. Agaricus campestris Lange was collected in September 1986 near Lyon, France.

Localization of **3** *into the mushroom.* Crude aq. extracts of separated gills, stipes and caps were analysed by HPLC (Hypersil ODS 5 μm, 250 × 5 mm, 2% aq. HOAc, 1 ml/min); **3** was detected by its absorption at 313 nm (R_t: 13 min.).

Extraction and isolation of β-N-(γ-*glutamyl*)-4-*formylphenylhydrazine* (**3**). Fresh frozen mushrooms (250 g) were extracted with H_2O at 4°. After filtration, the solvent was evapd *in vacuo* to 100 ml. MeOH (150 ml) was added and the resulting soln stored overnight at 4°. After centrifugation, the supernatant was concd



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under red. pres. to a small vol. of H_2O, filtered twice through a Sephadex A-25 QAE column (28 × 2 cm). The aq. eluate was collected in 16 fractions (15 ml each). UV detection indicated that **3** was present in fractions nos 5–9. Final purification was completed by successive semi-prep. HPLC using Lichrosorb RP 18 7 μm (250 × 9 mm) and μ Bondapak NH_2 (300 × 9 mm) eluted with 0.05% aq. HOAc. *ca* 10 mg of **3** were obtained.

β-N-(γ-glutamyl)-4-formylphenylhydrazine (**3**). UV $\lambda_{max}^{H_2O}$ nm (log ε): 231 (3.79), 313 (4.19); + NaOH 248 (3.76), 385 (4.30); + HCl 231 (3.79), 313 (4.19); FAB$^+$ MS *m/z* (rel. int): 266 (MH^+, 100), 201 (62), 166 (16), 137 (20), 136 (15), 132 (39), 121 (16); ^{1}H NMR (400 MHz, D_2O) 9.73 (1H, *s*, -CHO), 7.91 (2H, *d*, *J* = 8.5 Hz), 7.03 (2H, *d*, *J* = 8.5 Hz), 3.91 (1H, *t*, *J* = 6 Hz, Hα), 2.67 (2H, *m*, Hγ), 2.31 (2H, *m*, Hβ).

Trifluoroacetyl derivative. 200–500 μg of **3** was dissolved in 40 μl of TFA. After 12 hr at room temp., the sample was ready for MS. EIMS (70 eV) *m/z* (rel. int): 649 $[M]^+$ ($C_{20}H_{11}F_{12}N_3O_8$) (2), 439 (59), 343 (18), 281 (21), 217 (13), 202 (19), 180 (100), 152 (85), 136 (13), 135 (28), 119 (10), 105 (25), 84 (13), 69 (100).

Acetyl derivative. 200–500 μg of **3** was dissolved in 20 μl of pyr. then 20 μl of $(Ac)_2O$ were added. The mixture was left 12 hr at room temp. EIMS (70 eV) *m/z* (rel. int.) 331 $(M - H_2O)^+$ (2), 289 (81), 247 ($C_{12}H_{13}N_3O_3$) (74), 202 (100), 154 (16), 136 ($C_7H_8N_2O$) (89), 121 (57), 92 (14), 84 (C_4H_6NO) (100), 56 (42).

Acid hydrolysis of **3**. *Ca* 0.5 mg of **3** was brought to ebulition under reflux for 5 hr in 20 mM HCl. The hydrolysis of **3** was followed in HPLC (Hypersil ODS 5 μm, 2% aq. HOAc, 1 ml/min), by the decrease of the *A* at 313 nm as well as by the appearance of gln and glu. These compounds were both identified by TLC SiO_2 ($CHCl_3$–MeOH–NH_4OH, 2:2:0.5) in presence of ninhydrin reagent and by HPLC of their OPA derivatives (Hypersil ODS 5 μm, MeOH–2% aq. HOAc, 40:60, 1 ml/min, UV 340 nm), by comparison with authentic specimens. OPA derivatives were prepd. according to [14].

Reduction of **3**. Excess of KBH_4 was added to *ca* 0.5 mg of **3** dissolved in 50 μl of H_2O. The quick transformation of **3** into agaritine (**4**), was followed in HPLC (Hypersil ODS 5 μm, 2% aq. HOAc, 1 ml/min) both by disappearance of **3** at 313 nm (R_t: 13 min) and appearance at 254 nm of a compound (R_t: 6.5 min) corresponding to agaritine (co-chromatography with an authentic sample). Reduction of **3** into **4** was achieved after 30 min.

Acknowledgements—We thank Dr D. Rast, Institute of Plant Biology, University of Zürich, Switzerland, for the gift of an authentic sample of agaritine, J. Garcia for recording ^{1}H NMR spectra, Centre Grenoblois de Résonance Magnétique, CENG, Grenoble, France.

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