the DDT/lindane-R, fenthion-R, OMS-15-R, dimethoate-R, and SK-R strains were intermediate, while the OMS-12-R was the most resistant of all strains tested.

Previous reports have indicated that the benzoylphenylureas act only as stomach poisons (Mulder and Gijswijt, 1973; Wellinga *et al.*, 1973). However, by topically applying TH 60-40 in tetrahydrofuran to white prepupae, we have demonstrated that entry *via* the stomach route is not an essential requirement for toxicity.

The relatively high levels of cross-resistance toward TH 60-40, demonstrated by this study, may be disconcerting. Extrapolation of susceptibility data for the strain with the lowest resistance, parathion-R, reveals the presence of tolerance of approximately tenfold at the ED_{50}. Resistance in the OMS-12-R strain is obviously several fold greater. It is especially significant that the field strain, SK-R, is among the most resistant toward TH 60-40. Studies currently in progress are expected to elucidate the mechanisms of resistance to this compound. The detection of high levels of resistance in the house fly serves to emphasize the need for judicious use of new chemicals against presently susceptible populations, under conditions which minimize the degree of selection pressure.

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A Specific Enzymatic Procedure for the Determination of Neurotoxic Components (Derivatives of L-α,β-Diaminopropionic Acid) in *Lathyrus sativus*

Lathyrus sativus seeds are used to adulterate some of the common dietary pulses such as *Cicer arietinum* (Bengal gram) and *Cajanus cajan* (Red gram) in India despite food laws. Chromatographic or other procedures available for detection of the neurotoxic or other components in *Lathyrus sativus* are nonspecific. In the present method, the neurotoxic components (derivatives of L-α,β-diaminopropionic acid) are separated by cation exchange chromatography and subjected to acid hydrolysis. The L-α,β-diaminopropionic acid formed was determined using the specific enzyme diaminopropionate-ammonia lyase. As little as 50 nm (5 μg) of L-α,β-diaminopropionic acid can be detected by the present method. The new procedure determines all acid-labile forms of bound L-α,β-diaminopropionic acid and extracts of *Cicer arietinum* and *Cajanus cajan* do not interfere in the procedure.

Lathyrus sativus (Kesari dhal) contains at least three neurotoxic components (Rao *et al.*, 1964; Rajamohan and Ramachandran, 1972; Rukmini, 1972) which may be partly responsible for the irreversible spastic paralysis among people whose diet contains substantial amounts of this legume (Ganapathy and Dwivedi, 1961; Nagarajan, 1969). Kesari dhal has been used as an adulterant despite food laws and a ban on interstate movement (Prevention of Food Adulteration Rules, 1955). Procedures to detect the presence of kesari dhal in Bengal gram flour (*Cicer arietinum*) or red gram (*Cajanus cajan*) have been reported in the literature (Nagarajan and Mohan, 1967; Dutta, 1965; Hartman *et al.*, 1973). The method based on detection of unique phenolic components present in *L. sativus* is nonspecific as other pulses such as *Vigna catang* (cow gram), *Lens culinaris* (Masur), and *Dolichos biflorus* (Horse gram) interfere. The chromatographic and electrophoretic method (Nagarajan and Mohan, 1967) based on detection of β-oxalyl-L-α,β-diaminopropionic acid (OXDAPRO), while being convenient and reasonably rapid, lacks the specificity of an enzymatic procedure and furthermore the ninhydrin reagent cannot detect α,β-dioxalyl-L-α,β-diaminopropionic acid (DIOXDAPRO). Five per cent adulteration of Bengal gram with *Lathyrus sativus* can be detected by the chromatographic method. The possibility that other ninhydrin positive components with the same chromatographic and electrophoretic mobility as β-oxalyl-L-α,β-diaminopropionic acid occur in natural material cannot be entirely ruled out although such an occurrence would be expected to be rare.

All the three neurotoxic components in Kesari dhal are derivatives of L-α,β-diaminopropionic acid (DAPRO) and are labile to acid hydrolysis yielding in the case of OXDAPRO and DIOXDAPRO nearly quantitative yields of DAPRO (Rao *et al.*, 1964; Rajamohan and Ramachandran, 1972). The "new toxic factor" (Rukmini, 1972) also contains DAPRO as an integral structural moiety but details about its complete chemical nature are not known as yet to predict its stability to acid hydrolysis. It was felt that a specific enzymatic procedure to determine DAPRO in acid hydrolysates of processed extracts of *Lathyrus sativus* would be helpful in determining the adulteration of other commonly used legumes with this pulse.

Furthermore, a combination of the paper chromato-

graphic method and the present enzymatic method when applied to the same sample will also indicate the presence of other bound acid labile derivatives of DAPRO and would perhaps be useful in a plant breeding program designed to evolve new varieties of Kesari dhal with low neurotoxin content (Swaminathan *et al.*, 1972). An abstract of the enzymatic procedure was presented earlier in a meeting (Rajagopal Rao *et al.*, 1972) and details of the procedure are now given.

EXPERIMENTAL SECTION

Materials. Several newly evolved varieties of *Lathyrus sativus* were obtained from the genetics division of the Indian Agricultural Research Institute, New Delhi. Samples of *Cicer arietinum* and *Cajanus cajan* were obtained from the local market. OXDAPRO was isolated by a modified procedure of Rao *et al.* (1964). DAPRO-ammonia lyase was purified from cell-free extracts of a *Pseudomonad* grown on DL-DAPRO (as the sole source of carbon and nitrogen) by protamine sulfate treatment, ammonium sulfate fractionation, and negative absorption on calcium phosphate gel (Rajagopal Rao *et al.*, 1970). The purified enzyme had a specific activity of 1.5 μmol/min per mg of protein and is highly specific for L-DAPRO. Lactic dehydrogenase (LDH) was prepared by the procedure of Kornberg and Pricer (1951). Sodium pyruvate (A.R. Grade) was obtained from E. Merck, Darmstadt, West Germany.

Procedure. *Lathyrus sativus* (Kesari dhal) or the suspected sample was ground to a fine powder (to pass through 100 mesh screen). One gram of the sample was shaken with 100 ml of 70% ethanol and left aside at room temperature overnight (about 18 hr). It was later filtered (Whatman No. 1 filter paper) and the residue washed with 50 ml of 70% ethanol. The combined filtrate was evaporated to near dryness in a porcelain dish on a boiling water bath and the residue thoroughly mixed with 5 ml of water. It was centrifuged in plastic tubes at high speed to remove insoluble matter. Aliquots of the supernatant were used for paper chromatography (Nagarajan and Mohan, 1967) to determine OXDAPRO.

One milliliter of the supernatant was supplied to a Dowex 50-H^+ (X-4, 100–200 mesh) 5 × 1.2 cm ion exchange column and washed with 40 ml of water. An aliquot of the water elute (2 ml) was taken in a Corning test tube (12.5 × 1.2 cm) and 0.5 ml of 2 *N* HCl was added. The tube was sealed and kept at 110° for 2 hr in an oven. The hydrolysate was neutralized with 1–2 *N* sodium hydroxide and made up to volume (5 ml). Aliquots (0.05–0.2 ml) were assayed for DAPRO content by the ammonia lyase reaction.

Two types of assays were used for the ammonia lyase reaction. In the assay system a the reaction mixture contained potassium phosphate buffer (pH 8.0), 100 μmol; pyridoxal 5-phosphate, 0.01 μmol; and a test sample containing DAPRO, 0.05–0.5 μmol in a final volume of 4 ml. The reaction was started by addition of DAPRO-ammonia lyase and the system was incubated at 37° for 45 min. Later 1 ml of 0.2% 2,4-dinitrophenylhydrazine in 2 *N* HCl was added followed after 10 min by 2 ml of 2 *N* sodium hydroxide. The absorbance at 520 nm was determined in a Beckmann spectrophotometer or a Klett colorimeter (Filter 520) against an identical sample containing inactivated enzyme. Although aliquots of acid-hydrolyzed OXDAPRO should serve as a standard in the enzymatic assay and subsequent color development with 2,4-dinitrophenylhydrazine, we have used sodium pyruvate as a standard as it is convenient and the results are not significantly altered. In the assay system b the reaction mixture contained potassium phosphate buffer (pH 8.0, 100 μmol), pyridoxal 5-phosphate (0.05 μmol), reduced nicotinamide adenine dinucleotide (0.3 μmol), a test sample containing 0.05–0.2 μmol of L-DAPRO, and 20 units of lactic dehydrogenase in a final volume of 3 ml. The blank contained all components except the ammonia lyase. The reaction was started by the addition of excess DAPRO-ammonia lyase and virtually goes to completion in 10 min as determined by a decrease in absorbance at 340 nm in a Beckman Model DK-2 spectrophotometer. The difference in the initial and final optical densities is used to calculate the DAPRO content after corrections for volume changes. An absorbance change of 0.207 (L = 1 cm) represents 0.1 μmol of L-DAPRO.

Table I. Recovery of DAPRO and OXDAPRO in the Assay Methods

	Estimated quantity, μmol			
	DAPRO		OXDAPRO[a]	
DAPRO/ OXDAPRO, μmol	Assay with LDH[b]	Colorimetric	Assay with LDH	Colorimetric
0.05		0.05		0.045
0.10	0.101	0.10	0.097	0.090
0.20	0.200	0.20	0.184	0.184
0.30	0.300	0.30		0.276
0.40	0.401	0.40		0.370
0.50	0.500	0.50		0.470

[a] OXDAPRO was hydrolyzed with 0.25 *N* HCl for 2 hr at 110°, neutralized, and then assayed enzymatically. [b] For concentrations exceeding 0.3 μmol of DAPRO, appropriately higher amounts of NADH were used in the assay.

Table II. Analysis of Newer Varieties of *Lathyrus sativus*[a]

Variety of *Lathyrus sativus*	OXDAPRO present, mg %		
	Chromatographic	Assay with LDH	Colorimetric
P_{10}	450 ± 27	460 ± 10.5	525 ± 17
P_{17}	406 ± 38	510 ± 5.2	541 ± 9
P_{24}	346 ± 20	423 ± 23	588 ± 26
P_{263}	456 ± 32	508 ± 32	546 ± 13
P_{638}	299 ± 16	390 ± 13	509 ± 14
P_{678}	347 ± 38	313 ± 10	514 ± 31

[a] Values are the mean of six individual analyses ± standard deviation (in duplicate).

RESULTS AND DISCUSSION

Several types of extraction procedure such as ethanol, ethanol with 2% acetic acid, or 2% perchloric acid, for varying periods of time from 3 to 72 hr, were used. The ethanol extraction method at room temperature was convenient as the later column procedures were easier. OXDAPRO and DIOXDAPRO are sufficiently acidic and not retained on low cross-link cation exchange resin columns. Nearly all of the common protein amino acids including free DAPRO are removed in this step. On acid hydrolysis authentic OXDAPRO yields DAPRO nearly quantitatively (Table I). Oxalic acid, a product of acid hydrolysis, does not interfere (1.25 m*M* concentration) either in the ammonia lyase reaction or in the coupled assay with lactate dehydrogenase.

The results obtained by three methods with newer varieties of *Lathyrus sativus* are given in Table II. The coupled assay procedure with lactate dehydrogenase is better than the 2,4-dinitrophenylhydrazone method as high

blanks are obtained with the natural material in the latter procedure. This is most probably due to the fact that the carbohydrate present in the extracts is not retained on the column and yields interfering carbonyl compounds on acid hydrolysis. Four-tenths per cent of OXDAPRO added to *Cicer arietinum* or *Cajanus cajan* flour can be recovered (85–90% yield). In about 570 different samples of *Lathyrus sativus* analyzed by Nagarajan and Gopalan (1968), the range of OXDAPRO content varied from 0.1 to 2.5%, about 10% of the samples having 1–1.2%, 3% of the samples having 2–2.5%, 38% in the range 0.5–1%, and 16% in the range 1.2–1.6%. The presence of up to 50% *Lathyrus sativus* in the flours of *Cicer arietinum* or *Cajanus cajan* did not cause any analytical problems in the present procedure. With very low neurotoxin content, larger samples (10 g) have to be taken so that even traces can be detected by the present procedure. It is probable that the method can be further scaled down and sensitivity increased by fluorimetric methods in the coupled lactic dehydrogenase assay. The present method is recommended only when absolute confirmation for the presence of bound DAPRO is needed.

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Anaerobic Degradation of 1,1,1,2-Tetrachloro-2,2-bis(*p*-chlorophenyl)ethane (DTE)

DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene) is the major product from the anaerobic degradation of 1,1,1,2-tetrachloro-2,2-bis(*p*-chlorophenyl)ethane (DTE). This is the same reaction that is observed with electrochemical reduction. It is postulated that electrochemistry may be used as a means of predicting the products formed from the anaerobic reduction of organochlorine compounds.

DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane) has been shown to be the major degradative product of DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) under anaerobic conditions (Guenzi and Beard, 1967). DDD is also the first product formed in the electrochemical reduction of DDT (Farwell, 1973; Rosenthal and Lacoste, 1959). We questioned whether or not other compounds might give the same products from anaerobic degradation as they do from electrochemical reduction and if, therefore, electrochemistry could provide insight into the environmental degradation of pesticides under anaerobic conditions. As a first step in this investigation we studied the degradation of a DDT analog, 1,1,1,2-tetrachloro-2,2-bis(*p*-chlorophenyl)ethane (DTE), under flooded soil conditions and compared the degradation products with the electrochemical reduction products of DTE.

EXPERIMENTAL SECTION

Air-dried Bowdoin soil (pH 7.9, 1.44% organic carbon, 0.8% sand, 30.6% silt, 68.6% clay) was passed through a 35 mesh sieve and treated with DTE (Aldrich, puriss grade) in acetone to give a total DTE concentration of 100 ppm. After air drying for 1 hr the soil was amended with ground alfalfa (5% w/w) and fresh soil (5% w/w), the latter to ensure the presence of microbes. After mixing the soil for 1 hr, 100-g portions were transferred to amber bottles, flooded with water to a depth of 1 in., plugged with a gauze stopper, and incubated at room temperature.

At 2-week intervals the soil samples were transferred to a soxhlet apparatus and extracted for 3 hr with redistilled acetone. The acetone was evaporated *in vacuo* and the residue partitioned into redistilled hexane. The hexane was concentrated and the extract passed through a Florisil column (100–120 mesh, Floridian Co.) while eluting with hexane.

We were unable to separate DTE from DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene) (Aldrich, 99%) by gas chromatographic procedures and therefore we used high-pressure liquid chromatography. A Waters Associates ALC 202 liquid chromatograph equipped with a U.V. detector (254 nm) was used. The detector response for DTE was less than a tenth of that for DDE. The operating conditions were as follows. The column was C_{18}/Corasil packed in a 2 ft × $^1/_8$ in. stainless steel column; the elution solvent was methanol–H_2O (67:33); flow of 1.2 ml/min.

All compounds were incubated in triplicate samples of soil.

RESULTS AND DISCUSSION

The first product in the sequential, electrochemical reduction of DTE is the formation of DDE (Farwell, 1973;