General Twenty-three of the 35 samples (66%) were correctly rated in relation to the nitrification index by all three solubility index procedures, 24 (69%) by neutral permanganate activity, 28 (80%) by activity index, *AI*, and 30 (86%) by the solubility pattern index.

Of the 12 samples incorrectly rated by one or more of the solubility indexes, 11 (92%) were incorrectly rated by neutral permanganate activity, seven (58%) by activity index, and five (42%) by the solubility pattern. Of these 12, nine exhibited inferior nitrification characteristics, with two of the nine being borderline cases with values of 27.7 and 28.6, respectively. Of the three exhibiting acceptable nitrification indexes, two were borderline cases with values of 33.1 and 33.2.

Of the eight nonborderline cases, one was superior, with a nitrification index of 37.3. All solubility indexes for this sample were borderline but slightly below the stated minima. The other seven nonborderline cases were definitely inferior with nitrification indexes in the range 5.7 to 22.7. Six of the seven were correctly rated by the solubility pattern index, four by the activity index, and none by the neutral permanganate activity

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GROWTH REGULATORS

Amino Acid Derivatives of 4-Chlorophenoxyacetic Acid and Their Plant-Regulating Effects in Preliminary Screening Tests

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Continued interest in elucidating the mode of action of plant-growth regulators and in compounds having greater selective activity has led to the preparation and evaluation of derivatives of amino acids and other plant constituents. A series of new D-, L-, and DL-amino acid derivatives of 4-chlorophenoxyacetic acid was prepared and screened for plant-growth regulating activity. In general, the derivatives of DL- and L-amino acids proved to be active plant-growth regulators when tested on Black Valentine bean, sunflower, cucumber, barley, and corn plants; those of D-amino acids were less active and more selective. A notable exception to this generalization was the D-alanine derivative, which was as active as the L-isomer. The reason for the high activity of the D-alanine derivative has not been explained, but it is recognized that certain microorganisms are able to utilize D-alanine.

CRITICAL EVALUATION OF 4-CHLOROPHENOXYACETIC ACID (referred to sometimes as 4-ClPOA, CPA, 4-C, and Parachloro) as a herbicide is difficult. Results of commercial testing have not been sufficiently conclusive to justify specific recommendations for herbicidal use. The activity of 4-chlorophenoxyacetic acid in this respect requires further investigation (*5*). It has been stated (*19*), however, that the use of this compound has not been as great as 2,4-dichloro- or 2,4,5-trichlorophenoxyacetic acid because its action is milder.

As a selective growth regulator, 4-chlorophenoxyacetic acid appears to hold forth considerable promise. Weintraub, Brown, and Throne (*24*) recently reported that the compound has high formative effects as measured by tests of molar leaf area repression activity (MOLARA).

Zimmerman and Hitchcock (*29*) were among the first to demonstrate the growth-modifying properties of 4-chlorophenoxyacetic acid. They described this compound as less active than 2,4-dichlorophenoxyacetic acid in inducing cell elongation of tomato, as having ability to induce adventitious roots, and ability to induce parthenocarpy when applied to flowers of tomato and cucumber. Marre and Murneek (*11*) found that 4-chlorophenoxyacetic acid, applied to the cut surface of the style of emasculated tomato flowers, produced effects similar to those induced by pollination and fertilization—i.e., it stimulated the accumulation of starch and sugar. Moore and Thomas (*15*) state that 4-chlorophenoxyacetic acid appears to be a practical aid to growers

in obtaining later summer or early fall tomatoes. Parsons and Davis (*17*) found that the low yield caused by excessive nitrogen fertilization of tomatoes was partially overcome by hormone spraying with 4-chlorophenoxyacetic acid. Coombe (*2*) found 4-chlorophenoxyacetic acid the most effective among a number of compounds tested in increasing fruit set, berry size, and bunch weight of Zante currants, its effect being comparable to cincturing. The compound has been used to increase the size of Thompson seedless grapes (*21*, *22*), to decrease the physiological breakdown and improve the quality of pineapple fruits (*12*), to induce gall and root formation and to produce parthenocarpic fruit (*9*), to hasten fruit set and increase the yield of greenhouse tomatoes (*8*, *18*, *28*), to prevent abscission of lemon leaves (*4*), to induce parthenocarpy in the Calmyrna fig (*3*), to delay petal abscission of dogwood and certain species of flowering cherries (*25*), to increase the water-retaining capacity of plant parts (*14*), and to delay breakdown of vitamin C in stored snap beans (*13*). Information on the toxicity of 4-chlorophenoxyacetic acid and a review of toxicity studies on other chlorophenoxy acids are given in a recent paper by Wilson (*26*).

In spite of much excellent research and thoughtful theorizing, the mode of action of the halogenated phenoxy acids is not fully understood. Some work in these laboratories (*10*, *27*), showing differences in growth-regulating properties among D- and L-forms of amino acid derivatives of 2,4-dichloro- and 2-methyl-4-chlorophenoxyacetic acids, suggests that a split in the amide linkage may be necessary before a growth regulator of this type can exert its effect. The halogenated phenoxyacetyl derivatives of L-amino acids were found to possess the same order of activity as the parent acids; the D-compounds possess a decreased activity. Weintraub (*23*) states that the amide bond of a natural (L-) amino acid derivative may be readily hydrolyzed by a cellular plant enzyme, whereas the unnatural (D-) form may be more resistant to attack. Such hydrolysis might be necessary in order to free the carboxyl group of the phenoxy-type compound for reaction with a plant protein.

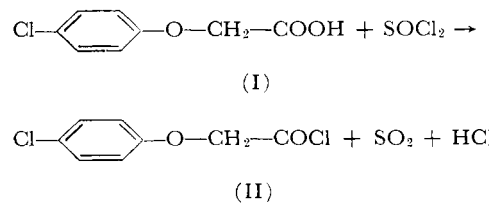
Especially because of the interesting growth effects reported for 4-chlorophenoxyacetic acid and the continued interest in the mode of action and specificity of plant-growth regulators, a series of D-, L-, and DL-amino acid derivatives of this compound has been synthesized and screened for growth responses. The new compounds are listed in Table I.

Experimental

The general procedure used for the preparation of the 4-chlorophenoxyacetyl derivatives of amino acids is similar to the one described by Krewson and others (*10*) for *N*-(2-methyl-4-chlorophenoxyacetyl)-D-leucine. The coupling was carried out using the Schotten-Baumann reaction.

Materials Used. The 4-chlorophenoxyacetic acid used in this work was supplied through the courtesy of the Dow Chemical Co. and the Monsanto Chemical Co. (melting point 156–8° C.) and was used without further purification. The amino acids were the best obtainable from commercial sources and were used as received.

4-Chlorophenoxyacetyl Chloride. This compound was prepared in 85% yield by the reaction of 4-chlorophenoxyacetic acid (I) (1 mole) with thionyl chloride (1 mole); the method used was essentially that described by Freed (*7*).



The acid chloride (II) boiled at 92.0° C. at 1.0 mm., with constant index of refraction (n_D^{25} = 1.5463).

Analysis calculated for $C_8H_6Cl_2O_2$: C, 46.86; H, 2.95; Cl, 34.58. Found: C, 47.07; H, 3.04; Cl, 35.40.

The following description is included to illustrate in detail the general procedure employed in the preparation of the amino acid derivatives.

***N*-(4-Chlorophenoxyacetyl)-L-phenylalanine.** L-Phenylalanine (III) (1.65 grams, 0.01*M*) was dissolved in 30 ml. of 1*N* sodium hydroxide (0.03*M*), and the mixture was chilled in an ice bath to 5° C. 4-Chlorophenoxyacetyl chloride (2.05 grams, 0.01*M*) was dissolved in 25 ml. of benzene; the mixture

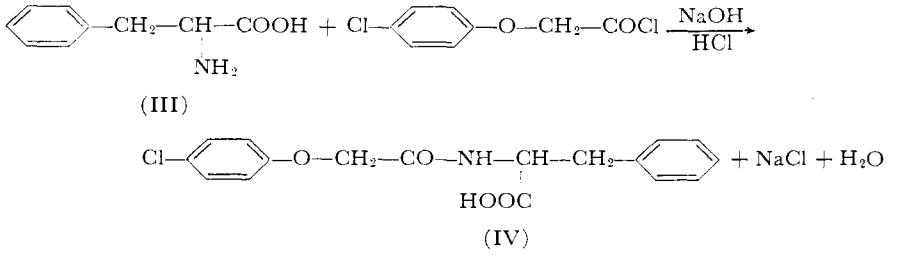
Table I. Yields, Physical Properties, and Analyses of 4-Chlorophenoxyacetyl Derivatives of Amino Acids

N-(4-Chlorophenoxyacetyl)-	M.P., °C.[a] (Corr.)	Yield, % Crude	Yield, % Refined	Formula	Analyses[b] Cl, % Calcd.	Cl, % Found	N, % Calcd.	N, % Found	Optical Rotation[c] $[\alpha]_D^{25}$	Optical Rotation[c] C (g./100 ml.) in pyridine
L-Alanine	144.0–145.0	66.0	47.9	$C_{11}H_{12}ClNO_4$	13.76	13.76	5.43	5.49	+16.92 ± 0.4	2.49
D-Alanine	142.5–143.5	63.5	40.2		13.76	13.82	5.43	5.26	−17.31 ± 0.4	3.40
DL-Alanine	157.0–158.0[d]	71.8	36.9		13.76	13.77	5.43	5.34		
L-Aspartic acid	144.0–145.0[d]	42.6	23.3	$C_{12}H_{12}ClNO_6$	11.75	11.73	4.64	4.64	+22.91 ± 0.5	3.12
D-Aspartic acid	144.0–145.0	66.2	31.5		11.75	11.70	4.64	4.60	−23.20 ± 0.5	2.97
DL-Aspartic acid	187.0–188.0	65.4	26.5		11.75	11.79	4.64	4.72		
L-Leucine	119.5–120.5[e]	83.7	66.6	$C_{14}H_{18}ClNO_4$	11.83	11.94	4.67	4.61	+ 8.23 ± 0.5	2.56
D-Leucine	122.0–123.0	68.3	60.0		11.83	11.97	4.67	4.61	− 8.04 ± 0.5	2.71
DL-Leucine	124.5–125.5[d]	45.5	41.6		11.83	11.95	4.67	4.54		
L-Methionine	111.0–112.0[d]	86.4	51.2	$C_{13}H_{16}ClNO_4S$	11.16	11.32	4.41	4.40	+ 7.13 ± 0.4	3.01
D-Methionine	112.0–113.0	66.0	59.7		11.16	11.06	4.41	4.29	− 5.96 ± 0.5	2.76
DL-Methionine	115.0–117.0	74.8	44.0		11.16	11.00	4.41	4.20		
L-Phenylalanine	144.0–145.0	82.3	67.3	$C_{17}H_{16}ClNO_4$	10.62	10.69	4.19	4.18	+15.77 ± 0.3	5.01
D-Phenylalanine	145.0–146.0	61.2	34.4		10.62	10.57	4.19	4.42	−16.04 ± 0.4	2.93
DL-Phenylalanine	151.8–152.5	65.8	27.2		10.62	10.69	4.19	4.08		
L-Threonine	131.0–132.0	64.8	23.4	$C_{12}H_{14}ClNO_5$	12.36	12.12	4.86	4.83	+25.55 ± 0.6	2.23
D-Threonine	131.0–132.0	71.3	20.0		12.36	12.70	4.86	4.78	−25.70 ± 0.3	3.34
DL-Threonine	150.5–151.5	56.0	19.4		12.36	12.54	4.86	4.72		

[a] Recrystallized from ethyl acetate–petroleum ether unless otherwise indicated.
[b] Analyses by M. J. Bythrow and R. B. Kelly.
[c] Optical rotations by J. S. Ard.
[d] Required one additional recrystallization from 50% alcohol.
[e] Recrystallized from 50% alcohol.

was chilled, and then added dropwise with mechanical stirring to the alkaline L-phenylalanine solution. The mixture was stirred for 3 hours while it was allowed to come to room temperature and was then extracted with three 40-ml. portions of ether in a separatory funnel. The ether fractions were combined and washed with 30 ml. of distilled water. The water washing of the ether fraction was added to the alkaline aqueous solution of the product. The alkaline solution was acidified to pH 2 with 1*N* hydrochloric acid. A white crystalline precipitate appeared during the addition of the hydrochloric acid at pH 3 to 4. After cooling in the refrigerator for 2 hours, the *N*-(4-chlorophenoxyacetyl)-L-phenylalanine (IV) was filtered off, slurried three times with water, filtered off after each slurry, and then left overnight in a vacuum desiccator which was continuously evacuated by vacuum pump. The crude yield was 2.75 grams (82.3%), melting point 126–8° (Kofler micro melting point apparatus). The product was dissolved in hot ethyl acetate and precipitated with petroleum ether (boiling range 63° to 70° C.). The final yield was 2.25 grams (67.3%), melting point 129–130° C.

Analysis. Calculated for $C_{17}H_{16}ClNO_4$: Cl, 10.62; N, 4.19. Found: Cl, 10.69; N, 4.18.



(III)

(IV)

Preliminary Screening Tests

4-Chlorophenoxyacetyl derivatives of the D-, L-, and DL-alanine, aspartic acid, leucine, methionine, phenylalanine, and threonine and the parent halogenated phenoxyacetic acid, were screened for plant-growth-modifying activity utilizing the dicotyledonous plants, Black Valentine bean, Mammouth Russian sunflower, and Arlington White Spine cucumber, and the monocotyledonous plants, Wong barley, and U. S. 13 Hybrid corn. The lanolin assay method (*10*) was used on the dicots and the coated sand assay method (*10*) was used on the monocots.

At intervals of 2, 4, 6, and 14 days following treatment, the degree of growth modification induced by the various compounds was estimated and scored according to the intensity of growth responses. The following responses were considered: stem curvature, epinasty, formative effects, induced cell proliferation (gall formation), and growth inhibition. In Table II the scores for the 14th day's observations are given; to condense the data, the other scores have been omitted. Although these data do not show the rate of response or relative progressive effectiveness of the compounds tested upon growth, they are representative. The percentage of plants killed by the compounds is also listed, but such figures are not intended to reflect herbicidal potentialities of the compounds, because the tests were not designed for this purpose. Corn and barley plants were not killed by these tests.

The scores covering second, fourth, sixth, and 14th day observations for the various responses to treatment with each D- and L-amino acid compound were added and expressed as a percentage of the maximum response possible in the manner described previously (*10*). Because of the voluminous nature of these results, the complete tabulation does not appear in this paper. However, the added scores for the various responses have been used to calculate index values, which are presented in Table III.

The index values for the DL-amino acid compounds have not been reported, for the reason that relatively high concentrations of each chemical were applied in order to obtain maximum effects in every case. At these high concentrations, the DL- compounds had index values which were comparable to the L-compounds.

Results of Plant Screening

The dicotyledonous plants tested showed a much greater response to the L-amino acid derivatives than to the D- derivatives with the notable exception of the D-alanine derivative, which was as active as its L- isomer.

Barley, a monocotyledonous plant, was slightly less affected by the D-amino acid derivatives. The response of corn, the other monocotyledonous plant tested, towards these amino acid derivatives is very similar to responses shown by corn in the previous series (*10*). The fact that corn responds to all the amino acid derivatives to the same or greater degree than it responds to the parent acid may be of some significance.

Discussion

Screening tests on the growth-modifying effects of 4-chlorophenoxyacetyl amino acids on monocotyledonous and dicotyledonous plants generally confirm those reported previously for 2,4-dichlorophenoxyacetyl and 2-methyl-4-chlorophenoxyacetylamino acids (*10*, *27*). Where there is plant-growth response in the case of these phenoxyacetyl derivatives of amino acids, the carboxyl group, depending upon the degree of hydrolysis of the amide bond, would be free to act with the terminal amino group of an enzyme protein in the manner suggested by Muir and Hansch (*16*). Although Muir and Hansch propose that, following this coupling, a cyclization occurs with the loss of hydrogen from the ortho position of the aryl ring and the sulfhydryl group, their theory does not explain the recent findings of Wain (*20*) that 2,4-dichloro-6-fluorophenoxyacetic acid is active, although it would explain the inactivity of 2,4,6-trichlorophenoxyacetic acid as reported by Weintraub, Brown, and Throne (*24*).

It seems likely from current results and those reported earlier (*10*, *27*) that the toxic effects of halogenated phenoxy acids are due in part to the free carboxyl group and not to the carbonyl of the potential carboxyl group. With the exception of D-alanine, the D-amino acid derivatives did not cause appreciable injury at the concentration level used in the present experiments, but they did show other types of response to varying degrees. It may be that the D-amino acid derivatives are competing with natural plant auxins, in which case it is possible that the carbonyl of the potential carboxyl group could play a significant role. In view, however, of the marked activity of the D-alanine derivative of the 4-chloro compound, it would appear that plant enzyme systems are sometimes capable of hydrolyzing the amide bond of D-amino acid derivatives, but such action may be preceded by enolization and racemization. Labeled D-amino acids could be used profitably in investigations of this type. In the plant, utilization of the D-amino acid may follow the course suggested (*1*) for its metabolism in the animal—that is, it may not be readily hydrolyzed by enzymes but may be attacked by D-amino acid oxidase to produce an optically inactive α-keto acid which may undergo inversion through amination of the latter to the L-amino acid configuration. Such an inversion may be slow and highly selective, depending upon the plant being treated, and the physical and chemical properties of the D-amino acid type growth regulator. The activity of an amino acid derivative of the halogenated phenoxy acid-type could be due to its action per se—that is, activity may depend upon the translocation and specificity of the whole intact molecule. Further investigations are in progress which seem to confirm this viewpoint.

Table II. Plant-Growth Regulating Activity of 4-Chlorophenoxyacetyl Derivatives of Amino Acids on Black Valentine Bean (VB), Sunflower (S), Cucumber (C), Barley (B), and Corn (Cn)

N-(4-Chlorophenoxyacetyl)-	Lanolin Method[a]																		Coated Sand Method[a]					
	Stem Curvature			Growth Inhibition			Epinasty		Formative Effects			Cell Proliferation: First internode	Cell Proliferation: Hypocotyl	Cell Proliferation: Treated area		Dead, %			Stem curvature		Growth inhibition		Formative effects,	Cell proliferation treated area,
	VB	S	C	VB	S	C	S	C	VB	S	C	VB	VB	VB	S	VB	S	C	B	Cn	B	Cn	Cn	Cn
Parent acid	4	4	4	4	4	4	4	4	4	4	4	4	4		4	100	100	100	0	0	3	0	0	0
L-Alanine	4	4	4	4	4	4	4	4	4	4	4	4	4		4	100	100	100	0	0	3	2	0	1
D-Alanine	3	4	4	3	4	4	4	4	4	4	4	3	3		4	67	100	100	0	0	3	2	1	2
DL-Alanine	4	4	4	4	4	4	4	4	4	4	4	4	4		4	100	100	100	0	0	3	2	1	1
L-Aspartic acid	1	4	4	3	4	4	4	4	4	4	4	3	3		4	0	100	100	0	3				
D-Aspartic acid	1	1	0	1	0	1	0	0	2	2	2			1	0	0	0	0	0	0	1	0	0	0
DL-Aspartic acid	2	2	2	3	3	3	1	0	4	3	3	3	0		3	0	0	33	0		2			
L-Leucine	3	4	4	3	4	4	4	4	4	4	4	3	3		4	33	100	100	0	0	2	1	0	0
D-Leucine	0	0	0	1	1	1	0	0	3	3	2	0	0	0	1	0	0	0	0	0	1	0	0	0
DL-Leucine	2	4	1	3	4	3	4	1	4	4	3	3	0		4	0	100	33	0	0	2	1	0	0
L-Methionine	3	4	4	3	4	4	4	4	4	4	4	3	3		4	33	100	100	0	1	1	2	1	0
D-Methionine	2	0	1	2	2	2	0	0	3	3	3	3	0		1	0	0	0	0	0	1	0	0	0
DL-Methionine	3	0	4	3	3	4	0	4	4	3	4	3	3		3	0	0	100	0	1	1	0	1	0
L-Phenylalanine	3	4	4	3	4	4	4	4	4	4	4	3	3		4	33	100	100	0	1	3	2	0	1
D-Phenylalanine	0	0	0	0	0	0	0	0	1	1	1	0	0		0	0	0	0	0	0	2	0	0	0
DL-Phenylalanine	3	4	4	3	4	4	4	4	4	4	4	3	3		4	0	100	100	0	0	3	1	0	0
L-Threonine	3	4	4	3	4	4	4	4	4	4	4	3	3		4	67	100	100	0	1	3	2	1	0
D-Threonine	0	0	0	2	0	1	0	0	3	2	2	0	0	0	0	0	0	0	0	0	1	2	0	0
DL-Threonine	3	3	4	3	3	4	2	4	4	3	4	3	3		3	0	33	100	0	0	3	1	1	0

[a] 14 days after treatment (*10*). 0, no effect; 1, slight effect; 2, moderate effect; 3, marked effect; 4, response could not be recorded because of inhibited growth or death of plants.

Table III. Index Values[a] of Growth-Modifying Properties of 4-Chlorophenoxyacetyl Derivatives of D- and L-Amino Acids

Amino Acid	Bean		Sunflower		Cucumber		Barley		Corn	
	D	L	D	L	D	L	D	L	D	L
Alanine	87	97	96	96	95	95	39	56	44	41
Aspartic acid	34	74	16	68	16	74	28	61	22	..
Leucine	21	84	16	86	11	92	17	50	15	37
Methionine	66	89	22	74	21	95	6	39	22	59
Phenylalanine	3	87	2	84	3	95	11	72	15	44
Threonine	16	87	4	86	8	97	22	72	22	30
Parent acid	100		96		100		78		19	

[a] Index values represent estimated percentage of maximum effectiveness based on stem curvature, epinasty, cellular proliferation, formative effects, and suppression of vegetative growth at 2, 4, 6, and 14 days (*10*).

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