17. Albersheim, P., Neukom, H. and Deuel, H. (1960) *Arch. Biochem. Biophys.* **90**, 46.
18. Albersheim, P., Neukom, H. and Deuel, H. (1960) *Helv. Chim. Acta* **43**, 1422.
19. Avigad, G. (1975) *Methods Enzymol.* **41**, 29.
20. Ernst, W. (1968) *Anal. Chim. Acta* **40**, 161.
21. Trevelyan, W. E., Procter, D. P. and Harrison, J. S. (1950) *Nature* **166**, 444.

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CELL-FREE CONVERSION OF 4-γ,γ-DIMETHYLALLYLTRYPTOPHAN TO 4-[4-HYDROXY-3-METHYL-Δ²-BUTENYL]-TRYPTOPHAN IN *CLAVICEPS PURPUREA* PRL 1980

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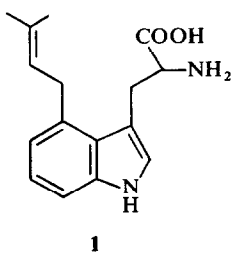
Key Word Index—*Claviceps* sp.; Clavicipitaceae; ergot; enzymatic study; ergot alkaloids; 4-dimethylallyltryptophan; 4-[4-hydroxy-3-methyl-Δ²-butenyl]-tryptophan.

Abstract—The conversion of 4-γ,γ-dimethylallyltryptophan to 4-[4-hydroxy-3-methyl-Δ²-butenyl]-tryptophan was catalyzed by the 60–80% ammonium sulphate fraction from *Claviceps purpurea* PRL 1980. The conversion was stimulated by NADPH. Two major unidentified products in the incubation mixture were not significantly incorporated into elymoclavine when they were added to cultures of *C. purpurea* PRL 1980.

INTRODUCTION

Although 4-γ,γ-dimethylallyltryptophan (DMAT) (**1**) has been established as the first intermediate in ergot alkaloid biosynthesis [1, 2], the next compound in the pathway has not been determined. Both *cis-trans* isomers of 4-[4-hydroxy-3-methyl-Δ²-butenyl]-tryptophan (HODMAT) (**2**) were converted to elymoclavine but not to agroclavine [3]. HODMAT is therefore not on the main pathway in which agroclavine is converted to elymoclavine [4, 5]. HODMAT was isolated from cultures of *C. purpurea* PRL 1980 [6]. The production of HODMAT indicates an alternate pathway for biosynthesis of elymoclavine which does not include agroclavine as an intermediate. We report the formation of HODMAT from DMAT in an $(NH_4)_2SO_4$ fraction from *C. purpurea* PRL 1980 and the cofactor requirement for the conversion.



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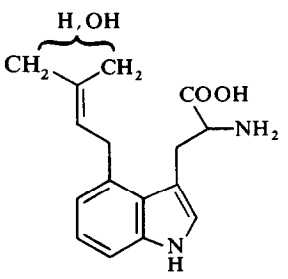

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RESULTS AND DISCUSSION

The HODMAT produced from DMAT with the 60–80% $(NH_4)_2SO_4$ fraction comigrated with reference HODMAT in the two Sil G solvent systems used for the PLC and in the polyamide TLC system. The conversion of DMAT to HODMAT was 0.2% (Table 1). The NADPH-generating system increased the conversion three to four fold. The stimulation of conversion by NADPH addition suggests that the hydroxylation involves a mixed function oxygenase. NADPH-dependent conversion of agroclavine to elymoclavine was previously observed in the 60–80% $(NH_4)_2SO_4$ fraction from *C. purpurea* PRL 1980 [5].

After TLC of the incubation mixture with $CHCl_3$–MeOH–HOAc (10:5:1), two prominent Van Urk's positive spots were observed below DMAT. The lower R_f compound X was fluorescent. The higher R_f compound Y was not. Conversion of DMAT at pH 6.5 was 25% to X and 12% to Y. X and Y were isolated by PLC and fed

Table 1. Conversion of 4-γ,γ-dimethylallyltryptophan (sidechain 3-^{14}C) to 4-[4-hydroxy-3-methyl-Δ²-butenyl]-tryptophan with 60–80% $(NH_4)_2SO_4$ fraction from *Claviceps purpurea* PRL 1980*

Additions	cpm 3-day culture	cpm 5-day culture
	47	52
NADPH-generating system	147	193
Liver concentrate	189	278

*The incubation mixture contained 0.15 μCi ^{14}C-DMAT (1.0 μCi/mg), 2 mg liver concentrate, and in the NADPH-generating system 5 μmol NADP, 5 μmol glucose-6-phosphate and 0.02 units of glucose-6-phosphate dehydrogenase, in 3.9 ml 0.1 M NaPi pH 7. Protein conc 3 mg/ml for the 3-day culture and 5.7 mg/ml for the 5-day culture. Half of the sample was spotted on the Cheng-Chin polyamide sheet for counting.

to *C. purpurea* PRL 1980. The specific incorporation was 0.38% into elymoclavine for both compounds. The specific incorporation of DMAT into elymoclavine in the same experiment was 26%. X and Y are therefore apparently not intermediates in alkaloid biosynthesis.

The conversion of DMAT to HODMAT demonstrated here plus the *in vivo* conversion of HODMAT [3] comprises an alternate pathway to elymoclavine which does not include agroclavine. An alternate pathway from chanoclavine I to elymoclavine has been previously suggested from cell-free studies [7]. The existence of alternate routes increases the number of possible intermediates between DMAT and elymoclavine. This indicates either a correspondingly larger number of enzymes involved or the ability of the enzymes to act on more than one substrate. The contributions of the alternate pathways to the biosynthesis of elymoclavine will be difficult to ascertain until the intermediates between DMAT and chanoclavine I in the main pathway have been determined.

EXPERIMENTAL

Culture conditions for *C. purpurea* PRL 1980 [8], method of synthesis of DMAT (sidechain 3-^{14}C) [9], and method of prepn of the 60–80% $(NH_4)_2SO_4$ fraction [5] were as previously described. Liver concentrate (catalog no. 202-20) was from Sigma. After the cell-free incubation HODMAT was purified with Dowex 50 cation exchange resin, PLC on Sil G with MeAc–isoPrOH–NH_4OH (9:7:5), and then PLC on Sil G with $CHCl_3$–MeOH–HOAc (10:8:5) with 10% formamide added. The sample was then cospotted with reference HODMAT and developed on a Cheng-Chin polyamide sheet with 80% HCO_2H–H_2O (1:2) and a radioautogram was made. The sheet was then either sprayed with Van Urk's reagent or the radioactive HODMAT spot was cut out into 0.5% 2,5-diphenyloxazole and the radioactivity measured with a liquid scintillation counter.

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REFERENCES

1. Plieninger, H., Fischer, R. and Liede, V. (1964) *Ann. Chem.* **672**, 223.
2. Robbers, J. E. and Floss, H. G. (1968) *Arch. Biochem. Biophys.* **126**, 967.
3. Pachlatke, P., Tabacik, C., Acklin, W. and Arigoni, D. (1975) *Chimia* **29**, 526.
4. Agurell, S. and Ramstad, E. (1962) *Arch. Biochem. Biophys.* **98**, 457.
5. Hsu, J. C. and Anderson, J. A. (1971) *Biochim. Biophys. Acta* **230**, 518.
6. Anderson, J. A. and Saini, M. S. (1974) *Tetrahedron Letters* 2107.
7. Ogunlana, E. O., Wilson, B. J., Tyler, V. E. and Ramstad, E. (1970) *Chem. Commun.* 775.
8. Saini, M. S., Cheng, M. and Anderson J. A. (1976) *Phytochemistry* **15**, 1497.
9. Bajwa, R. S. and Anderson, J. A. (1975) *J. Pharm. Sci.* **64**, 343.

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ENZYMATIC HYDROLYSIS OF α-CHACONINE AND α-SOLANINE

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Key Word Index—*Solanum tuberosum*; Solanaceae; potato; enzymes; steroid glycoalkaloids, α-chaconine; α-solanine.

Several authors have reported on the hydrolysis of potato (*Solanum tuberosum*) glycoalkaloids by enzymes of potato sprouts, blossoms and foliage and the isolation of partial hydrolysis products of α-chaconine and α-solanine [1–6]. We wish to report the results of our studies using enzyme preparations from potato sprouts and dormant tubers (Table 1).

We have confirmed a previous report of the apparently anomalous (non-stepwise) hydrolysis of α-chaconine by an enzyme mixture prepared from potato sprouts [1]. This enzyme mixture removed the rhamnose substituent at the 2-position of the glucose residue in α-chaconine and converted the β_2-chaconine thus produced to solanidine without the demonstrable production of γ-chaconine (the glucoside of solanidine, that would result from the hydrolysis of both rhamnose residues). From α-solanine, this same enzyme mixture first produced β-solanine (by removal of rhamnose), then γ-solanine (the galactoside of solanidine resulting from the loss of glucose from β-solanine), and finally solanidine. Our results confirm the presence of rhamnosidase, glucosidase and galactosidase activities in the enzyme mixture from sprouts.

Our enzyme preparation from dormant tubers produced β_1-chaconine (the 2-rhamnosylglucoside of solanidine), β_2-chaconine, γ-chaconine and solanidine from α-chaconine but only β-solanine and solanidine from α-solanine. This is the first report of the stepwise hydrolysis of α-chaconine and the apparently anomalous (non-stepwise) hydrolysis of α-solanine by potato tuber enzymes.

We also studied the action of these enzyme preparations on β_2-chaconine isolated from dried potato blossoms and on β_1-chaconine and γ-chaconine obtained by partial acid hydrolysis of α-chaconine. Both enzyme mixtures hydrolyzed the β-chaconines and