Phytochemistry, Vol. 21, No. 8, pp. 1975–1976, 1982.
Printed in Great Britain.

0031-9422/82/081975-02$03.00/0
Pergamon Press Ltd.

5-HYDROXYTRYPTOPHAN AND SOLUBLE PIGMENT FORMATION IN *CLAVICEPS* SP. PRL 1980

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(*Revised received* 11 *January* 1982)

Key Word Index—*Claviceps* sp. PRL 1980; Clavicipitaceae; ergot; 5-hydroxytryptophan; pigment; biosynthesis.

Abstract—5-Hydroxytryptophan (156 mg/l) was identified in 15-day-old cultures of *Claviceps* sp. PRL 1980. [side-chain 3-^{14}C] DL-Tryptophan and [side-chain 3-^{14}C] 5-hydroxytryptophan were incorporated into the brown pigment in cultures of the same fungus, 6% and 24%, respectively.

INTRODUCTION

The nonalkaloid indole derivatives identified in cultures of *Claviceps* sp. PRL 1980 are γ,γ-dimethylallyltryptophan (DMAT) [1–3], clavicipitic acid [1, 4], and *N*-acetyltryptophan [1]. In addition, there is an appreciable amount of a soluble brown pigment, which was shown to be derived from tryptophan in *C. purpurea* PRL 1578 [5]. This work reports the presence of another tryptophan metabolite, 5-hydroxytryptophan (5OHtry), in cultures of *Claviceps* sp. PRL 1980 and the relationship of this metabolite to the brown pigment.

RESULTS AND DISCUSSION

The neutral plus acidic fraction from cultures of *Claviceps* sp. PRL 1980 incubated with [^{14}C]tryptophan contained six radioactive compounds which separated on TLC. The product with the lowest R_f was a reddish-brown pigment and was Van Urk's negative. The other five compounds, which were Van Urk's positive, were identified by cospotting with reference compounds (in order of increasing R_f) as 5OHtry, tryptophan, DMAT, clavicipitic acid, and *N*-acetyltryptophan. All of these compounds except 5OHtry had been previously identified in cultures of *Claviceps* sp. PRL 1980 [1]. The presence of 5OHtry was not recognized in the earlier work because of poor separation of 5OHtry from tryptophan in the TLC solvent systems that were used. The amount of 5OHtry in a 15-day-old culture was 156 mg/l.

To verify the presence of 5OHtry in the cultures, 5OHtry was added to the neutral plus acidic fraction and the mixture was recrystallized to constant sp. act. The sp. act. for four recrystallizations were 4.88×10^3, 2.51×10^3, 2.16×10^3, and 2.12×10^3 cpm/mg, respectively. 5OHtry purified from the mother liquor by treatment with Dowex 50 cation exchange resin, CC on Sephadex G-10, and prep. TLC had an identical absorbance spectrum to reference 5OHtry. Maximum absorbance at pH 7 (aq.) was 275 nm. The fluorescence and excitation spectra of isolated and reference 5OHtry were also identical. In 2 N HCl with excitation at 295 nm, two emission maxima at 340 and 550 nm were observed with ratio of intensities 2.1:1. The peak at 550 nm disappeared when the pH was adjusted to 7. The reversible appearance of a fluorescence peak at 550 nm in acid solution is characteristic of 5-hydroxyindole compounds [6].

The absorbance maximum of the brown pigment eluted from the TLC plate and dissolved in MeOH–H_2O (4:1) had the same absorbance maximum as the mother liquor at 460 nm. Maximum rate of pigment formation was between one and 4 days after inoculation. The maximum concentration of pigment was reached after 12–14 days.

Radioactive brown pigment was obtained in the cultures after addition of [^{14}C]tryptophan or [^{14}C] 5OHtry. With the latter compound the per cent incorporation into brown pigment was 4 times that obtained when [^{14}C]tryptophan was added (24.0% vs 5.7%, Table 1). Initial addition of [^{14}C]tryptophan resulted in lower radioactivity in the pigment compared to addition after 2 days incubation in production medium (3.6% vs 5.7%; 2.1% vs 2.9%) presumably because some of the tryptophan was utilized for growth. Addition of unlabeled tryptophan to the culture medium increased the conversion of [^{14}C]tryptophan to brown pigment (3.6% vs 2.1%; 5.7% vs 2.9%). This indicated that tryptophan induces the enzymes involved in formation of brown pigment.

No tryptamine, 5-hydroxytrytamine, or 5-hydroxyindoleacetic acid was found in the cultures. Cultures grown with 5-hydroxyindoleacetic acid had a bright red color rather than the normal reddish brown color. These results suggest that 5OHtry is not converted to the brown pigment via 5-hydroxytryptamine or 5-hydroxyindoleacetic adid. The side-chain C-1 carbon is probably retained and the 5-hydroxyindole moiety of 5OHtry undergoes oxidation and condensation reactions to form the pigment.

A major metabolite of tryptophan in *Claviceps* sp.

Table 1. Incorporation of [^{14}C]tryptophan and [^{14}C] 5-hydroxytryptophan into brown pigment of *Claviceps* sp. PRL 1980

Radioactive addition			
Compound	Day added	Unlabeled tryptophan added†	% incorporation
Tryptophan	0	+	3.6
Tryptophan	0	−	2.1
Tryptophan	2	+	5.7
Tryptophan	2	−	2.9
5-Hydroxytryptophan	2	+	24.0

*See Experimental for details.
†Unlabeled tryptophan added in medium (day 0).

PRL 1980 is 5OHtry. The formation of 5OHtry could compete significantly with formation of DMAT for alkaloid production.

EXPERIMENTAL

Culture conditions. Sucrose–succinic acid medium (100 ml) [6] was inoculated from a slant of *C. purpurea* PRL 1980. After the medium turned reddish-brown (8–9 days), 25 ml was added to 100 ml of the same growth medium and shaken until a reddish-brown color began to appear (4–5 days). The resulting mycelium was washed ×2 with sterile H_2O and suspended in H_2O. A mycelial suspension equivalent to 50 ml growth medium was mixed with 100 ml mannitol–tryptophan–succinic acid medium [8] in a 500-ml flask (production medium) and shaken at 300 rpm at 25°. The incubation was continued for 10–15 days.

Extraction and TLC. The cultures were homogenized and filtered. The pH of the mother liquor was adjusted to pH 11 with 30% NH_3 and extracted with Et_2O to remove alkaloids. The aq. layer was neutralized to pH 7 with 20% HOAc, lyophilized and the dry material dissolved in MeOH–H_2O (4:1). An aliquot of the extract was subjected to TLC on Si gel G. The solvent system was BuOH–HOAc–H_2O (4:1:1) (BAW) for sepn of 5OHtry from tryptophan or MeOAc–*iso*-PrOH–30% NH_3 (9:7:4) (MIA) for sepn of brown pigment. The plates with radioactive products were scanned with a radioactivity scanner and the radioactivity determined by comparing the area under the curve with plates containing radioactive standards.

Quantitative determination of 5-hydroxytryptophan. The amount of 5OHtry was determined as described in ref. [9]. The 5OHtry region of a TLC plate was scraped off, the compound eluted and reacted with Van Urk's reagent.

Recrystallization of 5-hydroxytryptophan. Claviceps sp. PRL 1980 was grown in 10 ml production medium containing [side-chain 3-^{14}C] DL-tryptophan (90 μCi, 3.76 mCi/mmol) as described previously [10]. The mother liquor was adjusted to pH 10.5–11 with 30% NH_3 and the soln extracted with Et_2O. The aq. layer was lyophilized and the residue dissolved in MeOH–H_2O (4:1). H_2O (1 ml) was added to 0.3 ml of the MeOH–H_2O soln. The soln was heated to boiling, satd with unlabeled 5OHtry, decolorized with activated charcoal and filtered through a preheated funnel. The filtrate was cooled at 0° and the suspension centrifuged. The crystals were collected and dissolved in a minimum vol. of hot H_2O. An aliquot of the soln was removed for the determination of radioactivity and amount of 5OHtry. The remainder was cooled, the crystals collected and the procedure repeated.

Conversion of [^{14}C]tryptophan or [^{14}C]5-hydroxytryptophan to brown pigment. For study of radioactive incorporation into brown pigment 10 ml of growth medium was washed with H_2O and transferred to 10 ml of production medium with 3.5 mg tryptophan or without tryptophan in 50 ml conical flasks. Cultures were incubated for 12 days. Alternatively, after 2 days of incubation, the mother liquor was removed by centrifugation and the mycelium suspended in 10 ml production medium without tryptophan plus 5 ml [side-chain-3-^{14}C]DL-tryptophan (10 μCi, sp. act. 50.6 mCi/mmol) or [side-chain-3-^{14}C]DL-5-hydroxytryptophan (10 μCi, sp. act. 59.2 mCi/mmol). The cultures were incubated for an additional 10 days.

Purification of 5-hydroxytryptophan and measurement of absorbance and fluorescence emission spectra. The 5OHtry in the mother liquor from a 15-day-old culture of *Claviceps* sp. PRL 1980 was purified using Dowex-50 cation exchange resin, Sephadex G-10 CC and prep. TLC on Si gel G with MIA solvent [11].

Acknowledgements—The support of the Robert A. Welch Foundation Grant No. D-117 and of the National Institutes of Health Grant No. GM27617 is acknowledged. The technical assistance of Miss Pat Krocak is acknowledged.

REFERENCES

1. Liang, H.-J. and Anderson, J. A. (1978) *Phytochemistry* **17**, 597.
2. Robbers, J. E. and Floss, H. G. (1968) *Arch. Biochem. Biophys.* **126**, 967.
3. Agurell, S. and Lindgren, J.-E. (1968) *Tetrahedron Letters* 5127.
4. Robbers, J. E. and Floss, H. G. (1969) *Tetrahedron Letters* 1857.
5. Vining, L. C. and Taber, W. A. (1963) *Can. J. Microbiol.* **9**, 291.
6. Udenfriend, S., Bogdanski, D. F. and Weissbach, H. (1955) *Science* **122**, 972.
7. McDonald, J. K., Cheldelin, V. H. and King, T. E. (1960) *J. Bacteriol.* **80**, 61.
8. Taber, W. A. (1962) *Develop. Ind. Microbiol.* **4**, 295.
9. Kiepert, S. and Voigt, R. (1972) *J. Chromatogr.* **64**, 327.
10. Kim, I.-S., Kim, S.-U. and Anderson, J. A. (1981) *Phytochemistry* **20**, 2311.
11. Anderson, J. A. and Saini, M. S. (1974) *Tetrahedron Letters* 2107.