Conversion of Agroclavine to Setoclavine and Isosetoclavine in Cell-Free Extracts from *Claviceps* sp. SD 58 and in a Thioglycolate–Iron (II) System

RAGHBIR S. BAJWA and JOHN A. ANDERSON ˣ

Abstract □ Agroclavine was converted to setoclavine and isosetoclavine in crude extracts from *Claviceps* sp. SD 58. The ratio of setoclavine to isosetoclavine was 0.95. The conversion with boiled crude extract was 68% of the conversion with unboiled extract. In a thioglycolate–iron (II) system at 40° for 5 hr, 45.5% of agroclavine was converted to setoclavine and isosetoclavine. At 70° for 4 hr in the thioglycolate–iron (II) system, 4% of 4-dimethylallyltryptophan was converted to clavicipitic acid.

Keyphrases □ Agroclavine—conversion to setoclavine and isosetoclavine, cell-free extracts from *Claviceps* sp. SD 58 and thioglycolate–iron (II) □ Setoclavine and isosetoclavine—converted from agroclavine, cell-free extracts from *Claviceps* sp. SD 58 and thioglycolate–iron (II) □ Thioglycolate–iron (II)—conversion of agroclavine to setoclavine and isosetoclavine □ *Claviceps* sp. SD 58—conversion of agroclavine to setoclavine and isosetoclavine

Clavine alkaloid-producing strains of ergot fungus produce setoclavine, isosetoclavine, penniclavine, and isopenniclavine. These alkaloids are derived from agroclavine and elymoclavine (1). Beliveau and Ramstad (2) proposed that the hydroxylation was nonenzymatic, since both isomers, *i.e.*, setoclavine–isosetoclavine and penniclavine–isopenniclavine, were formed in most fungal species that carried out the conversion.

Shough and Taylor (3) showed that horseradish peroxidase in the presence of hydrogen peroxide could catalyze the 8-hydroxylation of agroclavine and elymoclavine. However, Jindra *et al.* (4) reported that *Claviceps* sp. SD 58 had high catalase activity but contained very little peroxidase. Wilson *et al.* (5) observed that mammalian cytochrome P-450 converted agroclavine to setoclavine and isosetoclavine in addition to other products, and NADPH was required as a cofactor.

This article reports the 8-hydroxylation of agroclavine and elymoclavine in a thioglycolate–iron (II) system, which may resemble the system for nonenzymatic formation of setoclavine, isosetoclavine, penniclavine, and isopenniclavine, in cultures of *Claviceps* sp. SD 58.

EXPERIMENTAL[1]

Materials—Radioactive agroclavine and elymoclavine were prepared by feeding ^{14}C-methylene-tryptophan to *Claviceps purpurea* PRL 1980. After 5 days of growth in mannitol medium (6) supplemented with 400 mg/liter L-tryptophan and 2.0 m*M* niacinamide, 100 μCi of ^{14}C-tryptophan was added to 25 ml of culture. After 15 days, the culture was homogenized and filtered. The residue was washed with water, and washings and the filtrate were extracted with ammonia–ether.

The ether extract was extracted with 0.1 *N* sulfuric acid, and the aqueous extract was made basic with sodium carbonate and then extracted with ether. The ether extract was evaporated to dryness, and the residue was dissolved in ethanol. The extract was fractionated on TLC with ethyl acetate–acetone–dimethylformamide (5:5:1).

Agroclavine and elymoclavine were further purified by preparative TLC, using chloroform–methanol (4:1) and chloroform–diethylamine (9:1) solvents. Setoclavine and isosetoclavine were also separated from the alkaloid extract by preparative TLC as described for the incubated systems.

4-Dimethylallyl-[3-^{14}C]-tryptophan was synthesized according to procedures of Plieninger and coworkers (7–9). The decarboxylation of 4-bromoindole-2-carboxylic acid was carried out as described by Kohler and Anderson (10). ^{14}C-Formaldehyde was used in the conversion of 4-dimethylallylindole to the gramine. The specific activity of the final product was 4.9 μCi/μmole.

Procedures—*Claviceps* sp. SD 58 was grown in yeast extract–mannitol medium NL-406 (11) for 10–16 days, and the culture was filtered on a buchner funnel. The cells were washed with 0.02 *M* phosphate buffer (pH 7.0), suspended in the same buffer, and homogenized[2] for 3–6 min at maximum speed. The mixture was centrifuged at 10,000×*g* for 20 min, and the supernate (crude extract) was collected. All operations for preparation of the crude extract were carried out at 0–5°.

The incubation mixture consisted of crude extract (7.0 mg/ml), 1.9×10^{-4} *M* agroclavine (8.4 μCi), and 1.0 mg streptomycin sulfate in a final volume of 2.2 ml. Crude extract and chemically defined mixtures were incubated on a shaker[3] at 25°, unless another temperature is indicated.

After incubation, alkaloids were extracted as described for the preparation of labeled alkaloids, and preparative TLC was carried out with chloroform–triethylamine (9:1). After elution of setoclavine and isosetoclavine with 1.0% ammonium hydroxide in ethanol, the samples were dried, dissolved in 0.5% 2,5-diphenyloxazole in toluene, and counted[4]. The percent conversion was the percent of radioactivity applied to the TLC plate recovered from the region of the plate containing setoclavine or isosetoclavine. Radioactive penniclavine and isopenniclavine were fractionated by preparative TLC with chloroform–methanol (4:1).

Conversion of agroclavine to setoclavine and isosetoclavine in the chemically defined systems was also measured fluorometrically. After incubation, 8 ml of methanol was added to 2 ml of incubation mixture. The fluorescence was then measured[5] with excitation at 325 nm and emission at 425 nm. These wavelengths corresponded to the excitation and emission maxima. Reference setoclavine and isosetoclavine had excitation and emission maxima at the same wavelengths. The intensity of fluorescence correlated well with the amounts of setoclavine and isosetoclavine estimated visually by TLC.

After reaction of dimethylallyl-[3-^{14}C]-tryptophan in the thioglycolate–iron (II) system, the incubation mixture was adjusted to

[1] *Claviceps* sp. SD 58 was provided by Dr. J. E. Robbers and reference clavicipitic acid was provided by Dr. H. G. Floss of Purdue University, La-Fayette, Ind. Reference setoclavine, isosetoclavine, and penniclavine were gifts from Dr. Von Wartburg and Dr. Stadler, Sandoz Ltd., Basle, Switzerland.

[2] Virtis "45" homogenizer.

[3] *Dubnoff.*

[4] Beckman model L 200 liquid scintillation counter.

[5] Aminco-Bowman spectrophotofluorometer.

Table I—Effect of pH on Conversion of Agroclavine to Setoclavine and Isosetoclavine in Thioglycolate–Iron (II) System[a]

pH	Conversion, %	
	Setoclavine	Isosetoclavine
4.0	20.7	19.7
4.5	22.8	22.7
5.0	18.9	18.3
5.5	15.1	13.9
6.0	14.5	14.6
7.0	9.1	8.3
8.0	7.6	6.6
9.0	7.2	7.6
5.5[b]	1.7	3.6

[a] The incubation mixture contained 3.5×10^{-4} *M* ^{14}C-agroclavine (0.064 μCi), 4.0×10^{-4} *M* iron (II) sulfate, 0.02 *M* sodium thioglycolate, and buffer in final volume of 2.4 ml. Sodium acetate buffer (0.5 *M*) was used for pH 4.0, 4.5, 5.0, and 5.5, 0.2 *M* sodium phosphate buffer was used for pH 6.0, 7.0, and 8.0, and 0.2 *M* tromethamine hydrochloride buffer was used for pH 9.0. The reaction was carried out for 5 hr at 40°. [b] Buffer only.

pH 5.0 with dilute hydrochloric acid and passed through a column of cation-exchange resin[6]. Alkaloids and amino acids were eluted from the resin with 5% ammonium hydroxide solution. The eluate was dried under vacuum on a rotary evaporator, and the residue was dissolved in 0.5 ml of 95% ethanol containing 1.0% ammonium hydroxide for TLC.

RESULTS AND DISCUSSION

The percent of setoclavine and isosetoclavine of the total alkaloid extract from cultures of *Claviceps* sp. SD 58 was 1.42 and 1.79%, respectively. The percent conversion of agroclavine to setoclavine was 1.87% with crude extract and 1.12% with crude extract that had been boiled for 10 min. The percent conversion of agroclavine to isosetoclavine was 1.97% with crude extract and 1.48% with boiled crude extract.

Several systems were tested for 8-hydroxylation of agroclavine, and the most effective system was thioglycolate[7]–iron (II). Under optimum conditions, conversion to setoclavine and isosetoclavine combined was 45.5% (Table I). The conversion was favored at low pH. The ratio of setoclavine–isosetoclavine was 1 throughout the pH range of 4.0–9.0. ^{14}C-Elymoclavine was converted to penniclavine (5.8%) and isopenniclavine (6.0%) in this system.

The efficient conversion of agroclavine to setoclavine and isosetoclavine in a nonenzymatic system, the ratio of setoclavine–isosetoclavine near 1 in the cultures as well as in crude extracts, and the stability of most activity to boiling indicate that the majority of the formation of setoclavine and isosetoclavine in cultures of *Claviceps* sp. SD 58 is nonenzymatic.

Dimethylallyl-[3-^{14}C]-tryptophan was also incubated in the thioglycolate–iron (II) system. At 40°, there was little conversion of dimethylallyltryptophan to Van Urk's positive products. The concentrations of thioglycolate and iron (II) were varied, and higher temperatures were used to increase conversion. Conditions that appeared to give the best conversion to Van Urk's positive products were 4.0×10^{-3} *M* sodium thioglycolate, 2.0×10^{-4} *M* iron (II) sulfate, 3.8×10^{-4} *M* dimethylallyl-[3-^{14}C]-tryptophan (0.2 μCi), and 0.5 *M* sodium acetate (pH 4.0) in a final volume of 2.2 ml incubated for 4 hr at 70°.

The products were fractionated on cation-exchange resin and on TLC with developing solvents of chloroform–methanol–acetic acid (70:20:5 and 8:4:1) and methyl acetate–isopropanol–ammonia (45:35:20). A radioactive spot corresponded to reference clavicipitic acid in these three solvents. The percent conversion to clavicipitic acid was 4.0%. Fifty percent of the radioactivity was recovered in a number of spots below dimethylallyltryptophan on TLC.

The 8-hydroxylation of agroclavine and elymoclavine and the conversion of dimethylallyltryptophan to clavicipitic acid in the thioglycolate–iron (II) system probably proceed by similar mechanisms. A reasonable initial step would be radical formation through removal of the allylic hydrogen. Activation at the allylic carbon of dimethylallyltryptophan is consistent with the recently revised structure of clavicipitic acid, in which a C—N bond connects the α-amino nitrogen and the allylic carbon (12). Further studies are necessary to determine the reactions involved in formation of the products in this system.

In contrast to the failure to obtain significant enzymatic 8-hydroxylation of agroclavine and elymoclavine, dimethylallyltryptophan is actively converted to clavicipitic acid in extracts from *Claviceps* sp. SD 58[8]. The thioglycolate–iron (II) and related systems should be useful models for the enzymatic reaction.

REFERENCES

(1) S. Agurell and E. Ramstad, *Arch. Biochem. Biophys.*, **98**, 457 (1962).

(2) J. Beliveau and E. Ramstad, *Lloydia*, **29**, 234(1966).

(3) H. R. Shough and E. H. Taylor, *ibid.*, **32**, 315(1969).

(4) A. Jindra, E. Ramstad, and H. G. Floss, *ibid.*, **31**, 190(1968).

(5) B. J. Wilson, E. Ramstad, I. Jansson, and S. Orrenius, *Biochim. Biophys. Acta*, **252**, 348(1971).

(6) W. A. Taber, *Develop. Ind. Microbiol.*, **4**, 295(1963).

(7) H. Plieninger, T. Suehiro, K. Suhr, and M. Decker, *Chem. Ber.*, **88**, 370(1955).

(8) H. Plieninger and H. Sirowej, *ibid.*, **104**, 2027(1971).

(9) H. Plieninger, H. Sobel, and V. Liede, *ibid.*, **96**, 1628(1963).

(10) R. D. Kohler and J. A. Anderson, *Synthesis*, **1974**, 277.

(11) H. Kaplan, U. Hornemann, K. M. Kelly, and H. G. Floss, *Lloydia*, **32**, 489(1969).

(12) G. S. King, P. G. Mantle, C. A. Szczyrbak, and E. S. Waight, *Tetrahedron Lett.*, **1973**, 215.

ACKNOWLEDGMENTS AND ADDRESSES

Received June 6, 1974, from the *Department of Chemistry, Texas Tech University, Lubbock, TX 79409*

Accepted for publication September 11, 1974.

The financial support of the Robert A. Welch Foundation (Grant D-117) and the National Institutes of Health (Grant GM-17830) is gratefully acknowledged.

[x] To whom inquiries should be directed.

[6] Dowex 50(H$^+$) (100–200 mesh), Dow Chemical Co.

[7] Sodium thioglycolate, Sigma Chemical Co.

[8] Unpublished results.