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REDUCTION OF ANTHRANILIC ACID AND RELATED AMINO ACIDS IN FRUIT-BODIES OF *HEBELOMA SACCHARIOLENS*

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Key Word Index—*Hebeloma saccharioliens*; Agaricales; biosynthesis; reduction of aromatic 2-aminocarboxylic acids; anthranilic acid; 2-aminobenzaldehyde.

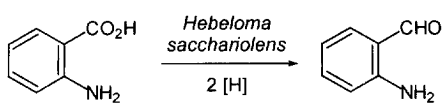
Abstract—Fruitbodies of the mushroom *Hebeloma saccharioliens* reduce anthranilic acid (**1a**) and some of its analogues to the corresponding aromatic aminoaldehydes with high efficiency. © 1997 Elsevier Science Ltd

INTRODUCTION

2-Aminobenzaldehyde (**2a**) is the source of the sweet odour of *Hebeloma saccharioliens* Quél. (Agaricales), a mushroom found in damp woodland [1]. Compound **2a** has also been detected in various plants [2, 3], honey [4] and some fungi [5, 6]. In this publication we report on studies regarding the biosynthesis of **2a** in *H. saccharioliens*.

RESULTS AND DISCUSSION

In order to clarify the biosynthetic origin of 2-aminobenzaldehyde (**2a**), aqueous solutions of [carboxy-^{13}C]- and [^{15}N]anthranilic acid (**1a*/1a****) were applied *via* a syringe to young fruit-bodies of *H. saccharioliens* in their natural habitat. After three to five days the fruit-bodies were collected and extracted with ether. GC/MS analysis indicated a very high incorporation§ of the labelled anthranilic acids (**1a*/1a****) into 2-aminobenzaldehyde (**2a*/2a****) (Table 1).

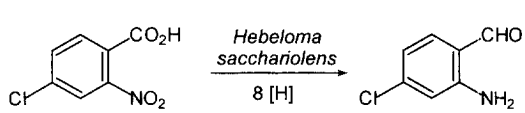


1a **2a**

The successful conversion of anthranilic acid (**1a**) prompted us to carry out experiments with unnatural precursors to test for the specificity of the enzymes. As can be seen from Table 2, the enzymes tolerate a variety of aromatic 2-aminocarboxylic acids **1** and reduce them with high efficiency.

Feeding of 2-amino-4-fluorobenzoic acid (**1b**) to *H. saccharioliens* yielded 2-amino-4-fluorobenzaldehyde (**2b**) which was identified by its characteristic mass spectral fragmentation pattern and the high resolution of its molecular ion. Due to the great similarity of anthranilic acid (**1a**) with its 4-fluoro derivative **1b**, the acceptance by the mushroom was excellent. Even *in vitro* reduction of 4-fluoro- (**1b**) and 5-fluoro-anthranilic acids (**1c**) to the corresponding aldehydes by homogenized fruit-bodies took place.

Whereas 3-amino-2-naphthoic acid (**1d**) afforded the aldehyde **2d** in high yield, reduction of the sterically hindered 2-amino-6-methylbenzoic acid (**1e**) was not observed. Electron-deficient pyridine analogues of anthranilic acid were readily accepted. Thus, 2-aminonicotinaldehyde (**2g**) and 3-amino-2-pyridinecarbaldehyde (**2h**) were identified by GC mass spectrometry after feeding of the corresponding aminopyridinecarboxylic acids **1g** and **1h**. Since *N*-trifluoroacetyl-anthranilic acid (**1f**) is not reduced by *H. saccharioliens*, a free amino group seems to be a prerequisite for the conversion to the aldehyde. In the case of 4-chloro-2-nitrobenzoic acid (**3**) a small amount of 2-amino-4-chlorobenzaldehyde (**2i**) could be detected by GC mass spectrometry.



3 **2i**

These results show that intact fruit-bodies of *H. saccharioliens* are able to reduce a variety of aromatic 2-aminocarboxylic acids to the corresponding alde-

† HR-GC/MS investigations.

‡ Author to whom correspondence should be addressed.

§ Determination of the incorporation rate with the $[M]^+$- and $[M+1]^+$-peak intensities from the mass spectra (subtraction method) [7].

Table 1. Reduction of [carboxy-^{13}C]- and [^{15}N]anthranilic acid (**1a*/1a****) by *Hebeloma saccharioiens* (• indicates the position of the label)

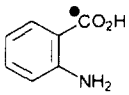
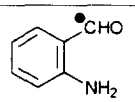
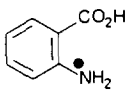
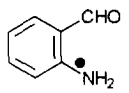
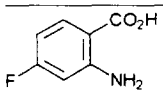
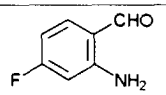
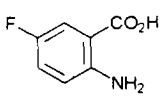
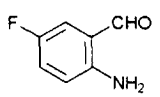
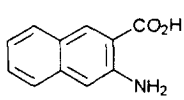
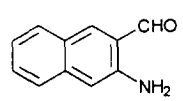
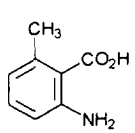
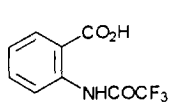
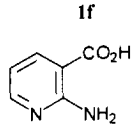
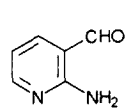
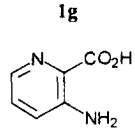
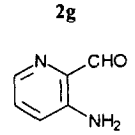
Precursor anthranilic acid (**1a*/1a****)	Product 2-aminobenzaldehyde (**2a*/2a****)	Incorp. rate	Analytical methods
		94%	GC/MS HR-GC/MS
		39%	GC/MS HR-GC/MS

Table 2. Conversion of aromatic 2-aminocarboxylic acids **1** into the corresponding 2-aminobenzaldehydes **2** by *Hebeloma saccharioiens*

Precursor anthranilic acid **1**	Product 2-aminoaldehyde **2**	Relative amount*	α†	Analytical methods‡
 **1b**	 **2b**	16§	1.0	GC/MS HR-GC/MS
 **1c**	 **2c**	3.2§¶	1.0	GC/MS
 **1d**	 **2d**	2.7	1.8	GC/MS HR-GC/MS
 **1e**	no reduction	—	—	GC/MS
 **1f**	no reduction	—	—	GC/MS
 **1g**	 **2g**	1.2	0.96	GC/MS
 **1h**	 **2h**	0.72	1.1	GC/MS HR-GC/MS

* Relative amount = {integral(unnatural **2**)}/{integral(natural **2a**)}. Integrals of the GC-peaks.

† Relative retention time α. Dead time t_0, $\alpha = \{t(\text{unnatural } \mathbf{2})\text{-}t_0\}/\{t(\text{natural } \mathbf{2a})\text{-}t_0\}$.

‡ All 2-aminobenzaldehyde derivatives **2** showed the typical $[M]^+$-, $[M-CO]^+$- and $[M-CO-HCN]^+$- peaks in their mass spectra.

§ Separation with special column temperature programming. Incorporation rate for the *in vivo* experiment. Reduction to **2b** and **2c** was also observed with a mushroom homogenate.

¶ Fruit-bodies in very bad condition.

hydes, a reaction which can not be achieved by chemical means due to the deactivation of the carboxyl group and the instability of the resulting amino aldehydes. Overreduction to the benzylic alcohols was not observed. Our studies indicate that fruit-bodies of basidiomycetes can be used as small bioreactors for efficient enzymatic transformations.

The reduction of benzoic acid and its hydroxy and methoxy derivatives to the corresponding aldehydes has been described for several wood-rotting fungi [8, 9]. Gross and Zenk [10, 11] isolated an enzyme from mycelia of *Neurospora crassa*, which catalyses the reduction of aromatic acids to the respective aldehydes in the presence of ATP and NADPH. The only reduction of anthranilic acid (**1a**) to 2-aminobenzaldehyde (**2a**) reported so far was achieved with cultures of *Aspergillus niger* [12]. It is interesting to note that in higher plants 2-aminobenzaldehyde (**2a**) is probably formed by oxidative cleavage of indole *via* 2-(formylamino)benzaldehyde [3, 13].

EXPERIMENTAL

General. GC/MS was carried out on a Varian Model 3400 GC with a split-splitless capillary injector coupled to a Finnigan MAT 95Q sector mass spectrometer (column 1) or a Finnigan MAT MAGNUM™ ion trap instrument (column 2) using EI at 70 eV. The He flow was 1 ml min^{-1}, the injector and coupling temp. 250°. Fused-silica capillary columns were used: SE-S4-CB 25 m × 0.25 mm id 0.25 μm df, Chromatographic Service (column 1) or Optima-1 DF-0.25 30 m × 0.25 mm id, Macherey–Nagel, Düren, Germany (column 2). HR-GC/MS were recorded on the Finnigan MAT 95Q.

Column temp. programming. Heating to 50° for 2 min, then to 300° at a rate of 10° min^{-1}, then to 300° for 3 min (column 1, 2). Programming for the fluoro compounds: heating to 50° for 1 min, then to 110° at a rate of 12° min^{-1}, 120° (1° min^{-1}), 280° (50° min^{-1}), 280° for 3 min (column 2).

Plant material. The feeding experiments were performed in September 1995/96 at the campus of the University of Regensburg. Voucher samples of *Hebeloma sacchariolens* Quél. are deposited at the Institut für Organische Chemie der LMU München (Germany); leg. F.v. Nussbaum, det. Dr N. Arnold.

Feeding experiments. Anthranilic acid and its analogues **1** (0.1–0.3 mmol) were dissolved in 0.6–1.2 ml of a 1:1 mixt. of H_2O and dimethyl sulphoxide. The solns were injected with a syringe in 3–5 young fruit-bodies (0.1–0.4 ml/fruit-body) of *H. sacchariolens* (cap diam. 1–2 cm). After 3–5 days the mushrooms (cap diam. 1–4 cm) were worked up or stored in a refrigerator at −18°.

Preparation of GC/MS-samples. Each fruit-body was extracted for 1 hr with 100 ml of pure Et_2O in an ultrasound bath. The extracts were dried (Na_2SO_4), filtered, cautiously concd under red. pres. and filtered over a Chromabond®-SiOH cartridge (Macherey–Nagel).

[*Formyl-*^{13}*C*]-2-*aminobenzaldehyde* (**2a***). HR-GC/MS: *m/z* (rel. int.) = 122.0557 $[M]^+$ (91) {calcd. 122.0561}; GC/MS: *m/z* (rel. int.) = 123 (4), 122 $[M]^+$ (90), 121 (11), 93 $[M-{}^{13}CO]^+$ (100), 92 (92), 66 $[M-{}^{13}CO-HCN]^+$ (28), 65 (26).

[^{15}*N*]-2-*Aminobenzaldehyde* (**2a****) {39% ^{15}N}. GC/MS: *m/z* (rel. int.) = 123 (4), 122 $[M]^+$ (66), 121 (92), 94 $[M-CO]^+$ (59), 93 (100), 66 $[M-CO-HC^{15}N]^+$ (35), 65 (33).

2-*Amino*-4-*fluorobenzaldehyde* (**2b**) [15]. HR-GC/MS: *m/z* (rel. int.) = 139.0459 $[M]^+$ (100) {calcd. 139.0433}, 111 $[M-CO]^+$ (98), 84 $[M-CO-HCN]^+$ (30), 83 (21). GC/MS: *m/z* (rel. int.) = 139 $[M]^+$ (100), 111 $[M-CO]^+$ (68), 84 $[M-CO-HCN]^+$ (14), 83 (15).

2-*Amino*-5-*fluorobenzaldehyde* (**2c**) [16]. GC/MS: *m/z* (rel. int.) = 139 $[M]^+$ (100), 111 $[M-CO]^+$ (98), 84 $[M-CO-HCN]^+$ (54), 83 (47).

3-*Amino*-2-*naphthalenecarbaldehyde* (**2d**) [17]. HR-GC/MS: *m/z* (rel. int.) = 171.0670 $[M]^+$ (100) {calcd. 171.0684}; GC/MS: *m/z* (rel. int.) = 171 $[M]^+$ (100), 143 $[M-CO]^+$ (90), 126 (33), 115 (33), 83 (21).

2-*Aminonicotinaldehyde* (**2g**) [18]. GC/MS: *m/z* (rel. int.) = 122 $[M]^+$ (98), 94 $[M-CO]^+$ (100), 93 (65), 67 $[M-CO-HCN]^+$ (28), 66 (16), 58 (19).

3-*Amino*-2-*pyridinecarbaldehyde* (**2h**) [19]. HR-GC/MS: *m/z* (rel. int.) = 122.0476 $[M]^+$ (100) {calcd. 122.0480}; GC/MS: *m/z* (rel.int.) = 122 $[M]^+$ (91), 94 $[M-CO]^+$ (100), 93 (29), 67 $[M-CO-HCN]^+$ (43), 66 (18).

2-*Amino*-4-*chlorobenzaldehyde* (**2i**) [20]. GC/MS: *m/z* (rel. int.) = 157 (18), 155 $[M]^+$ (61), 129 (31), 127 $[M-CO]^+$ (100), 102 (8), 101 (7), 100 $[M-CO-HCN]^+$ (23), 99 (16), 92 (41), 91 (21), 90 (15), 65 (41), 63 (34).

Acknowledgements—We thank Drs N. Arnold and H. Besl for their kind assistance in obtaining *H. sacchariolens* and Mr A. Nowak for providing a sample of [^{15}N]anthranilic acid. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 369).

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