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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ENZYMATIC HYDROLYSIS OF α-CHACONINE AND α-SOLANINE

ANSEL P. SWAIN, THOMAS J. FITZPATRICK, EUGENE A. TALLEY, SAMUEL F. HERB and STANLEY F. OSMAN
Eastern Regional Center, Agricultural Research Service, U.S. Department of Agriculture Philadelphia, PA 19118, U S.A

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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

Table 1. Action of enzyme preparations from potato sprouts and tubers on glycoalkaloids

Enzyme source	Substrate																					
	I α-Chaconine (α-C)						II α-Solanine (α-S)						III γ-Chaconine (γ-C)			IV β_2-Chaconine (β_2-C)				V β_1-Chaconine (β_1-C)			
	Incubation at 37° (hr)	Found by TLC					Incubation at 37° (hr)	Found by TLC					Incubation at 37° (hr)	Found by TLC		Incubation at 37° (hr)	Found by TLC			Incubation at 37° (hr)	Found by TLC		
		α	β_1	β_2	γ	S*		α	β	γ	S			γ	S		β_2	γ	S		β_1	γ	S
Sprouts	0	+	−	−	−	−	0	+	−	−	−		0	+	−	0	+	−	−	0	+	−	−
	0.02	+	−	+	−	+	0.5	+	+	−	−		3.0	−	+	0.1	+	−	tr	0.1	−	−	+
	0.25	−	−	+	−	+	3.0	+	+	−	+					17	+	−	+	2.0	−	−	+
	1.0	−	−	+	−	+	18	+	+	+	+					120	+	−	+	5.0	−	−	+
	96	−	−	tr	−	+																	
Tubers	0	+	−	−	−	−	0	+	−	−	−		0	+	−	0	+	−	−	0	+	−	−
	1	+	+	−	−	−	3	+	+	−	−		1	+	−	22	−	+	+	1	+	+	+
	3	+	+	tr	−	−	17	+	+	−	tr		5	+	+	72	−	−	+	5	+	+	+
	28	+	+	+	+	tr	24	+	+	−	+		22	−	+					22	−	−	+
	50	−	+	−	+	+	50	+	+	−	tr												

*S = Solanidine.
tr = trace.

produced solanidine from γ-chaconine. In the hydrolysis of the β-chaconines by the enzyme preparation from dormant tubers, the intermediate γ-chaconine appeared as expected. However, with the enzyme preparation from sprouts, no detectable γ-chaconine resulted from the hydrolysis of β_2-chaconine or, suprisingly, from β_1-chaconine.

EXPERIMENTAL

Enzyme prepns were obtained from chlorophyll-free sprouts of *S. tuberosum* L. cv Wauseon and dormant tubers of cv Kennebec. Sprouts or tubers were minced and pressed out through linen with the addition of a small amount of H_2O. Proteins were separated from the expressed juice by precipitating with $(NH_4)_2$ SO_4 (0.6 satn) and centrifuging at 14600 *g* for 60 min at 4° [1]. After dialysis against H_2O, the enzyme prepns were stored at 4° with the addition of a little toluene. Enzymatic hydrolysis of glycoalkaloids was carried out in buffer (pH range 4–7) in sealed glass capillaries [7]. Following incubation of enzyme prepns with glycoalkaloid substrates, products were separated on Si gel TLC plates which were developed with the lower layer of MeOH–$CHCl_3$–1% NH_4OH (2:2:1) and then visualized with I_2 vapor. α-Solanine, α-chaconine and β_2-chaconine were isolated from extracts of potato blossoms by precipitation from aq. soln made alkaline with NH_4OH at 70–80°, followed by column chromatography on dry Al_2O_3 [8]. Fractions were monitored by TLC on Si gel plates using Boll's solvent [9], the lower layer of EtOH–$CHCl_3$–1% NH_4OH (2:2:1). Glycoalkaloids isolated by this procedure were identified on the basis of the products of their hydrolysis, IR spectra, R_f comparison with authentic compounds on TLC plates, GLC retention values and MS of permethyl derivatives [10].

β_1-chaconine and γ-chaconine were isolated from a partial acid hydrolysate of α-chaconine by prep TLC.

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