Microbial Transformation of Aspidospermine

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Abstract □ Thirty actinomycetes were isolated from soil samples and screened for their ability to modify the structure of aspidospermine. One actinomycete culture converted aspidospermine into *O*-demethylaspidospermine but failed to modify *N*-deacetylaspidospermine, *N*-ethyl-*N*-deacetylaspidospermine, 7-methoxyindole, and 7-methoxytryptophan.

Keyphrases □ Aspidospermine—structural transformation (demethylation) by actinomycetes, UV, IR, and NMR spectral identification of conversion products □ Alkaloids—structural transformation by actinomycetes, UV, IR, and NMR spectral identification of conversion products □ Actinomycetes—structural transformation of aspidospermine, UV, IR, and NMR spectral identification of conversion products

The enzyme systems of microorganisms have been used to transform numerous organic compounds including steroids and other biologically active substances such as alkaloids, flavonoids, terpenoids, and antibiotics. Although interest in the modification of alkaloid structures by microorganisms seems to be increasing, the volume of work in this field is still not large. Typical alkaloids that have been been converted microbiologically include conessine (1), agroclavine (2), 14β-bromocodeinone (3), sparteine (4), nicotine (5), and indoles such as yohimbine (6) and vindoline (7).

These successes in modifying alkaloid structures and the unique advantages of microbial conversions (*e.g.*, reactions at chemically inactive positions, stereospecific substitutions, coupling of several reactions, and mild reaction conditions) led to the investigation of microbial transformations of other alkaloids.

Thirty actinomycete cultures were screened for their ability to modify indole alkaloids. One effectively modified aspidospermine, and the major transformation product was isolated and characterized. This report is the first in the literature concerning the cleavage of a phenyl methyl ether bond of an alkaloid by a microorganism.

EXPERIMENTAL[1]

Materials and Methods—The 30 actinomycetes employed in the screening work were isolated by the usual method (8) from soil samples collected locally. The pure cultures were maintained on sucrose nitrate slants. A crude aspidospermine fraction, previously obtained in this laboratory from the bark of *Aspidosperma quebracho-blanco* Schlecht (Apocynaceae) (9), was purified by recrystallization from ethanol.

N-Deacetylaspidospermine was prepared by acid hydrolysis of aspidospermine (10). *N*-Ethyl-*N*-deacetylaspidospermine was prepared from *N*-deacetylaspidospermine using ethyl iodide (10). 7-Methoxyindole was purchased[2].

Screening Procedures—The microorganisms were grown in 125-ml erlenmeyer flasks in a medium composed of 3% glycerol, 0.35% casamino acid[3], 0.1% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, and a trace of ferrous sulfate heptahydrate. The flasks were incubated on a rotary (240 rpm) shaker at 28°. After 2 days, 1 ml of each culture was transferred to new medium and incubated for 2 days.

Aspidospermine (I) was added to each culture (0.2 mg/ml), and the reaction was allowed to proceed for 48 hr. The culture broths were extracted for alkaloids in the usual (acid–base shakeout) manner, and the crude alkaloid mixtures were chromatographed on silica gel GF_{254} TLC plates and developed with benzene–acetone (1:1). After drying, the plates were sprayed with ceric ammonium sulfate reagent (11), followed by Dragendorff's reagent (12).

One isolate, designated as Culture 14823[4], gave a conversion product (different R_f value and chromogenic reaction) and was used for the larger scale production of this conversion product. Control experiments were performed in which no cultures were added. No transformation took place in these control experiments, indicating that the experiment with Culture 14823 was indeed an enzyme-mediated reaction.

Conversion Procedure in Fermentor—Culture 14823 was grown in a 14-liter stirring fermentor. Aspidospermine (200 mg/liter) was added to the culture fluid, and incubation was continued under the cultivating conditions. Samples of 50 ml each were taken at 8-hr intervals, extracted for alkaloids, and analyzed by TLC. The ratio of the amount of the product formed to that of the substrate remaining was determined semiquantitatively by observing the size and color depth of the spots after spraying with Dragendorff's reagent.

When the conversion appeared maximal (48 hr), the alkaloids were extracted and then purified on a silica gel PF_{254}[5] column by eluting with benzene–acetone (1:1). The major conversion product (32% yield) isolated exhibited the same R_f values in three different TLC solvent systems as did the product obtained from the small flask fermentation. Several additional experiments carried out in the fermentor yielded similar results. A few other fermentation products were detected, but the yields were too minute and no further isolation work was performed.

RESULTS AND DISCUSSION

Identification of Conversion Product—The UV spectrum of the major conversion product showed absorptions at λ_{max} (ethanol): 220 (log ϵ 4.39), 258 (3.93), and 293 (3.54) nm (indicative of a dihydroindole moiety) (9). A bathochromic shift to 308 nm (log ϵ 3.90) in 0.1 *N* KOH in ethanol suggested the presence of a phenolic group.

An IR spectrum of this conversion product taken in potassium bromide showed absorptions at ν 2930, 2780, 1635, 1601, 1580, 1475, 1445, 1380, 1360, 1262, 1182, 1132, 1106, 1062, 935, 770, and 740 cm^{-1}. The mass spectrum of the conversion product showed a molecular ion, M^+, at *m/e* 340 (25); other significant ions were observed at *m/e* 339 (20.0), 312 (8.3), 160 (4.1), 152 (5.4), 146 (3.3), and 124 (100.0). Thus, the conversion product showed the same base peak at *m/e* 124 as well as a common peak at *m/e* 152 as the substrate aspidospermine (13), indicating that the nonindole portion of both molecules is the same.

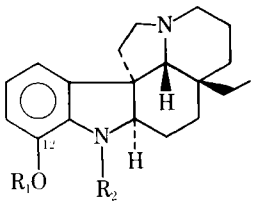
[1] The fermentor used was "Magnaferm" model MA-100 from New Brunswick Scientific Co., New Brunswick, N.J. UV spectra were taken using a Beckman model DB-G grating spectrophotometer, and spectra were examined from 200 to 340 nm. IR spectra were taken using a Beckman IR 18A spectrophotometer, and spectra were recorded from 4000 to 600 cm^{-1}. Mass spectra were determined using a single-focusing Hitachi Perkin-Elmer model RMU-6D mass spectrometer, operating at 70 ev. Proton NMR spectra were taken using a Varian A-60 instrument operating at 60 MHz.

[2] Aldrich Chemical Co. Inc., Milwaukee, Wis.

[3] Difco Laboratories, Detroit, Mich.

[4] Culture 14823 was identified by Dr. T. G. Pridham, Agricultural Research Service, Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Ill., as a strain of subspecies of *Streptomyces griseus* (Krainsky) Waksman and Henrici.

[5] E. Merck, Darmstadt, West Germany.



I: $R_1 = CH_3$, $R_2 = COCH_3$
II: $R_1 = H$, $R_2 = COCH_3$
III: $R_1 = CH_3$, $R_2 = H$
IV: $R_1 = CH_3$, $R_2 = CH_2CH_3$

The molecular ion of the conversion product was at 14 mass units less than that observed for aspidospermine. This finding and the bathochromic shift observed in the UV spectrum suggested that the methoxy at C-12 of aspidospermine was converted to a hydroxyl. If so, the remaining peaks at *m/e* 339, 312, 160, and 146 can be readily identified to be the corresponding peaks of *m/e* 353, 325, 174, and 160, respectively, observed in the spectrum of aspidospermine (13).

An NMR spectrum in deuterochloroform, with tetramethylsilane as an internal standard, confirmed the conversion product to be *O*-demethylaspidospermine (II). An intramolecularly hydrogen-bonded phenolic proton at C-12 appeared as a singlet at δ 10.89, three aromatic protons appeared as a multiplet centered at δ 6.90, the proton at C-2 appeared as a quartet at δ 4.15, the *N*-acetyl group appeared as a three-proton singlet at δ 2.35, and the three protons of the ethyl side chain appeared as a triplet at δ 0.67. The methoxy proton present in the NMR spectrum of aspidospermine at δ 3.89 was absent in the spectrum of the conversion product.

The IR and NMR spectra were superimposable with those of semisynthetic *O*-demethylaspidospermine, prepared according to the method of Witkop and Patrick (10) from aspidospermine.

It appears that the enzyme systems in Culture 14823 cleave the phenyl methyl ether linkage without affecting the neighboring acetyl group. To determine if this type of reaction could be applied to other compounds with the phenyl methyl ether moiety, the substrate specificity of the enzyme system was investigated. Culture 14823 was tested for its ability to transform the following compounds utilizing the same screening procedure: 7-methoxyindole, 7-methoxytryptophan[6], *N*-deacetylaspidospermine, and *N*-ethyl-*N*-deacetylaspidospermine. No significant transformations occurred.

SUMMARY

Aspidospermine was demethylated during the fermentation process by Culture 14823. The phenyl methyl ether bond is stable and requires drastic chemical means for fission, *e.g.*, heating with aluminum chloride at 140° or fusion with sodium hydroxide above 240° (10). However, the cleavage of this bond can be achieved by enzymatic action under very mild conditions.

This culture failed to demethylate *N*-deacetylaspidospermine, *N*-ethyl-*N*-deacetylaspidospermine, 7-methoxyindole, and 7-methoxytryptophan. Since these substances all lack the acetyl group on the indole nitrogen, this group may be essential for this enzymatic reaction to take place.

A literature search revealed that only a few examples of the microbial cleavage of phenyl methyl ether bonds have been reported, *e.g.*, demethylation of griseofulvin by fungi (14), of vanillic acid by bacteria (15), and of 7-methoxyisoflavone by *Penicillium cyclopium* (16).

[6] Supplied by Dr. A. Kalir, Department of Organic Chemistry and Pharmacology, Israel Institute for Biological Research, Ness-Zionn, Israel.

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