

Tryptophol Formation by *Zygosaccharomyces priorianus*

J. P. ROSAZZA, R. JUHL, AND P. DAVIS

Pharmacognosy Research Laboratories, The University of Iowa, College of Pharmacy, Iowa City, Iowa 52242

Received for publication 26 February 1973

*Zygosaccharomyces priorianus* converted L-tryptophan to tryptophol and to small quantities of indole-3-acetic acid. Neither tryptophol nor indole-3-acetic acid was metabolized when added separately to growing cultures. The possible intermediacy of indole-3-pyruvic acid, indole-3-acetaldehyde, and tryptamine in the degradation of L-tryptophan was tested by feeding these compounds to *Z. priorianus* and *Saccharomyces cerevisiae*. Indole-3-pyruvic acid and indole-3-acetaldehyde were converted to tryptophol and indole-3-acetic acid, with the latter accumulating only in small amounts. Tryptamine was converted to its N-acetyl derivative by these organisms. A qualitative study was made on the metabolism of L-phenylalanine, L-tyrosine, and L-5-hydroxytryptophan by these organisms. Like L-tryptophan, these amino acids were metabolized to their respective alcohol and acid derivatives. Of a large number of organisms tested, the yeasts possessed the highest capacity for degrading L-tryptophan to tryptophol.

The metabolic disposition of L-tryptophan (Trp) by microorganisms has been widely studied. Besides being incorporated nearly intact into secondary metabolites such as violacein (5, 31), the ergot alkaloids (24), neoechinuline (3), and other compounds (28, 54), numerous degradation products of this amino acid are also formed by microorganisms. Common metabolic reactions which lead to the accumulation of Trp degradation products include tryptophanase (6, 19, 32, 42), deamination (2, 4, 14, 15, 26, 43), and oxidative fission of the pyrrole ring of the indole nucleus (23, 36, 47, 48, 52, 55). A few bacterial systems metabolize Trp by means of decarboxylation as the primary step (30, 34).

Yeasts accomplish the degradation of amino acids principally via the Ehrlich mechanism (18, 37, 44, 53). In this general metabolic pathway, amino acids undergo transamination to their respective keto acids. These are decarboxylated, and the resulting aldehydes are reduced to their alcohol equivalents (44). Ehrlich was the first to demonstrate the formation of tryptophol (Trp-ol) (11), tyrosol, and phenethyl alcohol (10) from their respective amino acid precursors. These alcohol derivatives have been found in the higher-boiling fractions of fusel oils and are generally thought to be formed by way of the Ehrlich mechanism (37, 50, 53).

In our laboratories, preliminary studies on the metabolism of Trp by shaken cultures of *Zygosaccharomyces priorianus* resulted in the identification of Trp-ol and indole-3-acetic acid (IAA) as major metabolites. According to initial qualitative data, Trp-ol was formed to a much greater extent than was IAA by this organism. We became interested in the potential use of yeasts for the synthesis of Trp-ol and some Trp-ol derivatives which have been found to possess interesting pharmacological activity (13, 51).

However, results of other studies on the metabolism of Trp by yeasts indicated that Trp-ol and IAA were indeed formed, but that the reaction could be complicated by the formation of other Trp metabolites such as indole-3-lactic acid (LA) (15), indole-3-carboxylic acid, and indole-3-pyruvic acid (IPA) (14). These studies also indicated that the ratios of various Trp metabolites obtained was dependent on fermentation conditions.

This work was therefore undertaken to determine the stoichiometry of the degradation of Trp by *Z. priorianus*. An attempt was also made to determine whether the accumulation of Trp-ol and IAA was peculiar to particular organisms or to groups of organisms. The abilities of *Z. priorianus* and *Saccharomyces*
cerevisiae to convert known Trp metabolites and certain other amino acids to their respective alcohol derivatives was also examined.

**MATERIALS AND METHODS**

Melting points are corrected and were obtained in open-ended capillary tubes. Infrared spectra were obtained on a Beckman 10 spectrophotometer by using KBr disks. Nuclear magnetic resonance spectra were obtained with a Varian T-60 instrument in CDCl₃ with tetramethylsilane as an internal standard.

A number of chemicals were purchased. The identity of these compounds was confirmed either by melting point or by the preparation of suitable derivatives. Each compound was also examined by thin-layer chromatography (TLC) to verify purity. Chemicals used in this study were: Trp and phenethyl alcohol (Eastman); IAA and L-tyrosine (Tyr., (Fisher); L-5-hydroxytryptophan (5-OH-Trp), Trp-ol, 5-hydroxyindole-3-acetic acid (5-OH-IAA), IPA, LLA, tryptamine (Trp-amine), indole-3-acetaldehyde (I-ace-
tald), L-phenylalanine, phenylacetic acid, and 4-hy-
droxyphenylacetic acid (Sigma Chemical Co.) and 5-
hydroxytryptophol (5-OH-Trp-ol) (Regis).

Tyrosol (4-hydroxyphenethyl alcohol) was prepared from 4-hydroxyphenylacetic acid according to previ-
ously described methods (20, 41). This compound was crystallized from chloroform (mp 88–90 C; reported mp 91.5 C (41)).

Synthetic N-acetyltryptamine was obtained by refluxing 500 mg of Trp-amine-hydrochloride with a mixture of 1 ml of glacial acetic acid, 1 ml of acetic anhydride, and 10 mg of zinc dust for 30 min. The hot reaction mixture was poured into 25 ml of cold water, and the crude solid product was filtered. Crystallization was achieved with a benzene-hexane mixture (mp 76.5–77 C, reported 77 C [46]; nuclear magnetic resonance (CDCl₃) δ 1.96 (s, 3, COCH₃), 2.9 (q, 2, 
—CH₃—NH—), 3.6 (q, 2, Ar-CH₂), 7.32 (m, 5, Aromatic); infrared (KBr disk) μ 2.97, 3.26, 6.25, 6.41, 9.17, 9.41, 12.55, 13.48).

The benzyl ester of Trp was prepared by known procedures (mp 71–72 C, reported 71 C (33)).

**Cultures and general growth procedures.** Cultures used extensively in this study were Zygosac-
charomyces priorianus SP-WISC 22, which was ob-
tained from the culture collection of C. J. Sib at the University of Wisconsin, School of Pharmacy; and Saccharomyces cerevisiae NRRL Y-2034. Organisms were maintained on Sabouraud-maltose agar slants and stored in a refrigerator at 4 C prior to use. All organisms mentioned in this work are maintained in the culture collection at the College of Pharmacy of the University of Iowa.

 Cultures were grown in a soybean meal-glucose medium of the following composition: soybean meal, 5 g; glucose, 20 g; yeast extract, 5 g; NaCl, 5 g; K₂HPO₄, 5 g; distilled water, 1 liter; pH 7.0, adjusted with 5 N HCl. Media were autoclaved at 121 C for 15 min.

Fermentations were conducted on rotary shakers (model G-25, New Brunswick Scientific Co.) (250 rpm, at 27 C) usually in 25-ml amounts of medium contained in 125-ml cotton-plugged Erlenmeyer flasks. The 48-h growth started from slants was used as inoculum for the fermentation proper. The inoculum volume was 10% of the volume of fermentation medium in all cases. Substrates were added to these secondary cultures after 24 h of growth. Trp and other amino acids were added as their HCl salts, and all other substrates were added in dimethylformamide solutions (0.1 ml/25 ml of medium).

**Procedure for the separation of Trp-ol, IAA, and Trp.** Samples of fermentations (5 ml) were adjusted to pH 2 with 5 N HCl and extracted three times with 5-ml portions of ethyl acetate. The extracts were combined, and 10-ml samples were partitioned with 5 ml of 0.1% NaHCO₃. The resulting ethyl acetate solution (extract A) was assayed for Trp-ol. The NaHCO₃ layer was adjusted to pH 2 and extracted with two 5-ml portions of ether, the ether extracts were combined, and samples were assayed for IAA (extract B). The aqueous portion which remained after the initial ethyl acetate extractions was used for analysis of Trp (extract C).

This overall extraction procedure was slightly modified for the quantitative determination of Trp. Media samples were diluted to known volumes with 0.01 N HCl and extracted three times with ethyl acetate (as above) to remove all Trp-ol and IAA. Suitable samples of the remaining aqueous portions were then assayed for Trp.

Each extract was examined by TLC to estimate its purity. Extracts A, B, and C were found to contain only Trp-ol, IAA, and Trp, respectively. No other indole compounds were detected in any of these extracts in the experiments to be described.

**Trp-ol assay.** Trp-ol was determined by a modification of the procedure of Harbo and Aashiemi (17). Suitable samples of extract A were evaporated to dryness in a test tube under a stream of air. To the resulting residue was added 2.75 ml of methanol and 1.5 ml of a solution containing 2 g of p-dimeth-
ylaninobenzaldehyde (PDAB) in 84 ml of 95% ethanol and 16 ml of concentrated HCl. The test tubes were placed in a water bath maintained at 95 C for 2 min. The samples were removed from the water bath, 0.25 ml of 0.1% NaNO₂ was added, the solutions were allowed to cool for 30 min at room temperature, and absorbances were measured at 530 nm. The Trp-ol standard curve was linear in the concentration range of 0 to 50 μg. Recoveries of added samples of Trp-ol through the entire extraction procedure averaged 100 ± 4%.

**IAA assay.** IAA was determined by the spectroflu-
rometric procedure of Stoessel and Venis (49). Re-
ative fluorescent intensities were measured in an Amino-Bowman spectrophotofluorometer at an exci-
tiation wavelength of 400 nm and an emission wave-
length of 490 nm. A slit width of 3 was used through-
out. Concentrations were related to a standard curve which was linear in the range of 0 to 30 μg of IAA. Recoveries of added IAA samples through the extrac-
tion procedure averaged 100 ± 10%.

**Trp assay.** A modification of the procedure of Udenfried and Peterson was used (56). Suitable
volumes of extract C were diluted to 0.5 ml with 5 N HCl, PDAB reagent (3.0 ml of a 0.5% solution in concentrated HCl) was added, samples were allowed to stand for 10 min, and 0.25 ml of 0.1% NaNO₂ and 3.0 ml of anhydrous ethanol were added to them. After 10 min, absorbances were measured at 620 nm. The standard curve was linear between Trp concentrations of 10 to 100 µg, and recoveries of added Trp through the extraction process averaged 100 ± 5%.

TLC. TLC was performed on 0.25-mm thick silica gel GC-254 (Merck) plates. Modifications of published TLC solvent systems (35) were used to develop chromatograms. Indolic compounds were visualized by spraying developed plates with 0.1% PDAB in concentrated HCl, drying with a heat gun, and spraying with 0.1% aqueous NaNO₂. Most indoles gave blue to purple spots on chromatograms. Non-indolic compounds were visualized with a 3-mianisaldehyde-H₂SO₄-MeOH (1:1:48, vol/vol/vol) spray reagent. Different compounds displayed a variety of colors when sprayed plates were heated. Table 1 shows the solvent systems, and Rf values of compounds involved in this work.

A routine procedure was used in the TLC analysis of fermentations. Samples were adjusted to pH 2 with 5 N HCl and extracted with 0.25 volume of ethyl acetate, and approximately 50 ml of the extracts was spotted on TLC plates. Extracts were always co-chromatographed with standards to facilitate identification of unknowns. In most cases, two or three different solvent systems were used to verify the identity of metabolites.

RESULTS

Production and isolation of the major metabolite of Trp esters. Z. priorianus was grown in 500-ml Erlenmeyer flasks containing 100 ml of medium. The benzyl ester of Trp (1.22 g) was distributed evenly among 24 flasks, and one was kept as a control. Culture samples were taken at 24, 48, and 72 h after substrate addition and were examined by TLC (solvent system A) for the presence of the unknown indole. At 72 h, most of the substrate had been consumed, and the unknown product was the major indolic metabolite. The control contained a trace of this metabolite.

The cultures were filtered through a Celite pad, and the filtered solids were washed with 500 ml of distilled water. The combined filtrates (3 liters) were adjusted to pH 8.25 with 1 N NaHCO₃ and extracted three times with 800 ml of ethyl acetate. The extract was dried over Na₂SO₄ and evaporated to a viscous brown residue (0.934 g) which was adsorbed onto alumina (Fisher A-540, 100 g, column dimensions 2.5 by 26 cm). Elution of the column was accomplished with benzene-95% ethanol (25:1, vol/vol), and 10-ml fractions were collected at a flow rate of 1 ml/min, fractions 36 to 90 yielding 0.257 g of the indole product. The metabolite was crystallized from a mixture of equal parts of benzene and hexane: mp 58 to 59°C, reported for Trp-ol, 59 C (45); infrared (KBr disk) µ 2.92, 3.0 to 3.35 (broad band), 6.13, 6.74, 6.89, 7.04, 7.43, 7.49; nuclear magnetic resonance (CDCl₃) δ 1.17 (s,1,OH), 3.0 (t,2,CH₂OH), 3.9 (t,2,Ar-CH₃), 6.9-7.78 (m,5,aromatic), 8.13 (s,broad, NH). The peak at 1.17 disappeared upon equilibration with D₂O. Elemental composition was: calculated for Trp-ol, C 74.51, H

| Compound                     | Rf values in Solvent systems* |
|------------------------------|------------------------------|
|                              | A    | B    | C    | D    | E    | F    |
| Tryptophol                   | 0.50 | 0.40 | 0.90 | 0.71 |
| Indole-3-acetic acid         | 0.30 | 0.54 | 0.20 | 0.75 |
| Indole-3-Propionic acid      | 0.68 | 0.85 | 0.55 |      |
| Indole-3-acetdehyde          | 0.62 | 0.72 | 0.90 | 0.89 |
| Tryptamine                   | 0.15 |      | Many |      |
| Indole-5-pyruvic acid        | 0.54 | Many |      |      |
| Indole-3-Lactic acid         | 0.08 | 0.12 | 0.19 | 0.23 |
| N-Acetyltryptamine           | 0.40 | 0.20 | 1.00 | 0.60 |
| 5-Hydroxytryptophol          | 0.35 | 0.51 | 0.85 | 0.45 |
| 5-Hydroxyindole-3-acetic acid| 0.10 | 0.95 | 0.35 | 0.20 |
| Phenylethyl alcohol          | 0.73 | 0.96 | 0.92 | 0.90 |
| Phenylacetic acid            | 0.62 | 0.57 | 0.46 | 0.54 |
| Tyrosol                      | 0.30 | 0.96 | 0.91 | 0.50 |
| 4-Hydroxyphenylacetic acid   | 0.40 | 0.38 | 0.69 | 0.35 |

* Solvent systems used (vol/vol/vol ratios): A, benzene-ethanol (4:1); B, chloroform-glacial acetic acid, (95:5); C, ethyl acetate-isopropanol-25% NH₄OH (9:5:4); D, chloroform-glacial acetic acid-95% ethanol (90:5:5); E, ethyl acetate-acetone (10:1); F, benzene-methanol (5:1).
of

6.88, N 8.69; found, C 74.99, H 6.78, N 8.68. The metabolite was spectrally and chromatographically (solvent systems A, B, and C) identical to a purchased sample of Trp-ol.

Quantitative analysis of the degradation of Trp to Trp-ol and IAA. According to TLC analysis (solvent systems A, B, and C), IAA was identified as a minor metabolite in the previous experiment, and it was the only other indole compound detected. We learned that Z. priorianus could convert free Trp to Trp-ol and IAA in proportions similar to those obtained with the benzyl and ethyl esters. Thus, Trp was used as substrate in all subsequent work. This quantitative study was conducted to determine the stoichiometry of the conversion of Trp to Trp-ol and IAA during the course of a fermentation.

Z. priorianus was grown according to the usual procedure except that the fermentation proper was conducted in 1-liter Erlenmeyer flasks containing 200 ml of the Soybean-glucose medium. Trp was added as substrate to obtain a concentration of 4.90 μmol/ml (1 mg/ml) in each of three cultures. A fourth culture containing no Trp was kept as a control. Samples (5 ml) were withdrawn from the fermentations at time intervals between 0.5 and 77.5 h after substrate additions. These were stored at −10 C until required for analysis. Each sample was assayed for Trp, Trp-ol, and IAA by the methods described. (Separate analyses showed that 20 μg of Trp per ml was present as a normal component of the Soybean-glucose medium, presumably being introduced via the yeast extract. Traces of Trp-ol found in control flasks in earlier experiments were undoubtedly produced from this "endogenous" source of Trp. It should be noted that the amounts of Trp, Trp-ol, and IAA found in the control culture in this experiment were too low to be detected by the analyses described in Materials and Methods.) The results of this experiment are shown in Table 2.

Trp decreased steadily throughout the fermentation from 3.86 to 0.28 μmol/ml at the end. Trp-ol was rapidly formed during the first half of the fermentation, reaching a maximum of 3.62 μmol/ml at 46 h. Trp-ol levels decreased slightly until the end of the fermentation. Relatively small amounts of IAA were produced, and the ratio of Trp-ol to IAA at 32.5 h was nearly 100.

Assays at 0.5 h accounted for only 3.93 μmol of total indoles per ml, mostly as Trp. Trp was added to cultures in levels of 4.90 μmol/ml. This apparent low recovery may be attributed to the uptake of Trp from the medium by Z. priorianus cells. Based on the amount of Trp originally added to the cultures, the 46-h analyses gave a 96% recovery, expressed as total indoles. Of the Trp consumed in the fermentation at this time, 93% of it was converted to Trp-ol. In a separate, identical experiment, it was determined that the number of viable cells of Z. priorianus remained constant during the course of the fermentation and that the organism was in the stationary growth phase.

Metabolism of Trp-ol, IAA, I-acetald, IPA, and Trp-amine by Z. priorianus and S. cerevisiae. A qualitative study was made to determine the capacity of Z. priorianus and S. cerevisiae to metabolize Trp-ol, IAA, I-acetald, IPA, and Trp-amine to recognizable indole products. This was done to show whether IAA or Trp-ol were interconvertible and if I-acetald, IPA and/or Trp-amine could be utilized, thus supporting the general pathway for Trp degradation by Z. priorianus and other yeasts.

Table 2. Quantitative analysis of the degradation of Trp to Trp-ol and IAA by Zygosaccharomyces priorianus

| Hours after Addition of tryptophan | IAA | Trp  | Trp  | Trp  | Total |
|-----------------------------------|-----|------|------|------|-------|
|                                   |     |      |      |      |       |
| 0.5                               | 0.006 | 0.061 | 3.86 | 3.93 |
| 9.0                               | 0.016 | 0.856 | 3.39 | 4.26 |
| 22.0                              | 0.025 | 1.99  | 2.51 | 4.53 |
| 32.5                              | 0.035 | 3.38  | 1.34 | 4.75 |
| 46.0                              | 0.069 | 3.62  | 1.01 | 4.69 |
| 56.5                              | 0.117 | 3.55  | 0.60 | 4.33 |
| 69.5                              | 0.172 | 3.38  | 0.32 | 3.87 |
| 77.5                              | 0.191 | 3.27  | 0.28 | 3.74 |

* Values for IAA and Trp represent the averages of triplicate samples, while those for Trp-ol are duplicates.

* A 4.90-μmol amount of Trp per ml was added to the cultures at zero time.

Table 3. The metabolism of Trp-ol, IAA, IPA, I-acetald and Trp-amine by Zygosaccharomyces priorianus and S. cerevisiae

| Substrate* | Trp-ol | IAA | Other |
|------------|--------|-----|-------|
| Trp-ol     | −      | −   | −     |
| IAA        | −      | −   | −     |
| IPA        | ++     | +   | −     |
| I-Acetald  | ++++   | +   | −     |
| Trp-amine  | −      | −   | ++++  |

* Fermentations were conducted in duplicate with suitable controls. Substrates were added to cultures in concentrations of 0.5 mg/ml of medium. Samples taken at 48 h were examined by TLC (solvent systems A, B, C, and D).
mentations were conducted in duplicate with suitable controls, and substrates were added in concentrations of 0.5 mg/ml of medium. Samples taken at 48 h were examined by TLC (solvent systems A, B, and C, and D for tryptamine-containing cultures) (Table 3).

Z. priorianus and S. cerevisiae converted Trp-amine into an indolic compound different than Trp-ol or IAA. To obtain a quantity of the metabolite sufficient for identification, Z. priorianus was grown in 24, 500-ml Erlenmeyer flasks containing 100 ml of medium each. Trp-amine-hydrochloride (930 mg) was distributed equally among the flasks. Samples were taken at various time intervals after substrate addition, and the 48-h TLC analysis (solvent systems A and D) showed that all of the substrate was utilized.

The cultures were pooled, adjusted to pH 2 with 5 N HCl, and extracted with 3 liters of ethyl acetate. After drying, the extract was evaporated under vacuum to a tarry residue of 2.1 g. The tar was adsorbed onto silica gel (100 g, column dimensions 2.5 by 55 cm), and was eluted with benzene-95% ethanol (15:1, vol/vol). Fractions of 10 ml were collected at a flow rate of 2.5 ml/min, fractions 48 to 84 yielding a total of 214 mg of the indole metabolite. The compound was crystallized from a benzene-hexane mixture: mp 75°C, reported for N-acetyltryptamine 77°C (46); infrared (KBr disk) μ 2.97, 3.10, 3.26, 6.25, 6.41, 9.17, 12.55, 13.48; nuclear magnetic resonance (CDCl₃) δ 1.95 (s, 3,-COCH₃) 2.9 (q, 2, —CH₂NH—), 3.59 (q, 2, Ar-CH₂), 7.32 (m, 5, aromatic). The isolated product was spectrally and chromatographically (solvent systems A and D) identical to synthetic N-acetyltryptamine (Materials and Methods).

**Formation of Trp-ol and IAA by other microorganisms.** Although the formation of Trp-ol by yeasts and a few other microorganisms is known, no general investigation on the taxonomic distribution of this metabolic conversion has been reported. To determine if other microorganisms were capable of forming Trp-ol from Trp, 144 organisms representing five major classes and eight orders were grown in the presence of 1 mg of Trp per ml. Estimation of the relative amounts of Trp-ol and IAA produced was made by TLC (solvent systems A, B, and C) (Table 4).

Only 9 of the 144 cultures produced detectable quantities of Trp-ol. Two of 79 Moniliiales, and 1 of 3 Pezizales produced Trp-ol in this study. Six organisms of the Actinomycetales, 13 of Sphaerales, 7 of Eurotiales, and 8 Agaricales failed to produce Trp-ol. All cultures which formed Trp-ol also produced quantities of IAA as well. While the conversion of Trp to Trp-ol was not restricted entirely to the yeasts, they demonstrated a greater capacity for performing this metabolic transformation than other organisms examined.

**Metabolism of other amino acids by Z. priorianus and S. cerevisiae.** We examined the abilities of Z. priorianus and S. cerevisiae to metabolize L-phenylalanine, Tyr, and 5-OH-Tyr to their respective alcohol and acid derivatives. Fermentations were conducted in duplicate with controls, and the amino acid substrates were added as their HCl salts in levels of 0.5 mg/ml of culture medium. Samples of the fermentations were withdrawn at 72 h and examined by TLC (solvent systems E and F for the phenylalanine fermentation, and solvent systems C and F evaluate the 5-OH-Trp and Tyr containing fermentations). 5-OH-Trp-ol, 5-OH-IAA, phenethyl alcohol, phenylacetic acid, tyrosol, and p-hydroxyphenylacetic acid were used as standards for comparison on chromatograms. By estimation, these amino acids were converted into the expected products in approximately the same proportions as Trp-ol and IAA by these yeasts.

| Organism | Taxonomic order | Trp-ol | IAA |
|----------|----------------|--------|-----|
| Zygosaccharomyces priorianus (22) | Endomycetales | ++ + + | + |
| Saccharomyces cerevisiae (NRRL Y-2034) | Endomycetales | ++ + + | + |
| Saccharomyces sp. (6187) | Endomycetales | ++ + + | + |
| Schizosaccharomyces octosporus (NRRL Y-854) | Endomycetales | ++ + + | + |
| S. versatilis (NRRL Y-1026) | Endomycetales | ++ + + | + |
| N. speciosa (6188) | Endomycetales | ++ + + | + |
| Aspergillus stercorarius (373a) | Pezizales | + + + | + |
| Candida lipolytica (6189) | Moniliiales | + + + | + |
| Sporobolomyces species (1584) | Moniliiales | + + + | + |

* Fermentations were conducted in duplicate according to the usual procedure, and TLC assays were performed on samples taken 48 h after addition of substrate by using solvent systems A, B, and C. Microorganisms are designated by genus and species names where possible, culture collection numbers which are in parentheses, and taxonomic order.
DISCUSSION

This work began in connection with studies on the abilities of microorganisms to hydroxylate n- and carboxyl-blocked Trp derivatives. When incubated with certain esters of Trp, Z. priorianus produced relatively large amounts of a metabolite which reacted blue with PDAB- NaNO₂ spray reagents and gray with FeCl₃ on TLC plates. The identification of the metabolite was originally pursued because the microbial conversion of Trp esters to this compound was unusually complete and uncomplicated. Z. priorianus converted both the benzyl- and ethyl-esters of Trp to this unknown indole product, which was isolated and identified as Trp-ol.

The quantitative study showed that, when Trp was used as substrate, much larger amounts of Trp-ol were produced in comparison to IAA. The formation of Trp-ol by microorganisms has been reported (2, 7, 11, 14, 15, 22, 25, 27, 29, 38, 40), and its formation in greater amounts than IAA has also been observed before (14, 15, 25, 39, 40). Aeration rates are known to influence the ratios of IAA to Trp-ol (39, 40). In cell-free preparations of plant tissue, pH affected the ratios of IAA to Trp-ol produced from I-acetald (39). This finding indicated that two enzymes were capable of utilizing I-acetald—one which produces Trp-ol and a second which produces IAA. Two crude enzyme preparations were obtained which were capable of selectively forming either Trp-ol or IAA. The existence of two such separate enzymes in microorganisms has not yet been demonstrated.

ILA has also been reported as a major Trp-degradation product of yeasts (15, 16), where only minor amounts of Trp-ol and IAA were obtained. These results suggest that, in yeasts, the major pathway for Trp degradation involves transamination as the initial step and subsequent formation of I-acetald and other products as shown in Fig. 1. Dialyzed cell-free extracts from yeast have been shown to catalyze the transfer of the amino group from several amino acids, including Trp, to alpha-ketoglutarate (4). Pyridoxal has been shown to facilitate the reaction. Others have demonstrated that proposed intermediates such as IPA (14) and I-acetald (38, 40) serve well as substrates for yeasts in the formation of ILA, Trp-ol, and IAA.

The possible intermediacy of IPA, I-acetald, and Trp-amine in the degradation of Trp was tested by feeding these compounds to Z. priorianus and S. cerevisiae. I-Acetald served well as a substrate for these yeasts and was metabolized to Trp-ol and IAA in proportions similar to those obtained when Trp was used as substrate. IPA-containing cultures produced much larger amounts of IAA than Trp-ol and, by estimation, the ratio of IAA to Trp-ol was about 4. However, controls containing only culture medium and IPA were found to yield large amounts of IAA simply by agitating the solutions on the rotary shaker. No Trp-ol was formed in the control flask.

The great instability of IPA in aqueous media has been reported before (14, 15, 26, 29), and it has been shown that IPA spontaneously decomposes to IAA under acidic conditions (26), with as many as seven different spots on chromatograms under alkaline conditions (15, 29). In controlled experiments, it has been shown that IPA does convert to Trp-ol in yeasts (15).

Trp-amine was not metabolized to Trp-ol and IAA but was converted into N-acetyltryptamine by Z-priorianus and S. cerevisiae. Trp-amine is converted into its N-acetyl derivative by Bacillus cereus (34). Other microorganisms have been reported to N-acetylate D-tryptophan (16) and other amino acids (57). Ehrlich suggested that amines may represent the intermediates in the formation of Trp-ol, tyrosol, and other alcohols formed from amino acids (12). Others have demonstrated that Trp-amine is converted to Trp-ol and IAA by several microorganisms (8, 9). It is apparent that Trp-amine does not serve as a substrate in the formation of Trp-ol and IAA with Z. priorianus and S. cerevisiae.

N-Acetyltryptamine arises as an artifact dur-
ing ethyl acetate extraction of Trp-amine from biological samples, especially at alkaline pH (21). Our metabolite was isolated by ethyl acetate extraction at pH 2. A control study showed that N-acetyltryptamine could not have been formed by our extraction procedure.

Of a large number of organisms tested, the yeasts possessed the highest capacity for degrading Trp to Trp-ol. Only 9 of 144 microorganisms were found to produce Trp-ol when incubated with Trp. Rajagopal incubated 11 microorganisms and several plant tissues with I-acetal under aerobic conditions and showed that all of these living systems formed Trp-ol and that all of the microorganisms produced IAA as well (38). We used several microorganisms from within the same genera and orders as those in Rajagopal’s study and obtained Trp-ol only from the yeasts. We found no evidence indicating that other extractable indole metabolites were formed by microorganisms which did not produce Trp-ol. It is likely that these cultures either did not metabolize the Trp substrate or that they degraded it via other metabolic routes such as the kynurenine pathway.

*Z. priorianus* and *S. cerevisiae* metabolized L-phenylalanine, Tyr, and L-5,OH-Trp to their respective alcohol and acid derivatives. In agreement with other studies, these compounds accumulated in media without evidence of further degradation (1, 7, 27). Phenethyl alcohol and tryptophol accumulated in fermentations of *Candida albicans* (27), and tyrosol, Trp-ol, phenethyl alcohol, and histidol were formed by other yeasts (1, 7).

The conversion of 5-OH-Trp to 5-OH-Trp-ol and 5-OH-IAA is of significance because of the current interest in certain derivatives of Trp-ol which possess physiological activity, and are currently in demand for pharmacological research (13, 51). These fermentation systems could be used in the preparation of various substituted Trp-ols using available Trp derivatives as starting materials. It is noteworthy that *Z. priorianus* and *S. cerevisiae* were capable of converting the cheaper DL-5,OH-Trp into 5-OH-Trp-ol and 5-OH-IAA.

**ACKNOWLEDGMENTS**

Two of the authors, R.J. and P.D., acknowledge partial support as National Science Foundation summer fellows under grant GY 8734.

**LITERATURE CITED**

1. Ayrapaa, T. 1965. Formation of phenethyl alcohol from 3H-labeled phenylalanine. *J. Inst. Brewing* 71:341-347, Chem. Abstr. 63:14005a.
2. Bailey, G. B., and A. C. Gentile. 1962. Indole compounds synthesized by *Diploida natalensis*. Plant. Physiol. 37:439-445.
3. Barbetta, M., G. Casnati, A. Pochini and A. Selva. 1969. Norepinephrine: A new indole metabolite from *Aspergillus amstelodami*. Tetrahedron Letters, 1969:457-460.
4. Bigger-Gehring, L. 1955. Transamination reactions in *Saccharomyces fragilis*. J. Gen. Microbiol. 13:45-53.
5. DeMoss, R. D. 1967. Violacein. p. 77-81. In D. Gottlieb and F. D. Shaw (ed.), Antibiotics, vol. 2. Springer-Verlag, Berlin.
6. DeMoss, R. D., and K. Moser. 1969. Tryptophanase in diverse bacterial species. J. Bacteriol. 98:167-171.
7. Drews, B., H. Specht, and E. Schwartz. 1966. Über die bei der alkoholischen Gärung der Hefe entstehenden aromatischen Alkohole. Monatshr. Brau. 19:76-87.
8. Dvonikova, T. P., G. K. Skryabin, and N. N. Suvarov. 1968. Metabolism of tryptamine by *Aspergillus niger*. Mikrobiologiya 37:229-232, Chem. Abstr. 69:17012c.
9. Dvonikova, T. P., G. K. Skryabin, and N. N. Suvarov. 1970. Enzymic transformation of tryptamine by fungi. Mikrobiologiya 39:42-46, Chem. Abstr. 72:118652d.
10. Ehrlich, F. 1906. Über die Bedingungen der Pflanzenbildung und über ihren Zusammenhang mit dem Eiweißverbrauch der Hefe. Chem. Ber. 39:1027-1047.
11. Ehrlich, F. 1912. Über Tryptophol (N-Indolylylalcohol) ein neues Geparprodukt der Hefe aus Amiunösuren. Chem. Ber. 45:883-889.
12. Ehrlich, F., and P. Postchikumika. 1912. Überführung von Aminen in Alkohole durch Hefen- und Schimmelpilze. Chem. Ber. 45:1006-1012.
13. Feldstein, A., F. H. Chang, and J. M. Kurcharski. 1970. Tryptophol, 5-hydroxytryptophol and 5-methoxytrypophol induced sleep in mice. Life Sci. 9:323-329.
14. Glombitza, K. W. 1965. Dunschichtchromatographischer Nachweis der Indolyl-3-Benztraubensäure aus Biologischem Material. J. Chromatog. 19:320-325.
15. Glombitza, K. W., and T. Hartmann. 1966. Der Tryptophanabbau bei *Endomycopsis vernalis* und anderen Hefen. Planta 96:135-149.
16. Hagemann, F. 1965. Untersuchungen zum Tryptophanabbau dreier Hefearten. Naturwissenschaften 52:626-627.
17. Harbo, A., and T. Aasheim. 1962. A spectrophotometric method for the identification and assay of tryptophol and other indole derivatives. Physiol. Plant. 15:546-551.
18. Harris, G. 1958. Nitrogen metabolism, p. 438-533. In H. H. Cook (ed.), The chemistry and biology of yeasts. Academic Press Inc., New York.
19. Hoch, J. A., and R. D. DeMoss. 1965. Physiological effects of a constitutive tryptophanase in *Bacillus alrei*. J. Bacteriol. 90:604-610.
20. Hunter, J. H., and J. A. Hogg. 1949. Synthetic steroids. III. Isomers of 1-ethyl-2-methyl-7-methoxy-1,2,3,4,9,10,11,12-octahydrophenanthrene-2-carboxylic acid. J. Amer. Chem. Soc. 71:1922-1924.
21. Hutzinger, O. 1969. N-Acetyltryptamine: an artifact formed during the extraction of tryptamine. Anal. Biochem. 30:153-156.
22. Kaper, J. M., and H. Veldstra. 1958. Metabolism of tryptophan by *Agrobacterium tumefaciens*. Biochem. Biophys. Acta 30:401-420.
23. Kaplan, H. U. Hornemann, K. M. Kellery, and H. G. Floss. 1969. Tryptophan metabolism, protein and alkaloid synthesis in saprophytic cultures of the ergot fungus (*Claviceps*). Lloydia 32:489-497.
24. Kelleher, W. J. 1969. Ergot alkaloid fermentations, p. 211-244. In D. Perlman (ed.), Advances in applied microbiology, vol. 11. Academic Press Inc., New York.
25. Larsen, P., A. Harbo, S. Klungsoyr, and T. Aasheim. 1962. On the biogenesis of some indole compounds in *Acetobacter xylinum*. Physiol. Plant. 15:552-565.
26. Libbert, E., K. Brunn, A. Drawert, and R. Schröder.
1970. Pathways of indoleacetic acid production from tryptophan by plants and by their epiphytic bacteria. Metabolism of indolepyruvic acid and indolelactic acid. Biol. Plant. 12:246–255.

27. Lingappa, V. L., B. T., M. Prasad, and Y. Lingappa. 1969. Phenethyl alcohol and tryptophan: autoantibiotics produced by the fungus Candida albicans. Science 163:192–193.

28. Miller, M. W. 1961. The Pfizer handbook of microbial metabolites, p. 458–478. McGraw-Hill Book Co., New York.

29. Mino, Y. 1970. Studies on the destruction of indole-3-acetic acid by a species of Arthrobacter. V. Indole-3-acetic acid production from tryptophan. Physiol. Plant. 23:971–980.

30. Mitoma, C. M., and S. Udenfriend. 1960. A bacterial tryptophan decarboxylase. Biochim. Biophys. Acta 37:356–357.

31. Mitoma, C., H. Weissbach, and S. Udenfriend. 1966. 5-Hydroxytryptophan formation and tryptophan metabolism in Chromobacterium violaceum. Arch. Biochim. Biophys. 63:122–130.

32. Nakazawa, H., H. Enei, S. Ikumura, H. Yoshida, and H. Yamada. 1972. Enzymatic preparation of L-tryptophan, and 5-hydroxy-L-tryptophan. FEBS Lett. 25:43–45.

33. Otakuro, H., and K. Innouye. 1964. Synthesis of peptides related to the N-terminal structure of corticotropin. III. The synthesis of L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophan, the smallest peptide exhibiting the melanocyte-stimulating and the lipolytic activities. Bull. Chem. Soc. Jap. 37:1465–1469.

34. Perley, J. E., and B. B. Stowe. 1966. The production of tryptamine by Bacillus cereus (KVT). Biochim. J. 100:169–174.

35. Pillay, D. T. N., and R. Mehdi. 1968. Separation of simple indole derivatives by thin-layer chromatography. J. Chromatogr. 32:582–586.

36. Prasad, C., and V. R. Srivivasan. 1970. Tryptophan catabolism during sporulation in Bacillus cereus. Biochem. J. 119:343.

37. Rainbow, C. J. 1970. Brewer’s yeast, p. 179–199. In A. H. Rose and J. S. Harrison (ed.), The yeasts, vol. 3. Academic Press Inc., New York.

38. Rajagopal, R. 1967. Metabolism of indole-3-acetaldehyde. I. Distribution of indoleacetic acid and tryptophan forming activities in plants. Physiol. Plant. 20:882–900.

39. Rajagopal, R. 1968. Metabolism of indole-3-acetaldehyde. II. On dismutation. Physiol. Plant. 21:1076–1096.

40. Rigaud, J. 1970. La biosynthése de l’acide indoly1-3-acétique en liaison avec le metabolisme du tryptophol et de l’indolyl-3-acetaldehyde chez Rhizobium. Physiol. Plant. 23:171–178.

41. Rohman, C., and D. Miesel. 1961. Uber die Darstellung pharmakologisch interessanter Aminothé. Arch. Pharmazol. 294:538–546.

42. Roth, C. W., J. A. Hoch and R. D. DeMoss. 1971. Physiological studies of biosynthetic indole excretion in Bacillus alvei. J. Bacteriol. 106:97–106.

43. Senteshhannuganathan, S. 1960. The purification and properties of the tyrosine-3-exoglutarate transaminase of Saccharomyces cerevisiae. Biochem. J. 77:619.

44. Senteshhannuganathan, S., and S. R. Elsden. 1958. Formation of tyrosol by Saccharomyces cerevisiae. Biochem. J. 62:210–218.

45. Snyder, A. R., and F. J. Pilgrim. 1948. The preparation of 3-indoleacetic acid: a new synthesis of tryptophol. J. Amer. Chem. Soc. 70:3770.

46. Späth, E., and E. Lederer. 1930. Synthese der Harmala-Alkaloiode: Harmalin, Harmin und Harman. Chem. Ber. 63:120–123.

47. Stanier, R. Y., and O. Hayashi. 1951. The bacterial oxidation of tryptophan: a study in comparative biochemistry. Science 114:336–330.

48. Stanier, R. Y., O. Hayashi, and M. Tsuchida. 1951. The bacterial oxidation of tryptophan. I. A general survey of the pathway. J. Bacteriol. 62:355–366.

49. Stoessel, A., and M. A. Venis. 1970. Determination of submicrogram levels of indole-3-acetic acid. A new highly specific method. FEBS Lett. 23:929–930.

50. Swevarton, J. 1929. Higher alcohols of fermentation. Science 67:554–555.

51. Taborsky, R. G. 1971. 5-Hydroxytryptophol—evidence for its having physiological properties. Experientia 27:929–930.

52. Teuscher, G. 1967. Metabolic pathway of L-tryptophan in Streptomyces species. Phytochemistry 6:141–144.

53. Thorne, R. S. W. 1937. The assimilation of nitrogen from amino acids by yeast. J. Inst. Brew. 43:288–293.

54. Turner, W. B. 1971. Fungal metabolites, p. 315–332. Academic Press Inc., New York.

55. Tyler, V. E., Jr., K. Mothes, D. Gregor, and H. G. Floss. 1964. Conversion of tryptophan to 2,3-dihydroxybenzoic acid by Claviceps paspali/Stevens & Hall. Z. Naturforsch. 20:856.

56. Udenfriend, S., and R. E. Peterson. 1957. Assay of aromatic amino acids. III. Tryptophan, p. 613–614. In S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, Vol. 13. Academic Press Inc., New York.

57. Wallen, L. L., P. H. Stodola, and R. W. Jackson. 1959. Type reactions in fermentation chemistry, p. 441–442, bulletin ARS-71-13. U.S. Department of Agriculture, Washington, D.C.