extracted rice was washed with hexane and dried in vented hood. The dry rice was again extracted in a blender with 40° aq. MeOH (2 × 1 l. kg). The combined extracts of sq. MeOH were concd to a small vol. and them freeze-dried to a brown solid which was extracted with MeOH at room temp. for 1 hr. The MeOH was evaporated, and the residue was redissolved in a mimimum amount of MeOH and added to Me_2CO to ppt. insoluble material. The Me_2CO–MeOH solubles were repeatedly treated in this manner until no further ppt. occurred on Me_2CO addition (3 ×). The MeOH–Me_2CO soluble yellow oil was added to the top of a Si gel column (2 × 30 cm) presaturated with $CHCl_3$. The column was washed with 1 l. of $CHCl_3$ followed by elution of crude vomitoxin with 300 ml of 3% MeOH in $CHCl_3$. Further elution (100 ml) gave 220 mg of crude **1** per kg of rice substrate. Crude **1** was crystallized from 3% CH_3OH in $CHCl_3$ (170 mg, mp 140°). (Found- C, 50.3; H, 6.3; N, 9.98; parent ion *m/e* 143. Calc.: C, 50.34; H, 6.34; N, 9.78; MW 143).

Physical and chemical analyses. Mps are uncorrected. The IR spectra were recorded as films deposited on KRS-5 plate from Me_2CO solutions. NMR spectra were recorded with a Varian HA-100. CMR spectra were recorded with a Brüker-WH 90. The acetylamide **1** was esterified by treatment with ethereal CH_2N_2 for 1 hr at room temp. The resulting methyl ester of **1** (shown by NMR and parent *m/e* 152) was treated with ozone for 30 sec in CH_2Cl_2 and reduced with triphenylphosphite. The reaction mixture was taken to dryness and redissolved in Et_2O. The reduced ozonolysis product was analyzed on a Bendix 2600 GC (column- 2 m × 7 mm glass 3% Silar-5 cp) connected through a single-stage jet-type stainless-steel separator to a Dupont 492-1 mass spectrometer; MS (scan taken at 70.eV) were recorded every 4 sec/decade of the GC peaks. An authentic sample of methyl glyoxalate was used for comparison with the oxidation product and showed the same R_f and MS.

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EFFECT OF ETHREL AND CHLOROETHANOL ON PEA DIAMINE OXIDASE ACTIVITY

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Key Word Index—*Pisum sativum*; Leguminosae; pea; diamine oxidase; ethylene; ethrel; chloroethanol; 2,4-D.

Abstract—The activity of cotyledon and embryo diamine oxidase was reduced by feeding ethrel and chloroethanol to the seedlings. The inhibitory effect of 2,4-D on the activity of enzyme in the cotyledon which may be mediated through ethylene was reversed by exposure of seeds to red light.

INTRODUCTION

On feeding 2,4-D to intact pea seedlings, the activity of the cotyledon diamine oxidase was reduced [1]. This effect was mediated through the embryo since removal of the embryo after soaking in the 2,4-D for 14 hr abolished the inhibitory effect, suggesting the elaboration of an inhibitor of diamine oxidase activity or synthesis in the embryo. 2,4-D enhances the synthesis of ethylene in tissues [2, 3] and ethylene may therefore control this enzyme. Experiments were carried out using ethrel (2-chloroethyl phosphonic acid) and ethylene chlorohydrin (2-chloroethanol), substances known to form ethylene *in vivo* [3]. The results of this study are reported in the present communication.

RESULTS AND DISCUSSION

The data reported in Table 1 show that the activity of both cotyledon and embryo enzymes as decreased with increasing concentrations of ethrel when the seeds were germinated after soaking them in ethrel for 14 hr. Enzyme activity tends to return to the normal level after a further period of germination. Similar results were obtained with chloroethanol except that the concentration of chloroethanol required was only 10% of that of ethrel.

We have shown earlier [1] that the inhibitory effect of feeding 2,4-D to pea seedlings on the cotyledon enzyme was abolished if the embryo was removed from the seeds after soaking. However, with ethrel and chloroethanol the cotyledon enzyme was inhibited irrespective of whether the embryo was present or not after soaking since the ethylene produced would be directly available in the cotyledon.

Studies were also carried out to investigate whether chloroethanol would affect the enzyme when added 14, 38 and 62 hr after germination. In all the groups the enzyme activity was determined 48hr after adding chloroethanol. The data reported in Table 2 show that both cotyledon and embryo enzymes were inhibited. The inhibitory effect was maximum in the seeds when chloroethanol was added immediately after soaking and

Table 1. Effect of ethrel and chloroethanol on diamine oxidase activity in cotyledon and embryo of pea seedlings

Concentration* (mM)	Diamine oxidase (n kat/g. fr. tissue)					
	Cotyledon			Embryo		
	at hr					
	62	110	156	62	110	156
Ethrel						
0	11.5	12.7	12.8	13.9	13.5	14.2
13.8	7.4	12.5	12.8	6.7	13.2	13.8
20.7	2.8	11.0	12.2	3.2	6.5	10.5
27.6	0.5	3.7	9.5	0.8	8.9	9.8
Chloroethanol						
0	11.5	12.9	13.2	13.5	13.2	13.5
1.25	4.3	11.5	12.5	9.4	12.5	14.2
3.75	2.7	8.5	12.9	3.3	13.0	13.0
6.25	1.2	1.0	5.3	1.2	1.5	6.5

* Seeds were soaked in ethrel or chloroethanol for 14 hr.

was minimum when it was added 48 hr later. The presence or absence of embryo after soaking in the ethylene precursors does not seem to have any significant effect on the

Table 2. Effect of chloroethanol on pea cotyledon and embryo diamine oxidase when added at 14, 38 and 62 hr of germination

	Diamine oxidase (n kat/g fr. tissue)					
	Control			Chloroethanol treated*		
	at hr					
	62	86	110	62	86	110
Cotyledon (from seeds soaked and germinated with embryo)	12.2	13.8	14.0	0.5	4.5	8.8
Cotyledon (from seeds where embryo was removed after soaking)	12.8	15.8	15.9	0.5	4.5	14.2
Embryo	13.5	13.4	14.8	1.3	6.3	10.7

* Seeds were soaked in water for 14 hr and then kept in Petri dishes for germination. Chloroethanol (50 mM) was added to the soaked seeds at 14, 38 and 62 hr after the commencement of treatment. Enzyme activity was determined 48 hr after chloroethanol treatment.

Table 3. Reversal of 2,4-D inhibition of pea diamine oxidase by exposure to red light

	Diamine oxidase (n kat/g fr. tissue)	
	Cotyledon	Embryo
Control (dark)	10.4	12.5
Control (red light)	9.8	12.8
2,4-D treated (dark)	<0.08	5.8
2,4-D treated (red light)	6.9	9.4

Seeds were soaked in 5 ppm 2,4-D for 14 hr. Enzyme activity was determined 62 hr after the commencement of the treatment.

inhibition of cotyledon enzyme. The results indicate that the effect of chloroethanol was decreased in the seeds after enzyme synthesis has begun. Neither 2,4-D nor ethrel and chloroethanol show any inhibitory effect when added to the assay system.

The studies thus indicate that ethylene has an inhibitory effect on the cotyledon and embryo diamine oxidase. The enzyme activity *in vivo* was shown earlier [1] to be inhibited by 2,4-D which also enhances the synthesis of ethylene [2, 3]. The inhibitory effect of 2,4-D may thus be due to enhanced production of ethylene which takes place in the embryo but apparently not in the cotyledon. The enhanced synthesis of ethylene by 2,4-D is blocked by red light [2]. To investigate whether exposure of 2,4-D treated seeds to red light would affect the diamine oxidase activity, the seeds after soaking for 14 hr in 2,4-D were kept either in the dark or red light and the enzyme activity was assayed after germination for 62 hr. The data reported in Table 3 show that enzyme activity of the 2,4-D treated group was reversed by exposure to red light suggesting that the effect of 2,4-D was due to the synthesis of ethylene.

EXPERIMENTAL

Plant material. Pea seeds (*Pisum sativum*) were soaked and germinated in the dark or red light (11 lx) as in ref. [1].

Enzyme activity. Determined as described in ref. [1].

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