

PLANT GROWTH REGULATORS

Preliminary Screening Tests of Amino Acid Derivatives of 2-(2',4'-Dichlorophenoxy)propionic Acid

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Because the optical configuration of compounds affecting plant growth is important to their selectivity, a series of new amino acid derivatives of 2-(2',4'-dichlorophenoxy)propionic acid in their D-, L-, and DL- forms has been synthesized and screened for effectiveness as plant-growth regulators. This investigation was undertaken specifically to widen the possibilities for utilization of amino acids, and in general to elucidate the mode of action of growth regulators. The derivatives of DL- and L-amino acids proved generally to be active plant-growth regulators with high selectivity; those of the D-amino acids were almost completely lacking in growth-regulating properties during the test period. A variety of notable differences in behavior patterns are characteristic of this series, in sharp contrast to generalities reported for other halogenated phenoxy acid series. These derivatives are easily prepared and analyzed, and possess sharp melting points; hence they may be useful in the characterization of amino acids.

THE GROWTH-REGULATING COMPOUND 2-(2',4'-dichlorophenoxy)propionic acid (2,4-DP) is of exceptional interest, although its commercial production does not compare with that of other herbicides of this type (14). It was among the first of the original phenoxy compounds tested for effects upon plant growth by Hitchcock and Zimmerman (4) in 1942. At this time these investigators stated that 2-(2',4'-dichlorophenoxy)propionic and butyric acids possessed 30 to 100 times the root-inducing activity of indolebutyric, 1-naphthaleneacetic, and 2,4-dichlorophenoxyacetic (2,4-D) acids. Later these investigators (5) tested 63 phenoxy compounds and reported that only four had high root-inducing activity: 2-(2',4'-dichlorophenoxy)-, 2-(2',4'-dibromophenoxy)-, and 2-(2',4',5'-trichlorophenoxy)propionic acids, and 2,4,5-trichlorophenoxyacetic acid.

Several investigators (7, 15) have reported 2,4-DP to have formative effects upon plant growth.

Osborne and Wain (17) used the compound as one in a series of α -aryloxy derivatives to study their effects on plant growth, in an attempt to demonstrate that the hydrogen atom on the carbon atom adjacent to the carboxyl group is necessary for certain types of growth-regulating activity.

Simultaneously several groups of in-

vestigators (2, 8, 9, 12, 13) have reported stereochemical studies involving the separation of DL-2-(2',4'-dichlorophenoxy)propionic acid into its D- and L- optical isomers and the effects of these enantiomorphs upon plant growth. In general, these workers are in agreement that the D(+) configurative form has the greatest plant-regulating activity; the racemic DL-form has less [Collins and Smith (2) state that it has about one half the activity of the D-form], whereas the L(-) isomer possesses only negligible activity.

Because previous work in these laboratories (6, 7, 10, 16) had demonstrated the importance of optical configuration upon plant growth-regulating properties, it was thought advisable to synthesize a D-, L-, and DL-amino acid series of DL-2-(2',4'-dichlorophenoxy)propionic acid. [The melting point of *N*-(2-methyl-4-chlorophenoxyacetyl)-D-phenylalanine was erroneously reported as 125.5-126.5° C. (7). Correct melting point is 142.0-143.0° C.] The coupling of amino acids with this racemic compound produces derivatives containing an additional asymmetric carbon atom and a longer side chain. These new compounds, which are listed in Table I, were prepared to demonstrate the use of amino acids and to elucidate the mode of action and specificity of the aryloxyalkyl-carboxylic acids as plant-growth regulators.

Experimental

For the preparation of amino acid derivatives of 2,4-DP, procedures similar to those previously described (7) were employed. The amino acids used in this work were the best obtainable through commercial sources. The 2',4'-DP was supplied through the courtesy of the Monsanto Chemical Co. and the Dow Chemical Co. These compounds were utilized without further purification.

2-(2',4'-Dichlorophenoxy)propionyl Chloride. This intermediate was prepared by the reaction of 2-(2',4'-dichlorophenoxy)propionic acid (I) (1 mole) with thionyl chloride (1 mole) as described in detail by Freed (3).

The yield of acid chloride (II) was 90.4%; it boiled at 76° C. at 0.1 mm., with constant index of refraction ($n_D^{25} = 1.5440$).

Analysis	C	H	Cl
Calcd. for $C_9H_7O_2Cl_2$			
Cl ₂	42.59	2.74	41.96
Found	43.25	3.23	41.97

The following description is illustrative of the procedure employed in the preparation of all the amino acid derivatives of 2-(2',4'-dichlorophenoxy)propionic acid.

2-(2',4'-Dichlorophenoxy)-N-propionyl-D-alanine. To D-alanine (III) (4.45 grams, 0.05M) dissolved in 150

Table I. Yields, Physical Properties, and Analyses of 2-(2',4'-Dichlorophenoxy)propionyl Derivatives of Amino Acids

2-(2',4'-Dichlorophenoxy)- <i>N</i> -propionyl-	M.P., ° C. ^a (Corr.)		Yield, %		Formula	Analyses ^b				Optical Rotation ^c	
						Chlorine, %		Nitrogen, %			
						Crude	Refined	Calcd.	Found	Calcd.	Found
L-alanine	144.5-146.5	82.6	42.5	C ₁₂ H ₁₃ Cl ₂ NO ₄	23.16	22.64	4.57	4.57	-1.30 ± 0.6	3.04	
D-alanine	143 -144	81.9	45.6		23.16	23.35	4.57	4.43	+0.83 ± 0.5	2.96	
DL-alanine	156 -158 ^d	83.0	24.5		23.16	23.22	4.57	4.48			
L-aspartic acid	184 -187	50.7	11.0	C ₁₃ H ₁₃ Cl ₂ NO ₆	20.25	20.12	4.00	3.95	+28.11 ± 0.6	2.74	
D-aspartic acid	192 -195	50.3	10.0		20.25	19.96	4.00	4.00	-28.7 ± 0.5	3.04	
DL-aspartic acid	184 -187 ^d	55.4	31.0		20.25	19.83	4.00	3.78			
L-leucine	145 -146	71.6	9.0	C ₁₅ H ₁₉ Cl ₂ NO ₄	20.30	20.35	4.01	3.96	-33.3 ± 0.5	3.23	
D-leucine	145 -147 ^d	60.0	24.8		20.30	20.41	4.01	4.00	+34.3 ± 0.6	3.00	
DL-leucine	147 -148.5 ^d	72.8	23.1		20.30	20.36	4.01	3.75			
L-methionine	95 -97 ^e	67.2	5.0	C ₁₄ H ₁₇ Cl ₂ NO ₄ S	19.36	19.20	3.82	3.85	-11.2 ± 0.6	2.20	
D-methionine	101 -103 ^e	64.7	5.0		19.36	19.30	3.82	3.82	+22.0 ± 0.6	2.16	
DL-methionine	95 -97 ^d	...	14.5		19.36	19.00	3.82	3.80			
L-phenylalanine	150 -152	66.3	16.1	C ₁₈ H ₁₇ Cl ₂ NO ₄	18.55	18.49	3.66	3.52	+7.63 ± 0.5	3.19	
D-phenylalanine	149 -150	70.1	10.8		18.55	18.64	3.66	3.67	-7.48 ± 0.5	3.16	
DL-phenylalanine	156 -158 ^d	72.8	23.1		18.55	18.53	3.66	3.59			
L-threonine	138 -140	62.5	9.0	C ₁₃ H ₁₅ Cl ₂ NO ₅	21.09	20.94	4.16	4.18	-24.9 ± 0.5	3.07	
D-threonine	138 -140	20.1	9.1		21.09	21.26	4.16	4.09	+22.6 ± 0.5	3.11	
DL-threonine	140 -142 ^e	76.0	21.9		21.09	21.26	4.16	3.91			

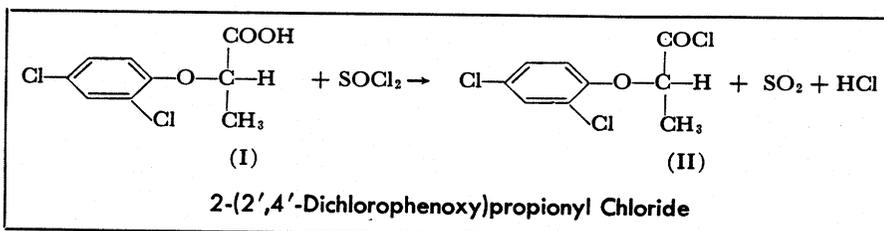
^a Recrystallized from 50% ethyl alcohol and once or more from ethyl acetate-petroleum ether unless otherwise indicated.

^b Analyses by Joan Farquhar.

^c Optical rotations by J. S. Ard.

^d Recrystallized once or more from ethyl acetate-petroleum ether.

^e Recrystallized from ethyl acetate-petroleum ether and from methylene chloride-*n*-hexane.



ml. of 1*N* sodium hydroxide was added dropwise, at ice-bath temperature, with continuous stirring, the 2-(2',4'-dichlorophenoxy)propionyl chloride (II) (12.7 grams, 0.05*M*) dissolved in 125 ml. of benzene.

After the benzene solution was added, the mixture was stirred mechanically for 3 hours, as it was warmed to room

temperature. The reaction mixture was then extracted with three 50-ml. portions of ether in a separatory funnel. The ether portions were combined and washed with 50 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 with 1*N* hydrochloric acid

solution using Congo red test paper as an indicator. After being cooled for 2 hours in a refrigerator, the white crystalline product of 2-(2',4'-dichlorophenoxy)-*N*-propionyl-*D*-alanine (IV) was filtered off, slurried three times with water, filtered off following each slurry, and finally left overnight in a vacuum desiccator continuously evacuated by a vacuum pump. The product was ground and washed three times with small portions of warm petroleum ether. The crude yield was 12.53 grams (81.9%), melting point 112° to 121° C. Following recrystallization from 50% ethyl alcohol, the melting point was 136° to 144° C. The product was then dissolved in a minimum of hot ethyl acetate and precipitated with petroleum ether (boiling range 63° to 70° C.). The final

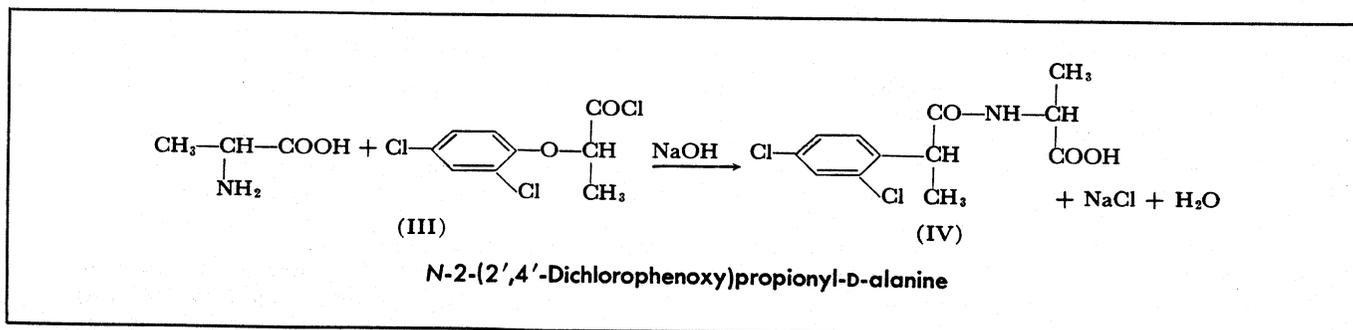


Table II. Plant Growth-Regulating Activity of 2-(2',4'-Dichlorophenoxy)propionyl Derivatives of Amino Acids on Black Valentine Bean (VB), Sunflower (S), Cucumber (C), Barley (B), and Corn (Cn)^a

2-(2',4'-dichlorophenoxy)-propionyl	Lanolin Method															Coated Sand Method												
	Stem curvature			Growth inhibition			Epinasty			Formative effects			Cell Proliferation															
	VB	S	C	VB	S	C	S	C	S	VB	S	C	VB	S	C	VB	S	C	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	4	4	4	4	100	100	100	2	1	3	1	1	0	3
L-alanine	4	3	4	4	3	4	3	4	4	4	4	4	4	4	3	4			100	60	100	2	1	2	1	0	0	3
D-alanine	0	0	0	2	0	0	0	0	0	3	3	0				0	1	0	0	0	0	0	0	1	1	0	1	
DL-alanine	3	4	4	3	4	4	4	4	4	4	4	4	3	3		4	4		80	100	100	1	1	2	1	0	0	3
L-aspartic acid	0	0	0	2	0	0	0	0	0	3	3	1	0	0		2	0	0	0	0	0	0	0	0	1	0	1	
D-aspartic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0		1	0	0	0	0	0	0	0	1	1	0	1	
DL-aspartic acid	0	0	0	0	0	0	0	0	0	1	2	0	0	0		2	0	0	0	0	0	0	0	1	1	0	2	
L-leucine	4	4	4	4	4	4	4	4	4	4	4	4	4	4		4	4	4	100	100	100	0	2	2	2	1	0	2
D-leucine	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	1	
DL-leucine	3	3	4	3	3	4	1	4	4	4	4	4	3	3		4	3		80	60	100	1	0	2	1	0	0	3
L-methionine	2	2	2	3	2	3	0	0	0	0	1	4	3	0		3	3	0	0	0	50	0	0	0	1	0	3	
D-methionine	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	1	
DL-methionine	2	2	2	3	2	3	0	0	0	4	1	4	3	0		3	3	0	0	0	80	0	0	0	1	0	3	
L-phenylalanine	0	0	0	2	1	0	0	0	0	3	3	2	0	0		2	1	1	0	0	0	0	0	0	0	0	1	
D-phenylalanine	0	0	0	0	0	0	0	0	0	0	0	0	0	0		1	0	0	0	0	0	0	1	1	1	0	2	
DL-phenylalanine	0	0	2	3	0	2	0	0	0	1	0	0	0	0		3	3	1	0	0	0	0	1	0	1	0	3	
L-threonine	4	1	4	4	3	4	1	4	4	4	3	4	4	4		4	3	100	40	100	2	1	2	1	0	0	3	
D-threonine	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	1	0	0	1	
DL-threonine	3	2	4	3	2	4	1	4	4	3	4	3	3	3		4	3	80	0	100	1	1	1	1	1	0	3	

^a 14 days after treatment. See (7) for description of method. 0, no effect; 1, slight effect; 2, moderate effect; 3, marked effect; 4, response not recorded because of marked reduction of growth or death of plants.

yield was 5.56 grams (45.6%), melting point 143.5-144.5° C.

Analysis	Cl	N
Calcd. for C ₁₂ H ₁₃ Cl ₂ NO ₄	23.16	4.57
Found	23.35	4.43

In most preparations the crude products separated either as crystalline white solids or colorless oils which solidified to crystalline solids on standing at refrigerator temperatures. Exceptions were the D- and L-methionine and the D- and L-threonine derivatives, in which oils formed that did not crystallize at low temperatures. These oils were washed with water and formed solids following

continuous overnight evacuation with a pump. Most difficult to prepare were the D- and L-threonine derivatives, which finally gave products only after repeated attempts. Repeated recrystallizations of some derivatives were necessary to obtain products of satisfactory chemical and optical purity. The solvent combinations required are indicated in the footnotes in Table I.

Preliminary Screening Tests

D-, L-, and DL-alanine, aspartic acid, leucine, methionine, phenylalanine, and threonine derivatives of 2-(2',4'-dichloro-

phenoxy)propionic acid and the parent halogenated phenoxypropionic acid were screened for plant growth-regulating activity utilizing the dicotyledonous plants, Black Valentine bean, Mammoth Russian sunflower, and Arlington White Spine cucumber, and the monocotyledonous plants, Wong barley and U. S. 13 Hybrid corn. The lanolin assay method (7) was used on the dicots and the coated sand assay method (7) 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. Responses studied were: stem curvature, growth inhibition, epinasty, formative effects, and induced cell proliferation (gall formation). Table II shows the responses observed 14 days after treatment; to conserve space the other scores have been omitted. These data are representative, although they do not show the rate of response or relative progressive effectiveness of the compounds tested. The experiments were not designed to indicate the herbicidal potentialities of the compounds, and the results of testing for herbicidal properties will be reported elsewhere. However, the percentage of plants killed at

Table III. Index Values^a of Growth-Regulating Properties of 2-(2',4'-Dichlorophenoxy)propionyl Derivatives of D-, L-, and DL-Amino Acids

Amino Acid	Bean			Sunflower			Cucumber			Barley			Corn		
	D	L	DL	D	L	DL	D	L	DL	D	L	DL	D	L	DL
Alanine	21	83	79	8	87	87	0	91	91	22	61	56	26	63	56
Aspartic acid	0	17	2	0	8	6	2	7	5	11	11	17	15	26	37
Leucine	0	79	71	0	88	73	0	100	91	0	44	44	4	70	41
Methionine	0	50	60	0	46	37	0	57	57	0	44	44	7	37	41
Phenylalanine	0	21	26	0	17	6	2	9	41	2	20	17	33	4	56
Threonine	0	88	81	0	58	60	0	91	84	6	61	44	11	63	52
Parent acid		100			98			100			78			70	

^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 (7).

the concentration level used is reported as a matter of preliminary interest.

Scores covering the second, fourth, sixth, and fourteenth day observations for the various responses to treatment with each D-, L-, and DL-amino acid compound were added and expressed as a percentage of the maximum response possible in the same manner as in a preceding paper (7). Again, because of the voluminous nature of these data, the complete tabulation does not appear in this paper. However, the added scores for the various responses have been used to calculate index values as previously described (7) and these are presented in Table III.

In previous papers (6, 7) the index values for the DL-amino acid compounds have not been reported because the DL-compounds had index values comparable to the L-compounds. However, in this 2,4-DP series, index values for the DL-compounds are given because these values (see Table III) are in several cases unexpectedly and noticeably lower than those of corresponding L-isomers.

Results of Plant Screening

The most striking feature to be noted in an examination of the data in Tables II and III is the almost complete lack of growth-regulating properties of the D-amino acid derivatives of 2,4-DP during the 2-week test period. This is true for both the mono- and dicotyledonous plants, although some of the D-amino acid derivatives may have had a slight effect upon the former. Such behavior is in marked contrast to the selective effects observed for most D-amino acid derivatives of other aryloxyalkylcarboxylic acids, such as 2,4-dichloro- (70, 76), 2-methyl-4-chloro- (7), and 4-chlorophenoxyacetic (6). In these cases the D-isomers have varied considerably in their selectivity with both the particular amino acid used and the specific plant under test. D-Alanine derivatives of halogenated phenoxy acids have been exceptions, as these compounds have (with the exception of 4-chloro- and 2,4-D derivatives on cucumber) possessed activities about equal to their L-isomers.

It is to be noted from Tables II and III that most of the L- and DL-isomers of the amino acid derivatives of 2,4-DP possess moderate to high activity. In general the formative effects upon plant growth varied from slight, as shown by the L- and DL-aspartic acid derivatives, to marked, as illustrated by the L- and DL-derivatives of alanine, leucine, and threonine.

Although plant responses induced by the L- and DL-compounds were about equal, they were in general considerably less than those due to the parent acid. In the case of the phenylalanine derivatives, responses in the cucumber and corn plants were noticeably greater to the DL than to the L-amino acid compounds.

However, the reverse of this was shown by some plants—for example, the response of corn to the L-leucine isomer was appreciably greater than its response to the DL-derivative of leucine. While response of corn to the D-phenylalanine derivative of 2,4-DP was low, it was noticeably greater than response to the L-isomer.

Whereas the D-, L-, and DL-aspartic acid derivatives of 2,4-dichloro-, 2-methyl-4-chloro-, and 4-chlorophenoxyacetic acids have produced appreciable effects (6, 7, 70), similar derivatives of 2,4-DP showed almost negligible effects upon the growth of all the plants tested.

The low activities of L- and DL-phenylalanine and the moderate action of L- and DL-methionine derivatives of 2,4-DP on all plants were in sharp contrast to the high activities of the corresponding L- and DL-amino acid derivatives of 2,4-D (70), 2-methyl-4-chloro- (7), and 4-chlorophenoxyacetic acid (6) derivatives.

Discussion

Screening tests on the growth-modifying properties of amino acid derivatives of 2-(2',4'-dichlorophenoxy)propionic acids with respect to both monocotyledonous and dicotyledonous plants demonstrate that there is, in general, lower activity for all D-, L-, and DL-compounds of this series compared to activities previously reported for other series of halogenated phenoxy acids. However, the highly selective and widely variable behavior of the L- and the relatively cheap DL-amino acid forms of 2,4-DP in these preliminary studies makes these compounds exceptionally attractive from the standpoint of further studies, not only because they induced marked plant responses, but also because of their herbicidal potentialities.

In previous papers (6, 7, 76) considerable weight was given to the idea that plant-growth response in the case of L- and DL-amino acid derivatives of halogenated phenoxy acids depends upon the presence in the plant of cellular hydrolytic enzymes which are capable of splitting the amide linkage; this would free the carboxyl group (considered essential for activity) of the substituted phenoxy acid. Also, it has been thought that the assay plants may be partially or completely incapable of splitting the D-amide linkage. Results obtained with the amino acids of 2,4-DP to some extent seem to support these ideas. However, the almost complete inactivities of the D-isomers and the lowered but highly selective activities of the L- and DL-amino acid derivatives of 2,4-DP lend further support to the suggestions made in the preceding paper (6) concerning their mode of action—that is, activity may be due to action *per se*; it may depend upon the translocation and specificity of the whole intact molecule. Also, activity of a D-isomer may depend upon attack

by D-amino acid oxidase to produce an optically inactive α -keto acid which in the plant may undergo inversion through amination of the latter to the naturally occurring L-amino acid configuration. At present, however, there is no strong evidence to support any of these views. It is hoped that studies now in progress on other optically active compounds will further elucidate the mode of action of growth regulators of this type.

Derivatives of these halogenated phenoxy acids may be useful in the characterization of amino acids because of their relative ease of preparation, satisfactory yields, sharp melting points, and the accuracy with which chlorine and nitrogen content may be determined.

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