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Microbial Hydroxylation of Indole Alkaloids

R. E. HARTMAN,[1] E. F. KRAUSE,[2] W. W. ANDRES, AND E. L. PATTERSON

Biochemical Research Section, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York

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ABSTRACT

HARTMAN, R. E. (Lederle Laboratories Division, American Cyanamid Co., Pearl River, N.Y.), E. F. KRAUSE, W. W. ANDRES, AND E. L. PATTERSON. Microbial hydroxylation of indole alkaloids. Appl. Microbiol. **12**:138-140. 1964.—The hydroxylation of the indole-type alkaloids, yohimbine, α-yohimbine, β-yohimbine, and corynanthine, was achieved with several genera of higher fungi and species of *Streptomyces*. Microorganisms were found which monohydroxylated these compounds in three different positions. The site of hydroxylation was strain-specific for two strains of *Cunninghamella echinulata* and *C. bainieri*.

The well-known microbiological transformation of steroids has successfully served as a source of many new compounds. Other complex natural products as well have been examined as substrates for microbiological transformation. For example, the tropane (Niemer, Bucherer, and Kohler, 1959), morphine (Iizuka et al., 1960), ergot (Brack, Brunner, and Kobel, 1962), and indole-type (Godtfredsen et al., 1958; Loo and Reidenberg, 1959; Myers and Pan, 1961; Pan and Weisenborn, 1958) alkaloids have been used for this purpose. This report describes the hydroxylation of some yohimbine-type alkaloids by various genera of higher fungi and by several species of *Streptomyces*.

[1] Present address: Saint Bonaventure University, St. Bonaventure, N.Y.

[2] Present address: Corning Glass Works, Corning, N.Y.

MATERIALS AND METHODS

The alkaloids used were purchased from commercial sources and purified by chromatography and crystallization (Patterson et al., 1963). The cultures are listed in Table 1. Inoculum was prepared by growing each culture for 72 hr at 28 C on a reciprocating shaker in an inoculum-development medium containing 2% corn-steep liquor, 4% cerelose, 1% $(NH_4)_2SO_4$, 0.6% KH_2PO_4, 0.5% $CaCO_3$, and 0.025% of the alkaloid substrate in distilled water at pH 6.2. A 5% inoculum was used to seed the fermentation medium consisting of 2% corn-steep liquor, 2% cerelose, 1% $(NH_4)_2SO_4$, 0.06% Na_2HPO_4, and 0.05% $CaCO_3$ in distilled water at pH 6.2. The fermentation was incubated on a reciprocating shaker at 28 C. After 24 hr, the substrate, as an aqueous, autoclaved (15 min at 121 C) stock solution of the hydrochloride salt, was added to a final concentration of 0.2 mg/ml. The fermentation procedure utilizing *S. platensis* differed from the above procedure in that the inoculum-development and fermentation media consisted of 2% molasses, 1.5% soybean meal, 0.1% casein, and 0.5% sodium chloride in distilled water, and a rotary shaker was used to provide aeration. Controls were run in which either no substrate or no inoculum was added.

Routinely, the transformation products in the fermentations were detected by descending paper chromatography carried out at room temperature on 0.5-in. wide Whatman no. 1 paper strips. Two solvent systems were utilized: toluene–ethanol–triethylamine (100:30:0.5) and *n*-butanol

TABLE 1. *Microbial hydroxylation of yohimbine and some of its optical isomers*

Culture		Position hydroxylated			
Species	Strain	Yohimbine	α-Yohimbine	β-Yohimbine	Corynanthine
Calonectria decora	CBS Wollenweber	18α	None	None	None
Cunninghamella bainieri	ATCC 924	10	10	10	10
C. bainieri	Campbell X-48	11	11	Trace*	Trace*
C. bertholletiae	NRRL A-11497	11	11	None	11
C. blakesleeana	ATCC 8688a	10	10	None	None
C. echinulata	NRRL A-11498	10	10	10	10
C. echinulata	U. of Wisconsin	11	11	Trace*	Trace*
C. elegans	NRRL A-11499	11	11	Trace*	Trace*
Streptomyces platensis	NRRL 2364	10	10	10	10
S. rimosus	NRRL 2234	10	None	10	10
S. fulvissimus	NRRL B-1453	None	11	None	11

* A transformation product which was phenolic was detected but in such low yield that further characterization was inconclusive.

–toluene–acetic acid–water (200:100:4:100). Samples (10 ml) were removed 3, 6, and 10 days after the addition of substrate. These were rendered alkaline by the addition of 1 ml of concentrated ammonium hydroxide, and were extracted with 20 ml of ethyl acetate. After removal of the solvent by distillation at reduced pressure, the residue from each extract was dissolved in 0.2 ml of chloroform and a 5-μliter sample was chromatographed.

The compounds were detected on the developed chromatogram by two methods. One consisted of visual inspection upon exposure to ultraviolet light (Haines and Karnemaat, 1954), which revealed their fluorescence. When viewed through a zinc sulfide screen, the phenolic transformation products appeared black and the others, as well as the starting substrates, were purple. The second method depended on the reaction with an acidic potassium ferricyanide spray consisting of 7% acetic acid–1% ferric sulfate–0.4% potassium ferricyanide (1:1:1; Barton, Evans, and Gardner, 1952). All products and substrates formed blue spots on a white background. The phenolic transformation products were distinguished from the other products and the substrates in that they reacted rapidly with the acidic potassium ferricyanide spray reagent.

The ultraviolet-absorption spectra served as further identification of the products (Patterson et al., 1963). For this, samples of the fermentation filtrates at pH 9 were extracted with 1 volume of ethyl acetate. The material in the extract was further purified by partition chromatography in the system, ethyl acetate–*n*-butanol–1 N acetic acid (75:25:100).

RESULTS

Table 1 contains the data concerning the hydroxylation by microorganisms of yohimbine (Fig. 1) and its optical isomers, α-yohimbine, β-yohimbine, and corynanthine.

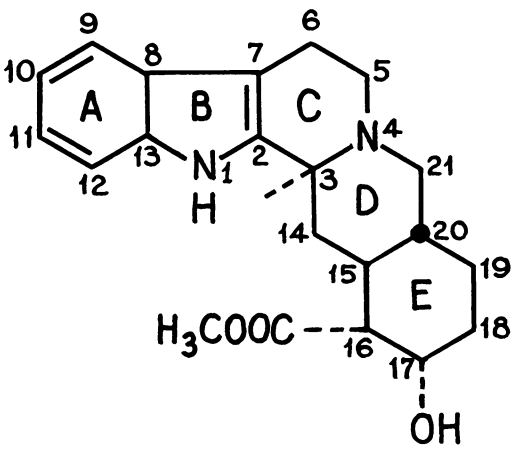

FIG. 1. *Yohimbine.*

Among the cultures that hydroxylated in the 10-position, differences in substrate specificity were observed. *Cunninghamella blakesleeana* hydroxylated yohimbine and α-yohimbine; *S. rimosus* transformed yohimbine, β-yohimbine, and corynanthine; and the remaining 10-hydroxylating cultures transformed all of the isomers to at least a small degree. Loo and Reidenberg (1959) previously isolated 10-hydroxy-yohimbine after fermentation of yohimbine by the strain of *S. platensis* used in this study. 10-Hydroxy-yohimbine was detected by Myers and Pan (1961) in fermentations of *S. rimosus*, *C. cunninghamella*, and *C. bainieri* to which yohimbine was added.

S. fulvissimus, which hydroxylated only α-yohimbine, was the only culture that showed a substrate specificity among the cultures that hydroxylated in the 11-position.

Calonectria decora, the one culture that produced 18α-hydroxy-yohimbine, was not able to hydroxylate the other isomers. *Cunninghamella bertholletiae*, which 11-hydroxylated yohimbine, did not modify 18α-hydroxy-yohimbine.

The site of hydroxylation of the yohimbine-type alkaloids was strain-specific, and was apparently also dependent on the fermentation conditions. With the two strains of *C. bainieri* and *C. echinulata*, one hydroxylated in the 10-position and the other in the 11-position. Although the strain of *S. rimosus* used in this study was the same as that reported by Pan and Weisenborn (1958) to 18α-hydroxylate yohimbine and α-yohimbine, under the conditions employed in this study only the 10-hydroxy derivative was isolated when yohimbine was the substrate, and α-yohimbine was not altered.

DISCUSSION

The concentration of the transformation products in most of the fermentations was low. At harvest, a considerable amount of starting material remained, and there was a net loss of alkaloid, indicating nonspecific destruction.

The most common transformation was 10-hydroxylation with respect to both the cultures and the substrates.

In general, the cultures that hydroxylated in the 10-position and the 11-position transformed yohimbine and α-yohimbine to a greater extent than β-yohimbine and corynanthine. No product was observed other than a monohydroxylated derivative. This suggested that the hydroxylated derivatives were not ready substrates, or that the more highly oxidized products which may have been formed were destroyed under the fermentation conditions used.

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