undergo decarboxylation to the phenyl ether 3 which in turn is transformed to the phenol 4. The direct conversion of the acid 2 to the phenol 4 may also be possible. The phenol 4 may then undergo complexing or binding to the soils and/or be subjected to further degradative processes involving ring fission to give such products as *m*-dichlorobenzene or 2,4-dichlorophenol. The former compound is very volatile with a boiling point of 172 °C and could thus be expected to volatilize very quickly from the moist soils; for this reason no attempts were made to effect its isolation; however, efforts were made to determine the presence of the latter compound in the appropriate extracts before and after methylation to 2,4-dichloroanisole, but none were observed. From these data it would also seem that the phenol 4 and the phenetole 3 do not build up to any great extent in the soils under investigation and are possibly broken down almost as fast as they are formed.

The persistence and fate of dichlorfop-methyl under field conditions is presently under study and will be reported.

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Metabolism of 2,4-Dichlorophenoxyacetic Acid. 11. Herbicidal Properties of Amino Acid Conjugates

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Twenty amino acid conjugates of 2,4-dichlorophenoxyacetic acid (2,4-D) were tested for their herbicidal effect upon green beans, sunflowers, peas, and soybeans in field and growthroom experiments. All conjugates possessed herbicidal properties. The most active compounds were the less polar amino acid conjugates such as leucine, isoleucine, valine, alanine, and methionine. In general the aromatic and polar amino acid conjugates exhibited poor herbicidal properties.

Previous investigations have demonstrated that amino acid conjugates of 2,4-dichlorophenoxyacetic acid (2,4-D) are formed in plants and in plant tissue cultures (Andreae and Good, 1957; Klämbt, 1961; Feung et al., 1971, 1972, 1973b, 1975). Amino acid conjugates of 2,4-D have been isolated from at least six plant callus tissue cultures (carrots, jackbean, sunflower, tobacco, corn, and soybean) (Feung et al., 1973b, 1975) and seven conjugates (Asp, Glu, Ala, Val, Leu, Phe, and Trp) have been found in soybean callus tissue cultures (Feung et al., 1973b). Nearly all of the potential 20 amino acid conjugates of 2,4-D have been shown to be biologically active and stimulate plant cell division and elongation (Feung et al., 1974).

Since 2,4-D is a widely used herbicide and its amino acid conjugates have been shown to be present in the tissue and have biological activity, it is important to investigate the toxicological effects of these conjugates on both plants and animals. Thus, this paper presents the herbicidal effects of 20 amino acid conjugates of 2,4-D on four plant species in field and growthroom studies.

MATERIALS AND METHODS

The 20 L-amino acid conjugates of 2,4-D used in these studies were synthesized previously in this laboratory (Feung et al., 1973a). The purified amino acid conjugates and 2,4-D were dissolved in 0.0005 M NaOH solution containing 0.2% Tween 20 immediately before spraying. Plants were sprayed with four levels of conjugates (0.5 lb/acre, 0.25 lb/acre, 0.125 lb/acre, and 0.1 lb/acre) with a compressed air hand sprayer in the field and an aerosol pressurized sprayer in the growthroom (conjugates were stable under these conditions).

Growthroom Study. Seeds of four plant species (Long Tender Green Bean, Russian Mammoth Sunflower, Alaska Pea, Amsoy-71 Soybean) were planted in six rows in a peat–vermiculite commercial potting mixture (Pro-Mix) in a 12 × 8 in. tray and grown in the greenhouse. All seeds were presoaked for 6 h prior to planting. Seedlings of each species were thinned to six plants per row 4 to 5 days following germination. Plants were treated 2 weeks following germination. An aerosol can sprayer was used to spray the plants with three different concentrations (0.5 lb/acre, 0.25 lb/acre, and 0.1 lb/acre) of each tested chemical and the control plants were sprayed only with diluent solution. Two growthroom experiments were conducted; both contained two replicates for each treatment. The treated plants were carefully watered to avoid washing off the tested chemicals. Injury was evaluated on the 7th, 11th, and 14th day following treatment by two individuals. Plants were arbitrarily judged as follows: 0, no effect; 1, slight effect; 2, moderate effect; 3, severe effect; and 4, dead. The ratings from the two replicates were averaged.

Field Studies. Seeds of the same four plant species tested in the growthroom were planted in six rows in a 6 × 6 ft field plot. All seeds were presoaked for 10 h prior

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Table I. Evaluation of Herbicidal Activity of Amino Acid Conjugates of 2,4-D on Four Plant Species Grown in Growthroom[a]

Compd	lb/acre	Green bean	Sun-flower	Pea	Soy-bean	Compd	lb/acre	Green bean	Sun-flower	Pea	Soy-bean
Gly	0.5	1.5	3.0	1.0	1.5	Lys	0.5	0.5	2.0	0.5	1.0
	0.25	0.5	1.5	0.0	0.0		0.25	0.5	1.0	0.0	0.0
	0.1	1.0	2.5	1.0	1.0		0.1	0.0	0.5	0.0	1.5
Ala	0.5	3.5	3.5	4.0	2.0	Gly	0.5	2.5	3.0	4.0	1.0
	0.25	2.0	3.0	3.5	2.0		0.25	1.0	3.0	2.0	0.5
	0.1	3.0	3.5	3.0	3.0		0.1	0.5	2.0	0.0	0.0
Ser	0.5	2.5	3.5	3.5	2.0	Met	0.5	1.5	3.0	4.0	2.5
	0.25	1.5	3.0	0.5	1.0		0.25	0.5	3.0	1.0	1.5
	0.1	1.0	2.0	1.0	0.5		0.1	0.5	2.5	1.0	0.5
Pro	0.5	3.0	3.5	1.5	2.0	His	0.5	1.5	3.0	1.0	0.5
	0.25	1.0	3.0	2.0	1.5		0.25	2.5	3.5	3.0	1.5
	0.1	0.0	1.5	0.0	0.0		0.1	1.0	3.5	1.0	0.0
Val	0.5	2.0	3.5	4.0	1.5	Phe	0.5	2.5	3.5	3.0	2.0
	0.25	2.0	3.0	3.5	1.0		0.25	1.5	3.5	3.5	1.5
	0.1	0.0	2.0	0.0	0.5		0.1	1.0	2.0	0.5	0.0
Thr	0.5	2.5	3.0	3.5	2.0	Arg	0.5	2.5	3.5	3.0	2.5
	0.25	1.0	3.0	2.5	1.0		0.25	1.0	3.0	0.5	0.5
	0.1	1.0	2.5	0.5	0.5		0.1	0.5	2.0	0.5	0.5
Cys	0.5	1.5	2.5	2.5	1.0	Tyr	0.5	3.0	3.5	4.0	3.0
	0.25	1.0	2.5	1.0	0.5		0.25	2.0	3.0	3.5	1.5
	0.1	1.0	2.5	1.0	0.5		0.1	0.5	2.5	0.5	0.5
Leu	0.5	3.0	4.0	4.0	2.5	Trp	0.5	1.5	3.5	1.5	1.5
	0.25	1.5	2.5	3.5	2.0		0.25	0.5	2.5	2.0	0.5
	0.1	0.5	2.0	3.0	0.5		0.1	0.0	1.5	0.5	0.0
Ile	0.5	3.0	4.0	4.0	3.0	Cys'	0.5	0.0	2.0	1.0	0.5
	0.25	2.5	3.0	4.0	2.5		0.25	0.5	1.5	0.0	1.0
	0.1	1.0	2.0	1.5	0.5		0.1	0.0	1.0	0.5	0.0
Hpr	0.5	0.5	3.0	2.5	1.0	2,4-D	0.5	4.0	4.0	4.0	4.0
	0.25	0.5	2.5	0.5	0.0		0.25	3.5	3.5	3.5	2.0
	0.1	0.0	1.0	0.0	0.0		0.1	1.0	3.0	0.5	0.5
Asp	0.5	1.0	2.5	3.5	0.0	Control	0.0	0.0	0.0	0.0	0.0
	0.25	0.5	2.0	0.5	0.0		0.0	0.0	0.0	0.0	0.0
	0.1	0.5	1.5	0.0	0.0		0.0	0.0	0.0	0.0	0.0

[a] Plants were germinated on Feb 8, 1974, sprayed on Feb 26. Above data represents herbicidal assay on Mar 12, 1974. Plants were arbitrarily judged by two persons as follows: 0, no effect; 1, slight effect; 2, moderate effect; 3, severe effect; and 4, dead.

to planting in the field. Field plots were 2 ft apart. Plants were treated 18 days after planting. With the aid of a compressed air hand sprayer (two nozzles), the plants in each field plot were separately sprayed with one of the three levels (0.5 lb/acre, 0.25 lb/acre, and 0.125 lb/acre) of each chemical, and the controls were sprayed with a diluent solution only. The rate of application was calibrated prior to spraying the tested chemicals and was ca. 40 gal/acre. Both experiments contained two replicates for each treatment. Responses were observed 1 day after treatment with chemicals. The measurement of herbicidal activity was on the 7th, 11th, and 14th day following chemical treatments. Field tests were conducted in the summers of 1974 and 1975.

RESULTS AND DISCUSSION

The results of the herbicidal activity of the amino acid conjugates of 2,4-D on green beans, sunflowers, peas, and soybeans grown in field and growthroom conditions are presented in Tables I and II. For simplicity the data presented in Tables I and II were recorded on the 11th day after treatment and represent rplicates from one experiment. The herbicidal effects were maximal at this time; however, similar results were observed on the 7th and 14th day after treatment.

The herbicidal activity of the conjugates was essentially the same in both field and growthroom experiments. All the conjugates possessed herbicidal properties; however, no conjugates were more active than 2,4-D on all plants. The results with green beans, sunflowers, peas, and soybeans were very similar. Two conjugates possessed exceptionally good herbicidal properties, these being isoleucine and leucine. On peas, the isoleucine and leucine conjugates exceeded the activity of 2,4-D. Other conjugates with good herbicidal properties were alanine, valine, proline, methionine, and arginine. The valine, methionine, proline, and arginine conjugates exhibited more herbicidal effects in the field experiments than they did in the growthroom. The aromatic amino acid conjugates all possessed poor herbicidal properties with the exception of tyrosine in the growthroom. Several polar conjugates such as hydroxyproline, lysine, aspartic, and glutamic also showed poor activity with the exception of treatment on peas where the latter two conjugates were fairly active. The conjugates that exhibited high herbicidal character also dramatically reduced yields. All the conjugates reduced yield especially at the higher application rate.

The herbicidal activity of the conjugates can be compared with the stimulation of growth of plant callus and the stimulation of elongation of *Avena* coleoptile sections as given in Table III. These data previously reported (Feung et al., 1974) have been reexamined and the biological activity of the conjugates are here expressed as their one-half maximum concentrations for ease of comparison. As is evident, the growth stimulation of callus tissue by the amino acid conjugates of 2,4-D parallels to some extent the herbicidal response of the conjugates demonstrated in this manuscript. The nonpolar amino acid conjugates such as leucine, isoleucine, alanine, valine, and methionine all were very active in stimulating growth of callus tissue. On the other hand, two polar conjugates that were exceptionally good at stimulating callus tissue growth (aspartic and glutamic) did not have strong herbicidal properties.

From these data one can conclude that the conjugates

Table II. Evaluation of Herbicidal Activity of Amino Acid Conjugates of 2,4-D on Four Plant Species Grown in the Field[a]

lb/acre	Compd	Green bean	Sun-flower	Pea	Soy-bean	% yield green bean	% yield pea	Compd	Green bean	Sun-flower	Pea	Soy-bean	% yield green bean	% yield pea
0.50	Gly	1.0	2.3	0.8	1.0	−55	−28	Lys	0.2	1.0	0.0	0.2	−14	−1
0.25		0.7	1.5	0.2	0.2	−61	0		0.0	0.2	0.0	0.2	+8	+3
0.125		0.2	0.7	0.0	0.0	−49	−5		0.0	0.0	0.0	0.0	−32	−8
0.50	Ala	2.3	3.0	1.3	2.2	−97	−67	Glu	2.0	2.3	1.2	1.3	−89	−38
0.25		0.7	2.0	0.5	0.8	−77	−17		0.8	1.2	0.0	0.0	−54	−2
0.125		0.5	1.5	0.0	0.2	−31	+13		0.7	0.8	0.0	0.0	−14	+30
0.50	Ser	2.0	3.0	1.2	1.7	−80	−38	Met	2.5	2.7	2.2	2.2	−91	−68
0.25		0.8	2.3	0.0	0.0	−93	+20		0.7	1.8	0.0	0.2	−28	−9
0.125		0.2	1.0	0.0	0.0	−55	−5		0.2	0.3	0.2	0.0	−45	−4
0.50	Pro	1.8	2.8	1.5	2.0	−94	−46	His	1.2	1.8	1.5	1.8	−56	−57
0.25		1.0	2.0	0.7	1.0	−25	−45		0.2	1.2	0.0	0.3	−37	0
0.125		0.8	1.0	0.0	0.0	−29	+32		0.2	0.7	0.0	0.0	−29	−5
0.50	Val	2.2	2.8	2.3	2.7	−86	−71	Phe	1.0	1.5	0.8	0.7	−66	−24
0.25		1.0	2.0	1.3	1.5	−57	−59		0.5	1.2	0.5	0.3	−41	−5
0.125		0.0	1.2	0.3	0.3	−36	−26		0.0	0.5	0.0	0.0	−21	+36
0.50	Thr	1.8	2.5	1.2	1.7	−92	−27	Arg	2.0	3.0	1.8	1.7	−96	−50
0.25		0.7	1.7	0.3	0.7	−58	−12		1.0	1.8	0.8	0.7	−49	−18
0.125		0.3	0.7	0.0	0.0	−35	−27		0.7	0.7	0.0	0.0	−24	+25
0.50	Cys	0.7	1.0	0.3	0.3	−73	−15	Tyr	1.0	2.0	0.5	0.2	−55	−7
0.25		0.0	0.5	0.0	0.0	−5	−3		0.5	1.0	0.0	0.0	−11	+8
0.125		0.0	0.0	0.0	0.0	−42	+13		0.3	0.2	0.0	0.0	−43	+13
0.50	Leu	2.2	2.2	2.3	2.3	−93	−88	Trp	0.7	1.0	0.3	0.2	−19	−16
0.25		1.0	2.2	1.3	1.5	−56	−65		0.0	0.3	0.0	0.0	−20	0
0.125		0.5	1.0	0.0	0.0	−7	−8		0.0	0.0	0.0	0.0	+10	+5
0.50	Ile	2.2	3.0	2.5	2.7	−91	−96	Cys′	1.5	2.0	1.7	1.7	−79	−46
0.25		1.5	2.0	1.5	1.3	−70	−69		1.3	1.7	0.7	0.5	−66	−5
0.125		0.5	1.7	0.5	0.3	−62	−12		0.0	0.8	0.2	0.5	−28	−5
0.50	Hyp	1.0	1.5	0.0	0.8	−69	−12	2,4-D	3.0	3.7	1.8	3.0	−98	−69
0.25		0.0	1.0	0.0	0.2	−47	0		2.5	3.5	1.3	1.8	−89	−28
0.125		0.2	0.2	0.0	0.0	−20	+7		0.7	1.5	0.3	1.0	−72	−18
0.50	Asp	0.8	1.3	0.5	0.8	−85	−49	Control	0.0	0.0	0.0	0.0	100	100
0.25		0.2	0.8	0.0	0.0	−80	−1		0.0	0.0	0.0	0.0	100	100
0.125		0.0	0.5	0.0	0.0	+7	+18		0.0	0.0	0.0	0.0	100	100

[a] Plants were planted on June 6, 1975 and sprayed on July 1, 1975. Above data represents herbicidal evaluations made on July 17, 1975; peas were picked on July 17, 1975 and beans were picked on July 31, 1975. Plants were arbitrarily judged by three persons as follows: 0, no effect; 1, slight effect; 2, moderate effect; 3, severe effect; and 4, dead.

Table III. One-half Maximum Concentration of Amino Acid Conjugates Which Stimulated Elongation of Avena Coleoptile Sections and Growth of Soybean Cotyledon Callus Tissue[a]

2,4-D or conjugate	Callus, 10^{-3} M	2,4-D or conjugate	Avena, 10^{-3} M
Leu	1.1	Gly	0.1
Glu	1.8	Cys	0.4
Asp	1.9	2,4-D	0.5
Ile	2.0	Pro	0.9
2,4-D	2.1	Ala	1.0
Met	2.1	Leu	1.0
Cys′	2.1	Ile	1.4
Val	2.3	Lys	1.7
Gly	2.4	Met	2.6
Ala	2.4	Phe	3.0
Pro	2.4	Thr	4.0
Thr	2.4	Val	8.0
Cys	2.5	Arg	11.0
Phe	2.5	Tyr	11.0
Tyr	2.6	Cys′	15.0
Arg	2.8	Asp	17.0
Trp	2.8	Trp	36.0
His	5.8	Ser	37.0
Hyp	17.0	Glu	40.0
Lys	20.0	His	130.0
Ser	25.0	Hyp	150.0

[a] Data calculated from *J. Agric. Food Chem.* **22**, 307 (1974).

with the most biological activity tend to be the more nonpolar amino acid conjugates. Perhaps this nonpolar property enhances their permeability which appears to be particularly important with spray application on intact plants. The aromatic and polar amino acid conjugates generally show poor biological activity. These data are also consistent with the biological activity expressed by amino acid conjugates of indolacetic acid (Feung et al., 1977).

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