of unripe mango fruit, after draining the duct content of the tissue, by blending fruit tissue in 0.1 M Pi buffer, pH 6.2 using 1 ml buffer/g fruit.

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PECTOLYTIC ENZYME ACTIVITY FROM *NICOTIANA TABACUM* POLLEN

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Key Word Index—*Nicotiana tabacum*; Solanaceae; pollen; pectolytic enzymes.

Abstract—Ungerminated pollen of *Nicotiana tabacum* contains a pectolytic enzyme which has its optimal activity between pH 5.5 and 6.5. Pectic lyase was not detected.

INTRODUCTION

Since the first light microscopic observations that pollen tubes grow within the pistil to the egg cells [1], it has been assumed that pollen tubes digest their way through the tissues by means of enzymes [2–5]. The results of electron microscopic studies of pollinated styles supported this view [6, 7]. Depending upon the plant species, pollen tubes grow in general in the intercellular substance of a stylar transmitting tissue or in a secretion product present in a stylar canal. Both materials consist mainly of carbohydrates which are related to complex pectic substances [8–10]. By means of labeling experiments, it has been shown that carbohydrate material of the secretion product of styles as well as of the intercellular material is used by growing pollen tubes for tube wall synthesis [9, 11, 12]. Up to now scant experimental evidence has been given for the presence of an enzyme system in pollen which digests pectin material [3, 13, 14].

The present study was undertaken to determine if pollen of *Nicotiana tabacum* contains enzymes which break down pectin.

RESULTS AND DISCUSSION

A crude, non-particulate preparation of *N. tabacum* pollen has been tested for pectolytic activity. When it was incubated with commercial citrus pectin, a decrease in viscosity of the reaction mixture as well as an increase in reducing groups released into the reaction mixture was observed over a 24 hr period (Fig. 1). The reducing groups released represent 1.2, 1.7 and 4.6% hydrolysis in 6, 12 and 24 hr respectively, based on the simplifying assumption that the units of pectin were anhydrogalacturonic acid. In the same time periods, the viscosity decreased 12, 26 and 42% respectively. There was no evidence of transeliminase activity in these reaction mixtures, since the samples did not show an increase in absorbance at 235 nm. The enzyme showed slightly lower rates of hydrolysis and viscosity decrease when it was incubated with polygalacturonic acid.

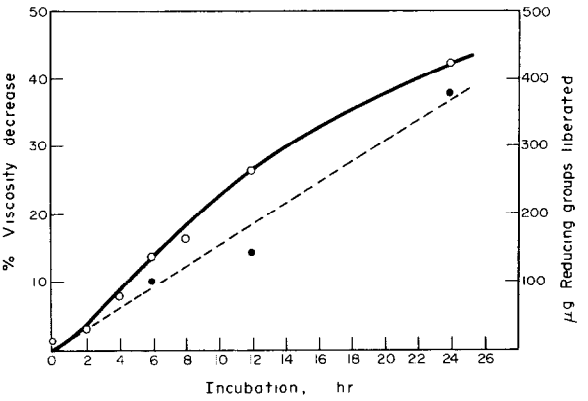

Fig. 1. Viscosity decrease (—○—) and liberation of reducing groups (—●—) of pectin by pollen enzyme. (The best straight line is drawn through the points for reducing groups).

The 6, 12 and 24 hr incubations of pectin and crude enzyme, after removal of protein by heat, were passed through Dowex 1-formate and the acidic components absorbed to the exchanger eluted with 0.1, 1 and 3 M

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formic acid. TLC of the pooled eluates from each incubation time revealed only galacturonic acid as the hydrolysis products after 24 hr. After 6 and 12 hr a very faint spot with an R_f less than that of monogalacturonic acid was observed, possibly digalacturonic acid. The appearance of galacturonic acid as the main breakdown product suggests the activity of these pollen extracts is due to an exopolygalacturonase. The ratio of viscosity loss to hydrolysis, however, is similar to that reported by Rexová-Benková and Marcovič [15] for endo-D-galacturonases from culture filtrates of the fungus *Coniothyrium diplodiella* and would suggest the presence in pollen of both exo- and endo-enzymes.

The pollen enzyme in its present state of purification has a specific activity of 0.129 mU/mg of protein (1 U = 1 μmol of reducing groups released per min) at 30°. This is equal in an average extraction to a yield of 24.9 mU/g of pollen. The pH optimum was determined by viscosity decrease, using 0.05 M sodium acetate buffer from pH 3 to 7 and 0.02 M Tris–HCl from pH 8 to 9. Optimal activity of the pollen enzyme occurred between pH 5.5 and 6.5 (Fig. 2).

The pectinase from *N. tabacum* pollen is now being purified and its activity studied on different substrates, including intercellular material from stylar transmitting tissue.

EXPERIMENTAL

Plant. Pollen was collected from *Nicotiana tabacum* L. var. Samsun plants grown under greenhouse conditions. The seeds of this species were kindly provided by Dr. J. Tupy, Czechoslovak Academy of Sciences, Institute of Experimental Botany, Department of Genetics, Praha. The pollen was stored at −15°.

Enzyme preparation. Ungerminated pollen was suspended in ice cold 0.0025 M phosphate buffer pH 6 (1.4 gm pollen/0.5 ml buffer) and homogenized in a glass homogenizer. The homogenate was centrifuged at 2300 *g* for 10 min and the residue washed several times with phosphate buffer until the supernatant fluid appeared clear. The combined supernatants were centrifuged for 10 min at 48 200 *g* and protein pptd from the supernatant with 80% satd $(NH_4)_2SO_4$ (51.6 gm/100 ml). The ppt. was taken up in a minimum of 0.0025 M phosphate buffer and dialysed for 4 hr with 3 changes of the same buffer. The protein concn in the dialysed material was determined by the method of ref. [16] and the enzyme prepn distributed in portions with 10 mg of protein and stored at −15°.

Enzyme assays. To determine pectic lyase activity, the reaction mixtures used for viscometric measurements were centrifuged for 10 min at 12 100 g after heat inactivation of the enzyme, and the absorption of the supernatant was measured spectrophotometrically at wavelengths between 230 nm and 240 nm [17, 18]. Polymethylgalacturonase activity was determined by incubating commercial pectin (polygalacturonic acid methyl ester from Citrus fruits, Grade 1, Sigma) with the enzyme prepn (final concn of pectin was 0.25%) and measuring the decrease in viscosity of the reaction mixture as well as the increase in reducing groups during the incubation. The standard reaction mixture contained 10 mg protein in 0.6–1 ml of enzyme prepn, 0.5 ml 1.5% pectin and 0.05 M NaOAc buffer, pH 6, to make a total vol. of 3 ml. The incubations were performed at 30° in the presence of thymol or 0.01% merthiolate. Polygalacturonic acid (GradeIII, Sigma) was used as a substrate at a final concn of 0.5%. Enzyme prepns heated at 100° for 10 min served as controls. Viscometric measurements were made with Ostwald viscometers. 100% decrease in viscosity referred to the viscosity measured for a soln of buffer plus an amount of galacturonic acid equivalent to the pectin added or to buffer alone. No detectable difference was found between these two controls. Controls were also run with buffered pectin and buffered enzyme solns.

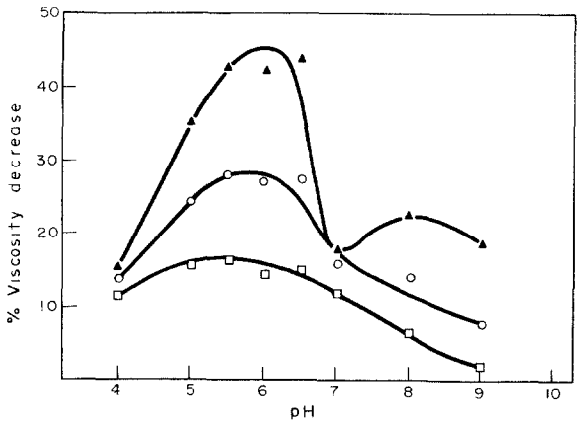

Fig. 2. Viscosity decrease at different pH values of pollen pectinase at 6 (—□—), 12 (—○—) and 24 hr (—▲—) of incubation.

Identification of uronides. The reducing groups released with time by the enzyme prepn were determined by the method of ref. [19]. At intervals aliquots were withdrawn from the incubation mixture and, after heat inactivation of the enzyme and centrifugation, the supernatants were passed through a Dowex 1-formate column. The acidic carbohydrate material was successively eluted from the resin by formic acid (0.1, 1 and 3 M) and the quantities of uronic acid in the pooled eluates determined [19]. The uronides in the pooled Dowex 1-eluates were identified by TLC in Me_2CO–*n*-BuOH–0.1 M NaH_2PO_4 (8:5:7) on a Merck Kieselguhr F-254 support [20]. Acidic carbohydrates were detected on chromatograms by spraying with alkaline $AgNO_3$ [21].

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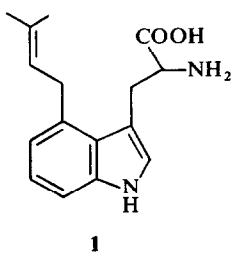
Key Word Index—*Claviceps* sp.; Clavicipitaceae; ergot; enzymatic study; ergot alkaloids; 4-dimethylallyltryptophan; 4-[4-hydroxy-3-methyl-Δ²-butenyl]-tryptophan.

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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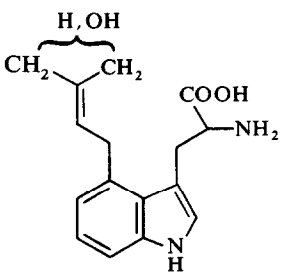

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RESULTS AND DISCUSSION

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.