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THE TRANSFORMATION OF TRYPTAMINE AND D-TRYPTOPHAN BY BASIDIOMYCETES IN SUBMERGED CULTURE

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Abstract—The basidiomycete *Cantharellus cibarius* converts D-tryptophan in submerged culture to *N*-acetyl-D-tryptophan. Another basidiomycete *Tricholoma nudum* converts tryptamine to indole-3-acetic acid and 5-hydroxyindole-3-acetic acid. *N,N*-Dimethyltryptamine is also converted to indole-3-acetic acid but the branched-chain secondary amines β-methyl- and β-ethyltryptamine are not metabolized.

INTRODUCTION

IN AN earlier publication[1] on a survey of the biochemical transformation of indole compounds, it was reported that a number of basidiomycetes convert simple β-substituted indoles to more acidic compounds. The survey relied on paper chromatography for the identification of products and except in the case of the formation of oxindole-3-acetic acid from indole-3-acetic acid by *Hygrophorus conicus*[2] none of the transformation products was isolated.

An extension of this study to include the isolation of the transformation products as crystalline compounds and the characterization of these compounds by classical chemical techniques has been made. The original assumption[1] that the product formed from tryptophan by *Cantharellus cibarius* was indole-3-lactic acid has been shown to be incorrect and the product is actually *N*-acetyl-D-tryptophan.

Indole-3-acetic acid (IAA) was isolated as a crystalline solid from a culture of *Tricholoma nudum* grown in the presence of tryptamine. Although crystalline IAA has been isolated from a number of cultures of fungi grown on media supplemented with tryptophan[3] this is the first reported instance of the isolation of IAA from a culture of a higher fungus or when tryptamine has been used as the substrate.

RESULTS AND DISCUSSION

Cantharellus cibarius

Indole-3-lactic acid and *N*-acetyltryptophan are difficult to resolve on a two-dimensional paper chromatogram using *n*-butanol–acetic acid–water in the first dimension followed by isopropanol–ammonia in the second. However, the two compounds are readily separated in a single dimensional benzene–propionic acid[4] solvent system. There is also a pronounced difference in the colors formed when a papergram containing the two compounds is sprayed

[1] E. C. SCHUYTEMA, M. P. HARGIE, I. MERITS, J. R. SCHENCK, D. J. SIEHR, M. S. SMITH and E. L. VARNER, *Biotech. Bioeng.* **8**, 275 (1966).
[2] D. J. SIEHR, *J. Am. Chem. Soc.* **83**, 2401 (1961).
[3] M. E. MACE, *Phytopathology* **55**, 240 (1965).
[4] M. D. ARMSTRONG, K. N. F. SHAW, M. J. GORTATOWSKI and H. SINGER, *J. Biol. Chem.* **232**, 17 (1958).

with Ehrlich reagent. The initial blue–gray color of indole-3-lactic acid fades to a gray while the blue–gray *N*-acetyltryptophan spot becomes more intensely blue with time.

When *Cantharellus cibarius* was grown in submerged culture containing 25 mg of D,L-tryptophan in 60 ml of culture, an acidic indole compound was detected on a two-dimensional papergram. If the L-isomer of tryptophan was used, no transformation product was observed. Approximately 15 mg of the crystalline unknown was isolated by means of preparative thin-layer chromatography (TLC) on silica gel. Acid hydrolysis of the unknown gave an indole which had the properties of tryptophan on paper chromatograms. Extraction of the aqueous hydrolysate with ether and subsequent separation of the ether extract on a gas–liquid chromatograph give a single peak with exactly the same retention time as a known sample of acetic acid in ether. A mixed melting point with authentic *N*-acetyl-D-tryptophan showed no depression. Recrystallization of the unknown from a solution containing *N*-acetyl-L-tryptophan gave crystals which melted at the same temperature as racemic *N*-acetyl-D,L-tryptophan. Thus, it is concluded that the mycelium of *C. cibarius* converts D-tryptophan in submerged culture to *N*-acetyl-D-tryptophan. There was no paper chromatographic evidence for the formation of kynurenine or anthranilic acid from D-tryptophan by *C. cibarius*.

Recently there have been several reports in the literature on the synthesis of *N*-acetyl-D-tryptophan by micro-organisms. Luckner[5] has reported that two *Penicillium* species convert D-tryptophan to *N*-acetyl-D-tryptophan. Several species of *Saccharomyces* have been reported to acetylate D-tryptophan[6] and Zenk and Schmitt[7] have isolated a cell-free enzyme preparation from yeast which will acetylate only the D-isomer of tryptophan. Zenk and Scherf[8] in a survey of plants and micro-organisms found that only the metabolism of D-tryptophan in fungi formed *N*-acetyltryptophan. Apparently the ability to acetylate D-tryptophan is not prevalent among basidiomycetes since *C. cibarius* is the only one of a relatively large number of basidiomycetes surveyed[1] which acetylated D-tryptophan. An unidentified species of *Coprinus* with which rather extensive studies are being conducted in this laboratory also acetylates D-tryptophan.

Tricholoma nudum

It had been observed earlier[1] that *Tricholoma nudum* converted tryptamine into IAA in submerged culture. It was a relatively simple matter to recover the crystalline product since evaporation of the ethyl acetate or ether extract of the acidified culture broth left behind a solid residue. When the residue was dissolved in hot benzene, needle-like crystals of IAA separated on cooling. The melting point, the movement on paper and thin-layer chromatograms and the i.r. and u.v. spectra of the recovered crystals were identical to those of an authentic sample of IAA. The conversion of tryptamine to indole-3-acetic acid by basidiomycetes is a rather common occurrence. There is also paper chromatographic evidence that tryptophan can be degraded to IAA by some of these organisms. In certain species studied, the IAA is further degraded to oxindole-3-acetic acid.[1,2] Evidence obtained in this laboratory indicates that, in *Hygrophorus conicus*, oxindole-3-acetic acid (OIAA) is degraded still further. Although the methyl oxindoles reported by Ray and Thimann[9] and Hinman and

[5] M. LUCKNER, *Z. Allgem. Mikrobiol.* **3**, 93 (1963).
[6] F. HAGEMANN, *Arch. Mikrobiol.* **49**, 150 (1964).
[7] M. H. ZENK and J. SCHMITT, *Naturwissenschaften* **51**, 510 (1964).
[8] M. H. ZENK and H. SCHERF, *Planta* **62**, 350 (1964).
[9] P. M. RAY and K. U. THIMANN, *Arch. Biochem. Biophys.* **64**, 175 (1956).

Lang[10] as end products from the enzymic inactivation of IAA may be among the products, at least two compounds formed by *H. conicus* from OIAA still contain the side-chain carboxyl group, as indicated by experiments with ^{14}C labeled IAA.

A second transformation product appeared on chromatograms of acid extracts of the culture broth of *T. nudum* to which tryptamine had been added. This second compound was assumed to be 5-hydroxyindole-3-acetic acid due to its behavior on a two-dimensional chromatogram. It gave a blue–gray color when sprayed with Ehrlich reagent and a brown-colored spot when sprayed with diazotized sulphanilic acid and sodium carbonate. No attempt was made to isolate this compound.

The ready conversion of tryptamine to IAA suggested that *T. nudum* contained an active amine oxidase. To check the specificity of the oxidase, three substituted tryptamines, *N,N*-dimethyltryptamine, β-methyltryptamine and β-ethyltryptamine were added to cultures of *T. nudum* instead of tryptamine. After a 2-day incubation of the branched-chain tryptamines, no other indoles could be detected by chromatography in the culture broth except the original starting material. However, IAA and 5-hydroxyindole-3-acetic acid were detected on papergrams of an extract from the culture to which *N,N*-dimethyltryptamine had been added. These observations indicate that the organism was unable to oxidatively deaminate branched-chain amines, although a methyl substituted amine was easily deaminated.

EXPERIMENTAL

The cultures of *Cantharellus cibarius*, *Tricholoma nudum* and *Hygrophorus conicus* were obtained from Abbott Laboratories, North Chicago, Ill. The unidentified species of *Coprinus* was obtained from Professor Alexander Smith, University of Michigan, Ann Arbor. Indole-3-acetic acid, D-tryptophan, L-tryptophan, D,L-tryptophan, *N*-acetyl-L-tryptophan, *N*-acetyl-D-tryptophan and *N*-acetyl-D,L-tryptophan were obtained from the Sigma Chemical Company, St. Louis, Missouri. 5-Hydroxyindole-3-acetic acid was obtained from Regis Chemical Company, Chicago, Ill. *N,N*-Dimethyltryptamine, β-methyltryptamine and β-ethyltryptamine were obtained from the Aldrich Chemical Company, Milwaukee, Wis. Silica gel G according to Stahl was obtained from Brinkman Instruments, Inc., Des Plaines, Ill. All solvents were reagent grade and were used without further purification. Melting points were taken on a Kofler hot stage, u.v. spectra were obtained using a Beckmann DK-2A recording spectrophotometer and i.r. spectra were obtained with a Beckmann IR5A spectrophotometer either as nujol mulls or KBr pellets.

Cultivation of Micro-organisms

Stock cultures were grown at approximately 25° in 250 ml Erlenmeyer flasks containing 70 ml of 4% malt extract on a rotary shaker (240 rev/min). For the microbial transformation of tryptamine, tryptophan or other indole compounds, 30 mg of the indole was added at the time that the flasks were inoculated with the organism.

Chromatography

Two-dimensional ascending paper chromatography, on Whatman No. 1 paper was used. The developing solvents used for single and two-dimensional paper chromatograms were: Solvent A: 2-propanol–water–ammonium hydroxide (sp. g. 0·88) (200:10:30). Solvent B: *n*-butanol–glacial acetic acid–water (120:30:50). Solvent C: benzene–propionic acid–water (100:70:5). For the location of indole compounds, the dried papergrams were dipped into, or sprayed with, Ehrlich reagent (1 g of *p*-dimethylaminobenzaldehyde in 10 ml HCl diluted with 40 ml acetone).

Indole-3-acetic acid on a papergram sprayed with Ehrlich reagent gave a purple color which turned blue on standing. The R_f values of IAA were 0·44 in Solvent A, 0·89 in Solvent B, and 0·86 in Solvent C. *N*-Acetyl-D-tryptophan gave a purple color on a papergram when sprayed with Ehrlich reagent. The spot turned blue on standing. The R_f values for *N*-acetyl-D-tryptophan were 0·81, 0·93, and 0·71 respectively in solvents A–C. Oxindole-3-acetic acid on a papergram developed with a solvent containing NH_3, when sprayed with Ehrlich reagent, gave a yellow–green color which became an intense blue–green on standing. The R_f values of oxindole acetic acid were 0·34 in Solvent A and 0·87 in Solvent B. When 5-hydroxyindole

[10] R. L. HINMAN and J. LANG, *Biochemistry* **4**, 144 (1965).

acetic acid on a papergram was sprayed with Ehrlich reagent it gave a purple color which turned blue on standing. It also gave a brown color when sprayed with diazotized sulphanilic acid followed by 10% aqueous sodium carbonate. The R_f values of 5-hydroxyindole acetic acid were 0·62, 0·60 and 0·30 respectively.

Survey of the Transformation of Indole Compounds by Basidiomycetes

After 4 days of growth at 25° the mycelium was removed by filtration and the filtrate was brought to pH 3 (Hydrion paper) with 10% HCl. The acidified solution was extracted with ethyl acetate (3 × 50 ml) and the ethyl acetate extract was concentrated to a gummy residue. The residue was dissolved in sufficient ethyl acetate to give a final concentration of about 10 mg of solid per ml of solution. 10 μl of this solution were spotted on an 11-in. square of Whatman No. 1 paper, which was developed in Solvent A in the first dimension and then in Solvent B in the second dimension, dried, and sprayed with Ehrlich reagent.

Isolation and Characterization of N-*acetyl*-D-*tryptophan*

A batch of five flasks each inoculated with *Cantharellus cibarius* and 30 mg of D-tryptophan were incubated for a period of 4 days on a rotary shaker at approximately 25°. The contents of the flasks were then filtered. The filtrate was brought to pH 3 with HCl and the acidified solution was extracted with three 50-ml portions of ethyl acetate. The ethyl acetate extract when concentrated almost to dryness left a black–brown pasty residue.

The residue was redissolved in sufficient ethyl acetate to give a final concentration of about 10 mg per ml. This solution was streaked onto three 20 × 20 cm plates of silica gel G 50 μ thick. Each plate carried approximately 40 mg of crude material. The plates were developed with Solvent A. A 5 × 20 cm silica gel G plate was spotted with the extract and developed. The indole bands on the plates were located by the use of a short wavelength u.v. light and further ascertained by spraying the small plate with Ehrlich reagent. The u.v. fluorescent bands corresponding to the desired indole were scraped off the glass plates and eluted with ethanol. The alcohol solution was evaporated nearly to dryness and then a small amount of ether was added. The ether solution was cooled and petroleum ether (b.p. 78–103°) was added until the solution became turbid. Cooling with simultaneous stirring brought about crystallization. The crystals were collected and then recrystallized twice from hot water. The m.p. of the recrystallized substance was 182°. A known sample of *N*-acetyl-D-tryptophan melted at 182°. The i.r. spectrum of the isolated material as a nujol mull was identical to that for authentic *N*-acetyl-D-tryptophan. When the unknown was mixed with an equal weight of known *N*-acetyl-L-tryptophan and recrystallized from water, the melting point of the crystals was 203°. The melting point of *N*-acetyl-DL-tryptophan reported in the literature was 206–207°.[11] The crystalline product could not be separated from synthetic *N*-acetyl-D-tryptophan by two-dimensional paper chromatography.

A small amount of the crystalline material was dissolved in 1 ml of 6 N HCl and heated under reflux for 6 hr. The presence of tryptophan in the hydrolysis mixture was indicated by co-chromatography on paper. The material obtained by extracting the hydrolysis mixture with ether and drying had the same retention time on a 6-ft silicone oil GLC column as a known sample of acetic acid in ether.

Isolation and Characterization of Indole-3-Acetic Acid

A batch of four 250-ml Erlenmeyer flasks were each inoculated with *T. nudum* and 30 mg of tryptamine and were incubated for a period of 4 days. The contents of the flasks were filtered and the filtrate was brought to pH 3 with HCl. The acidic aqueous phase was extracted with ethyl acetate and the extract concentrated nearly to dryness under vacuum. 10 ml of 95% ethyl alcohol were then added and the solution evaporated to dryness. The residue was air-dried and then crystallized from hot benzene. The recovered crystalline material melted at 165° (lit. m.p. 164–165°).[12] The i.r. absorption spectrum of the unknown was identical to that of an authentic sample of IAA. The isolated material could not be separated from a sample of known IAA by two-dimensional paper chromatography in Solvents A and B.

The ethyl acetate extract from the acidified culture fluid of *T. nudum* supplemented with tryptamine contained, as shown by paper chromatography, a second substance which gave a blue–gray spot when sprayed with Ehrlich reagent. The compound also reacted on a papergram with diazotized sulphanilic acid. This, together with the fact that it had the same R_f as 5-hydroxyindole-3-acetic acid in two solvent systems, was taken as proof that the compound was 5-hydroxyindole acetic acid.

[11] *Handbook of Chemistry and Physics*, 46th edition, Chemical Rubber Co., Cleveland, Ohio (1965).
[12] I. HEILBRON, *Dictionary of Organic Compounds*, Oxford University Press, New York (1953).