personnel. In some states the soil-testing laboratory is used as the training ground for graduate students. As soon as one completes his graduate work another moves in. Too, it may be difficult to keep well trained personnel. In one state testing laboratory there were six directors in 10 years, all Ph.D's. Fortunately, more emphasis on the key nature and the importance of the soil testing program is now being given by the administration in most states.

Marked changes in certain aspects of soil testing appear to be in store for the near future. It will take top-flight personnel to stay on top of the problems and to adjust with changing times.

This is a condensed version of the meeting paper. The complete article appeared in the October 1959 issue of Commercial Fertilizer and Plant Food Industry.

END OF SYMPOSIUM ON SOIL TESTING

PLANT GROWTH REGULATORS

Synthesis and Preliminary Evaluation of Amino Acid Derivatives of 2-(2,4,5-Trichlorophenoxy)propionic Acid

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A series of D-, L-, and DL-amino acids involving 2-(2,4,5-trichlorophenoxy)propionic acid has been prepared and evaluated. A wide variety of notable differences in behavior pattern in this new series is in sharp contrast to generalities reported for the halogen-substituted phenoxyacetic acid series. The derivatives of L- and DL-amino acids reported here proved generally to be active plant-growth regulators with high selectivity during the test period on the assay plants used; an exception was the derivative of L-tryptophan, which was completely inactive. The D-amino acid derivatives were almost completely lacking in growth-regulating properties with the exceptions of the D-alanine and D-tryptophan derivatives which showed some degree of activity. Most of these derivatives are easily prepared and purified and those with sharp melting points may be useful in characterization of amino acids.

Aryloxyalkyl carboxylic acids form an important group of synthetic growth-regulating substances now available; of these DL-2-(2,4,5-trichlorophenoxy)propionic acid, designated 2,4,5-TP, has achieved merit. Recently the optical forms of the amino acids derivatized with 2-(2,4-DP) (*4*) have shown a specificity in their mode of action on plant growth, indicating a varying relationship between the optical configuration of the amino acid and the biological activity. The need for further studies using an extended series of these amino acids was evident. Hence the reason for preparing this series of D-, L-, and DL-amino acid derivatives of 2,4,5-TP. This work with 33 new compounds represents an elaboration of previous studies (*4–6*) in which only 18 compounds were prepared for each series.

Many of the compounds in this and in series previously reported (*3–8*) have now been submitted to various agencies for evaluation as anticancer agents, estrogenic substances, fungicides, herbicides, insecticides, and nematocides.

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Experimental

The general procedure used in the preparation of the amino acid derivatives of 2,4,5-TP was that previously described (*5*) utilizing Schotten-Baumann techniques. The 2,4,5-TP used was of technical grade and the amino acids were the best obtainable from commercial sources.

2-(2,4,5-Trichlorophenoxy)propionyl Chloride. A 73% yield of this compound was obtained by the reaction of 2,4,5-TP (1 mole) with thionyl chloride (1.7 moles) essentially as described (*7*). The acid chloride boiled at 111–17° C. and 0.3 mm. of mercury, with a constant index of refraction (n_D^{26} = 1.5601).

An abbreviated description illustrates the general procedure employed (*5*) in the preparation of the amino acid derivatives of 2,4,5-TP.

***N*-[DL-2-(2,4,5-Trichlorophenoxy)-propionyl]-L-methionine.** In the cases involving the preparation of L-methionine, L-leucine, and L-phenylalanine derivatives of 2,4,5-TP, 0.03 to 0.04 molar ratios of amino acid to acid chloride were used. For cystine derivatives ratios were 2 to 1; all other derivatives were synthesized with 1 to 1 proportions. For synthesis of the L-methionine derivative 4.48 grams of the amino acid were used with 11.5 grams of 2-(2,4,5-trichlorophenoxy)-propionyl chloride; this produced a crude yield of 9.80 grams (81.5%) with a melting range of 120° to 128° C. The crude product was dissolved in boiling ethyl acetate and precipitated with petroleum ether (boiling point 63° to 70° C.). The final yield was 7.93 grams (66.0%) melting at 127° to 130.5° C. (Table I).

The most difficult amino acid derivatives to prepare were those of DL-aspartic acid, DL-cystine, and D-, L-, and DL-leucine. These separated as oils and

Table I. Yields, Physical Properties, and Analyses of Amino Acid Derivatives of DL-2-(2,4,5-Trichlorophenoxy) propionic Acid

N-[DL-2-(2,4,5-Trichlorophenoxy) propionyl]-	M.P., °C.[b] (Corr.)	Yield, % Crude	Yield, % Refined	Formula	Analyses[a] Chlorine, % Calcd.	Chlorine, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Optical Rotations	$[\alpha]_D^{25}$	C.g./100 ml., in pyridine
L-Alanine	161.5–163.5	48.3	18.0	$C_{12}H_{12}Cl_3NO_4$	31.23	31.36	4.11	4.09	Levo	13.2 ± 0.6	2.01
D-Alanine	157 –160	61.7	47.5		31.23	31.11	4.11	4.15	Dextro	13.31 ± 0.6	2.02
DL-Alanine	173 –176	54.9	43.8		31.23	31.07	4.11	4.15			
D-Asparagine	155 –157.5[c]	56.3	13.3	$C_{13}H_{13}Cl_3N_2O_5$	27.73	28.19	7.30	6.95	Levo	13.45 ± 0.6	2.00
L-Asparagine	152 –154[c]	33.0	16.9		27.73	27.70	7.30	7.16	Dextro	11.12 ± 0.6	2.55
DL-Asparagine	173 –178[d]	35.6	5.7		27.73	28.44	7.30	6.55			
DL-Aspartic acid[e]	178 –181[f]	24.3	11.4	$C_{13}H_{12}Cl_3NO_6$	27.66	27.63	3.64	3.65			
L-Hydroxyproline	202 –204[f]	32.2	16.7	$C_{14}H_{14}Cl_3NO_5$	27.80	27.32	3.66	3.73	Dextro	9.36 ± 0.4	2.00
L-Isoleucine	113 –116[g]	90.6	62.7	$C_{15}H_{18}Cl_3NO_4$	27.80	27.64	3.66	3.61	Levo	6.97 ± 0.5	2.01
D-Isoleucine	106 –113[g]	34.0	18.6		27.80	27.34	3.66	3.74	Dextro	6.66 ± 0.5	2.00
DL-Isoleucine	100 –104[f]	94.1	33.9		27.80	27.72	3.66	3.64			
L-Leucine	52 – 58[h]	88.0	38.7	$C_{15}H_{18}Cl_3NO_4$	27.80	27.73	3.66	3.65	Levo	17.02 ± 0.5	2.00
D-Leucine	44 – 49[i]		19.3		27.80	27.39	3.66	3.62	Dextro	15.27 ± 0.6	2.00
DL-Leucine	145 –148	76.7	35.1		27.80	27.56	3.66	3.65			
L-Methionine	127 –130.5	81.5	66.0	$C_{14}H_{16}Cl_3NO_4S$	26.55	26.36	3.50	3.47	Levo	7.80 ± 0.5	2.00
D-Methionine	129 –131	46.6	30.1		26.55	26.56	3.50	3.56	Dextro	7.40 ± 0.5	2.00
DL-Methionine	130 –132	57.5	35.1		26.55	26.46	3.50	3.51			
L-Phenylalanine	145 –150[f]	85.6	28.4	$C_{18}H_{16}Cl_3NO_4$	25.53	25.47	3.36	3.36	Dextro	5.87 ± 0.5	2.00
D-Phenylalanine	149 –152	25.2	13.0		25.53	25.48	3.36	3.37	Levo	0.27 ± 0.4	2.01
DL-Phenylalanine	145 –148	51.9	40.3		25.53	25.47	3.36	3.36			
DL-Serine	137 –140	95.3	53.3	$C_{12}H_{12}Cl_3NO_5$	29.83	29.27	3.93	3.86			
L-Threonine	156 –161[f]	85.4	8.4	$C_{13}H_{14}Cl_3NO_5$	28.70	29.03	3.78	3.56	Levo	9.26 ± 0.6	2.00
D-Threonine	153 –155	55.8	12.2		28.70	28.41	3.78	3.73	Dextro	9.82 ± 0.5	2.01
DL-Threonine	133 –135	41.7	32.6		28.70	28.56	3.78	3.81			
L-Tryptophan	171 –173[f]	73.2	17.4	$C_{20}H_{17}Cl_3N_2O_4$	23.34	23.29	6.15	6.20	Levo	4.75 ± 0.6	2.01
D-Tryptophan	169.5–170.5	69.3	32.5		23.34	23.09	6.15	6.08	Dextro	4.38 ± 0.6	2.05
DL-Tryptophan	203 –205	49.9	15.7		23.34	23.23	6.15	6.17			
D-Valine	121 –123[f]	93.2	69.4	$C_{14}H_{16}Cl_3NO_4$	28.85	28.89	3.80	3.79	Dextro	15.16 ± 0.5	2.01
DL-Valine	140 –145[f]	99.5	56.0		28.85	28.57	3.80	3.80			
N,N'-Bis[DL-2-(2,4,5-trichlorophenoxy) propionyl]-											
L-Cystine	121 –123	68.6	40.5	$C_{24}H_{22}Cl_6N_2O_8S_2$	28.62	28.28	3.77	3.65	Levo	92.97 ± 0.4	3.00
D-Cystine	109 –119	86.5	18.2		28.62	27.85[k]	3.77		Dextro	92.6 ± 1.0	3.40
DL-Cystine	136 –138[f]	28.2	17.9		28.62	28.08	3.77	3.76			
DL-Lysine	109 –116	73.7	44.4	$C_{24}H_{24}Cl_6N_2O_6$	32.77	32.25	4.32	4.27			

[a] Analyses by J. S. Ard.
[b] Recrystallized one or more times from ethyl acetate–petroleum ether unless otherwise indicated.
[c] Recrystallized from ethyl alcohol-water previous to ethyl acetate–petroleum ether.
[d] Recrystallized from ethyl alcohol-water and from methanol-water.
[e] Repeated attempts to prepare L- and D-aspartic acid derivatives were unsuccessful.
[f] And from ethyl alcohol–water.
[g] Not recrystallized, but washed with petroleum ether.
[h] And derivatized with cyclohexylamine (*2*).
[i] And from ethyl alcohol–water and from methylene chloride–petroleum ether.
[k] Value before final recrystallization.

hardened either after refrigeration or following evacuation treatment. To achieve chemical and optical purity of these compounds additional recrystallizations were necessary as indicated in the footnotes of Table I.

Preliminary Evaluation as Plant Regulators

Twenty-two of the 33 amino acid derivatives of 2,4,5-TP prepared (Table I) were evaluated for plant growth regulating properties on Pinto bean, large seeded sunflower, and Arlington White Spine cucumber. The lanolin assay technique previously described (*6*) was employed. The compounds evaluated were: the parent acid 2,4,5-TP, the DL-aspartic acid, and D-, L-, and DL-forms of the amino acids alanine, cystine, leucine, methionine, phenylalanine, threonine, and tryptophan derivatives of 2,4,5-TP.

Observations were made 7 to 9 days after treatment and the degree of growth modification induced by the various compounds was estimated and scored as 0, none; 1, slight; 2, moderate; and 3, marked. The responses studied were: stem curvature, growth inhibition, epinasty, formative effects, and induced cell proliferation (gall formation).

Results of Plant Tests

With all the derivatives of 2,4,5-TP except those of tryptophan, the L- and DL- forms were active, although less active, quantitatively, than the parent acid (Table II). In general the D-amino acid derivatives were only slightly active or inactive. Growth inhibition formative effects, and cell proliferation of the hypocotyls of plants treated with the derivatives indicated that translocation of the amino compounds or their biologically active metabolites was similar to that of the parent acid. In contrast, the L-tryptophan amino acid derivatives induced no apparent growth regulation,

Table II. Plant-Growth Modifying Activity of Amino Acid Derivatives of DL-2-(2,4,5-Trichlorophenoxy)propionic Acid on Pinto Bean (*B*), Sunflower (*S*), and Cucumber (*C*)[a]

N- DL-*2-(2,4,5-Trichloro-phenoxy)-propionyl* -	*Stem Curvature*			*Growth Inhibition*			*Epinasty*			*Formative Effects*			*Cell Proliferation*								
													First Internode			*Hypocotyl*			*Treated Area*		
	B	*S*	*C*	*B*	*S*	*C*	*B*	*S*	*C*	*B*	*S*	*C*	*B*	*S*	*C*	*B*	*S*	*C*	*B*	*S*	*C*
L-Alanine	3	1	3[b]	3	3	3	0	3	3	0[b]	3	3[b]	3	0[b]	0	3	2[b]	0	3	2[b]	0
D-Alanine	3	0	0	2	2	3	0	1	0	3	1	0[b]	2	0	0[b]	1	1	1	3	1	1
DL-Alanine	3	0[b]	3[b]	3	3	3	0	0[b]	3[b]	0	1[b]	3[b]	3	0[b]	0[b]	2	2[b]	2[b]	3	2[b]	2[b]
DL-Aspartic acid	3	1	1[b]	1	3	3	0	1	1[b]	0	3	0[b]	2	0	0[b]	2	0	1[b]	3	0	3[b]
L-Cystine[c]	3	3	2[b]	3	3	3	0	2	0[b]	0	3	0[b]	2	0[b]	0[b]	2	0[b]	2[b]	3	3	3[b]
D-Cystine[c]	0	0	2	2	0	2	0	0	0	0	1	1	0	0	0	0	1	1	1	0	0
DL-Cystine[c]	1	1	0[b]	1	2	3	0	1	0[b]	0	2	0[b]	1	1	0[b]	0	0	2[b]	2	0	2[b]
L-Leucine	3	3	3[b]	3	3	3	0	3	3[b]	0[b]	3	3[b]	3	3	0[b]	3	3	1	3	3[b]	0
D-Leucine	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
DL-Leucine	3	3	3[b]	3	3	3	0	3	3[b]	0	3	3[b]	2	2	0[b]	3	1	2[b]	3	2	3[b]
L-Methionine	3	3	1	3	3	3	0	1	1	0[b]	1	3[b]	3	3	0	3	2	0	3	3[b]	0
D-Methionine	0	0	0	2	1	3	0	1	0	0	2	0	1	0	0	0	0	0	2	0	0
DL-Methionine	3	3	2[b]	3	3	3	0[b]	1	1[b]	0[b]	3	0[b]	3	3	0[b]	3	0	2[b]	3	3	2[b]
L-Phenylalanine	2	1	2	2	2	3[b]	0	0	3	0[b]	3	3[b]	3	0	0	3	0	0	3	1	0
D-Phenylalanine	0	0	1	0	0	2	0	0	0	0	1	0	0	0	0[b]	0	0	1	0	0	2
DL-Phenylalanine	2	0	2[b]	1	0	3	0	1	0[b]	0	1	2[b]	0	0	0[b]	1	0	1[b]	3	0	1[b]
L-Threonine	2[b]	2	3[b]	3	3	3	0[b]	2	3[b]	0	1[b]	3[b]	0	0[b]	0[b]	2[b]	1	2[b]	3[b]	2	3[b]
D-Threonine	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DL-Threonine	3	3	2[b]	2	3	3	0	2	1[b]	0	0	3[b]	3	3	0[b]	3	0	1[b]	3	1	2[b]
L-Tryptophan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D-Tryptophan	0	2	2[b]	1	2	3	0	0	1[b]	0	1	3[b]	0	0	0[b]	0	0	1[b]	1	1	2[b]
DL-Tryptophan	1	1	2[b]	3	3	3	0	2	1[b]	3	3	2[b]	1	0	1[b]	0	0	1[b]	3	0	2[b]
Parent acid	2[b]	1[b]	3[b]	2[b]	2[b]	3[b]	1[b]	0[b]	3[b]	0[b]	2[b]	3[b]	0[b]	1[b]	0[b]	2[b]	2[b]	2[b]	3[b]	2[b]	3[b]

[a] Evaluations made 7 to 9 days after treatment. 0, no effect; 1, slight effect; 2, moderate effect; 3, marked effect.
[b] Response obtained with a mixture containing 1% of compound was so marked that evaluation of responses listed could not be made and it was necessary to retest the compound at 0.1% concentration. Figures, therefore, represent responses obtained with 0.1% concentration.
[c] *N,N'*-Bis[DL-2-(2,4,5-trichlorophenoxy)propionyl]-derivative.

while the D- and DL- forms were active.

Comparison of the tryptophan and threonine derivatives illustrates the wide difference of growth responses caused by variation of optical configuration of the amino acid derivatives.

Discussion

Growth-modifying properties of the amino acid derivatives of 2,4,5-TP demonstrate that there is, in general, lower activity for the majority of D-, L-, and DL- compounds of this series than for those reported for the halogenated phenoxyacetyl compounds such as 4-chloro- (*5*), 2,4-dichloro- (*8*), and 2-methyl-4-chlorophenoxyacetyl derivatives (*6*). The behavior pattern of the 2,4,5-TP compounds follows somewhat that of the amino acid series of the 2-(2,4-dichlorophenoxy)propionic acid, 2-(2,4-DP) (*4*), in that plants for the most part showed little or no response to D-amino acid derivatives of either series. However, the selective and widely variable behavior of the L- and the relatively inexpensive DL-amino acid forms makes these 2,4,5-TP compounds, like those of the 2-(2,4-DP) series, exceptionally attractive from the standpoint of further study.

In a previous paper (*4*) it was pointed out that considerable weight had been given to the idea that plant-growth response in the case of the L- and DL-amino acid derivatives of halogen-substituted phenoxyacetic acids (*5*, *6*, *8*) depends upon the presence of cellular hydrolytic enzymes which are capable of splitting the amide linkage to free the carboxyl group (considered essential for activity) of the substituted phenoxy acid. Also, it was mentioned that the assay plants may be partially or completely incapable of splitting the D-amide linkage. Although the results obtained with the amino acid derivatives of 2-(2,4-DP) (*3*, *4*) and 2,4,5-TP to some extent support these ideas, the data obtained with the phenoxypropionic acid series seem to lend some support tc another concept concerning mode of action—that is, activity may be due to effect per se; it may depend upon the translocation and specificity of the whole intact molecule. The almost complete inactivity of the D-amino acid isomers of the phenoxypropionic acid series evaluated, and the lowered, but selective, activity of the L- and DL-isomers are the contributory factors to this concept.

Acknowledgment

The authors appreciate and acknowledge the assistance of Eleanor Daly in conducting the plant tests involving the plant-regulating activity of these compounds.

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Received for review July 8, 1959. Accepted October 26, 1959.