Synthesis, Properties, and Biological Activity of Some Amino Acid Derivatives of Halogen-Substituted Phenoxy Acids

J. F. CARMICHAEL,[1] E. J. SAGGESE, J. S. ARD, and C. F. KREWSON

Eastern Regional Research Laboratory, Philadelphia, Pa. 19118

E. M. SHANTZ

Laboratory for Cell Physiology, Growth, and Development, Cornell University, Ithaca, N. Y.

Thirty-nine new amino acid derivatives of halogen-substituted phenoxyacetic acids have been synthesized and some of their properties reported. A progress report on preliminary results of plant growth regulator tests with 18 compounds of this type is included. At low concentrations ($10^{-5}M$), L-leucine and L-isoleucine derivatives of 2,4-dichloro- and 2-methyl-4-chlorophenoxyacetic acids accelerated growth of explants of potato tubers. Derivatives of D-leucine and D-isoleucine were without effect as were all leucine and isoleucine compounds of 2,3-dichlorophenoxyacetic acid. Extended studies are in progress relative to the effect of halogen substitution and optical configuration upon activity.

THE BIOLOGICAL EVALUATION of a variety of amino acid derivatives of chlorine-substituted phenoxy acids (*2–9, 11*) has established the fact that there are marked differences in the growth-regulating properties of these compounds. Varying the optical configuration of the amino acid has sometimes caused plant growth changes more drastic than a difference in the structure of the amino acid used. The studies referred to have been concerned with the relation of chemical structure to growth modification, especially the effect of amino acid coupling upon the herbicidal activity of the most widely used phenoxyalkylcarboxylic acids.

The 39 compounds prepared for this series were made chiefly for the purpose of evaluating the effects of aromatic halogen substitution on the plant growth–regulating activity of *N*-phenoxyacetyl derivatives of amino acids. Leucine in its D-, L-, and DL- forms was selected as a representative amino acid. These compounds are also receiving evaluation by the Cancer Chemotherapy National Service Center and pertinent biological data will appear in one of the *Cancer Research* supplements.

This report presents information on the preparation and properties of the amino acid derivatives of halogen-substituted phenoxyacetic acid which are listed in Table I, and it also includes a summary of preliminary data on the growth regulating activity of some compounds of this type selected for initial studies. More comprehensive work is in progress based on these preliminary results.

Experimental

The D-, L-, and DL-leucines used in this work were the best obtainable from commercial sources and were not further purified. The various chlorine-substituted phenoxyacetic acids were supplied through the courtesy of J. R. Bishop of Amchem Products, Inc., Ambler, Pa. These acids were converted to their corresponding acetyl chlorides with thionyl chloride using techniques similar to those previously described (*1, 7*). The quantities of reactants were varied between 0.008 and 0.5 mole, depending upon the amounts of phenoxy acids available. The following description illustrates the procedure used.

2,5-Dichlorophenoxyacetyl Chloride (I). This compound was prepared in approximately theoretical yield by the reaction of 8.8 grams ($0.04M$) of 2,5-dichlorophenoxyacetic acid with 6.4 grams ($0.05M$) of thionyl chloride. The mixture was refluxed on a steam bath for 4 hours, allowed to stand overnight protected from air and moisture, and then distilled under a reduced pressure of less than 1 mm. A main fraction of 8 to 10 ml., distilling at 138° to 140° C., n_D^{25} 1.54641, was collected following the discard of a small forerun fraction. The product (I) consisted of a colorless, supercooled liquid which crystallized upon refrigeration.

Analysis. Calculated for $C_8H_5Cl_3O_2$: Cl, 44.42%. Found: 44.38%.

The procedure used for the synthesis of the leucine derivatives is similar to that previously described for other amino acid derivatives (*11*); the following description is illustrative.

N-(2,5-Dichlorophenoxyacetyl)-L-leucine (II). The chloride (I) (2.39 grams, $0.01M$) was dissolved in 15 ml. of benzene, and L-leucine (III) (1.7 grams, $0.013M$) was dissolved in 30 ml. of $1N$ sodium hydroxide. To the chilled (5° C.) alkaline solution of III, mechanically stirred, the benzene solution of II was added dropwise. The addition was continued over a 1-hour period at a maintained temperature of 5° C. The reaction mixture was stirred for 3 hours while it warmed up to room temperature. The mixture was then extracted twice with 50-ml. portions of ethyl ether. The ether extracts were combined, washed with 50 ml. of water, and the water was added to the original alkaline solution; the ether portion was discarded. After the alkaline solution was filtered, pH was adjusted to 3 with $1N$ hydrochloric acid which caused the formation of the crude product, II, as a white precipitate. Following a 2-hour stand at 5° C., the precipitate was filtered from the solution and thoroughly washed with water. The crude II was dried under vacuum at room temperature to a constant weight of 2.94 grams (87.8%), (melting point 143° to 146° C.). The product, crystallized once from ethyl acetate–petroleum ether (boiling point of the petroleum ether, 63° to 70° C.), gave 2.51 grams (75.2%) of II melting at 151° to 153° C. Table I presents data on II and on the other leucine derivatives prepared.

Discussion of Chemistry

Some difficulty was experienced in purifying crude *N*-(2,5-dichlorphenoxyacetyl)-D-leucine which came down as an oil that did not solidify at 5° C. A solution of the oil in ethyl ether was thoroughly washed with water. After drying the ether solution over calcium sulfate, a white solid was obtained which melted at 109° to 120° C.; crude yield, 41.9%. Crystallization from ethyl acetate–petroleum ether did not improve its quality. However, recrystallization from methanol-water gave a satisfactory product which melted at 145° to 146° C. Several leucine derivatives of other phenoxy acids also came down in

[1] Present address: 5605 Sherrell Drive, NE, Atlanta 5, Ga.

Table I. Yields, Physical Properties, and Analyses of Leucine Derivatives of Halogen-Substituted Phenoxyacetic Acids

Compound	Formula	M.P., °C.[a] (Corr.)	Yield, % Crude	Yield, % Refined	Chlorine, % Calcd.	Chlorine, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Optical Rotation, $[\alpha]_D^{25}$ C, 2.00 Grams/100 Ml., in Pyridine
N-(2-chlorophenoxyacetyl)-									
L-leucine	$C_{14}H_{18}ClNO_4$	123 –124.5	72.3	58.7	11.83	11.69	4.67	4.67	*levo* 1.07 ± 0.4
D-leucine		125 –126.5[b]	70.7	48.3	11.83	11.98	4.67	4.62	*dextro* 4.36 ± 0.4
DL-leucine		124 –125.5	83.3	71.7	11.83	11.82	4.67	4.68	
N-(3-chlorophenoxyacetyl)-									
L-leucine	$C_{14}H_{18}ClNO_4$	127 –130[c]	77.4	66.6	11.82	11.90	4.67	4.73	*dextro* 11.29 ± 0.4
D-leucine		141 –142	77.4	50.5	11.83	11.94	4.67	4.55	*levo* 9.01 ± 0.4
DL-leucine		126.5–128.5[c]	79.7	58.4	11.83	12.13	4.67	4.55	
N-(4-fluorophenoxyacetyl)-[d]									
L-leucine	$C_{14}H_{18}FNO_4$	110 –111	...	67.5	...	...	4.97	4.93	*dextro* 1.88 ± 0.4
D-leucine		110 –111	...	64.3	...	...	4.97	4.99	*levo* 1.80 ± 0.5
DL-leucine		105.5–106.5	85.5	72.7	...	...	4.97	4.95	
N-(2,3-dichlorophenoxyacetyl)-									
L-leucine	$C_{14}H_{11}Cl_2NO_4$	157 –161	...	35.9	21.22	21.46	4.19	..[e]	*dextro* 9.9 ± 2[f]
D-leucine		162.5–164.5	95.8	68.6	21.22	21.24	4.19	4.05	*levo* 4.36 ± 0.4
DL-leucine		156 –157.5	...	77.3	21.22	21.01	4.19	4.10	
N-(2,5-dichlorophenoxyacetyl)-[g]									
L-leucine	$C_{14}H_{17}Cl_2NO_4$	151 –153	87.8	75.2	21.22	21.15	4.19	4.19	*levo* 8.14 ± 0.4
D-leucine		145 –146[h]	41.9	11.9	21.22	21.13	4.19	4.14	*dextro* 4.30 ± 0.4
DL-leucine		132 –133	64.0	56.9	21.22	21.59	4.19	4.14	
N-(3,4-dichlorophenoxyacetyl)-									
L-leucine	$C_{14}H_{17}Cl_2NO_4$	132 –134[c]	100.0	77.3	21.22	21.45	4.19	4.12	*dextro* 11.73 ± 0.4
D-leucine		145.5–148[c]	...	82.5	21.22	21.13	4.19	4.17	*levo* 8.96 ± 0.4
DL-leucine		133.5–135.5[i]	89.3	...	21.22	21.24	4.19	4.23	
N-(3,5-dichlorophenoxyacetyl)-									
L-leucine	$C_{14}H_{17}Cl_2NO_4$	171 –174[c]	...	74.4	21.22	21.23	4.19	4.08	*dextro* 9.35 ± 0.4
D-leucine		178.5–180.5[c]	...	81.6	21.22	20.76	4.19	4.23	*levo* 6.95 ± 0.4
DL-leucine		162 –164[c]	...	45.1	21.22	20.68	4.19	4.27	
N-(2-methyl-5-chorophenoxyacetyl)-[j]									
L-leucine	$C_{15}H_{20}ClNO_4$	163 –164[b]	61.4	55.5	11.30	11.23	4.46	4.44	*dextro* 3.34 ± 0.4
D-leucine		168 –169.5	75.2	54.0	11.30	11.39	4.46	4.49	*levo* 1.66 ± 0.4
DL-leucine		141 –142.5	65.7	35.1	11.30	11.32	4.46	4.46	
N-(2,3,6-trichlorophenoxyacetyl)-[k]									
L-leucine	$C_{14}H_{16}Cl_3NO_4$	151 –154[c]	80.3	67.4	28.85	29.08	3.80	3.76	*dextro* 2.19 ± 0.4
D-leucine		156 –160[c]	80.4	63.1	28.85	29.14	3.80	3.80	*dextro* 1.64 ± 0.4
DL-leucine		155.5–157.5[c]	78.2	64.8	28.85	29.06	3.80	3.82	*dextro* 0.11 ± 0.2[f]
N-(2,4,6-trichlorophenoxyacetyl)-									
L-leucine	$C_{14}H_{16}Cl_3NO_4$	172 –173[m]	88.6	57.3	28.85	28.73	3.80	3.71	*dextro* 1.83 ± 0.4
D-leucine		167 –169.5[m]	...	16.3	28.85	29.05	3.80	3.50	*levo* 0.80 ± 2[f]
DL-leucine		164 –166[d]	...	29.7	28.85	28.73	3.80	3.62	
N-(3,4,5-trichlorophenoxyacetyl)-									
L-leucine	$C_{14}H_{16}Cl_3NO_4$	186 –191[c]	69.1	21.8	28.85	28.81	3.80	..[e]	*dextro* 5.9 ± 2[b]
D-leucine		197 –200[c]	42.4	14.9	28.85	27.98	3.80	..[e]	..[e]
DL-leucine		170 –171.5[c]	61.7	35.3	28.85	28.71	3.80	3.81	
N-(2,3,4,6-tetrachlorophenoxyacetyl)-									
L-leucine	$C_{14}H_{15}Cl_4NO_4$	147 –150[n]	63.7	...	35.19	35.73	3.48	3.40	*dextro* 5.6 ± 2[f]
D-leucine		179 –181.5[n]	80.8	45.2	35.19	35.65	3.48	3.22	*levo* 1.51 ± 0.4
DL-leucine		181 –183.5[n]	83.7	56.8	35.19	35.92	3.48	3.49	
N-(2,3,4,5,6-pentachlorophenoxyacetyl)-									
L-leucine	$C_{14}H_{14}Cl_5NO_4$	201 –208	...	16.4	40.61	39.78	3.21	3.13	*dextro* 3.9 ± 2[f]
D-leucine		211 –216	...	24.5	40.61	40.32	3.21	3.08	*dextro* 2.8 ± 2[f]
DL-leucine		214 –216	...	21.9	40.61	40.01	3.21	3.09	

[a] Recrystallized once or more from ethyl acetate–petroleum ether unless otherwise indicated. [b] As in "a" plus one recrystallization from ethylene chloride. [c] Purified by thoroughly washing three times with warm petroleum ether (b.p., 63° to 70° C.). [d] *N*-(4-chlorophenoxyacetyl)- derivatives of D-, L- and DL-leucine previously reported (*5*). [e] Insufficient material available. [f] Micro. [g] *N*-(2,4-dichlorophenoxyacetyl)- derivatives previously reported (*9, 14*). [h] As in "a" plus one recrystallization from hot methanol-water. [i] No refinement required. [j] *N*-(2-methyl-4-chlorophenoxyacetyl)- derivatives previously reported (*6*). [k] *N*-(2,4,5-trichlorophenoxyacetyl)- derivatives previously reported (*11*). [l] A 1:1 mixture of the L- and D-leucine derivatives also gave a dextro value, 1.84 ± 0.4. Repetition of all determinations gave check values. [m] As in "a" plus one recrystallization from hot ethanol-water. [n] Recrystallized from hot ethanol-water.

the form of oils, but these solidified on storage at 5° C. Usually when the crude product was an oil, the yield was low. Solvent combinations used in the purification of products have been indicated in Table I.

No attempts were made to improve yields in the preparation of the compounds described either by conservation of mother liquor or by modification of procedures used.

The quality of *N*-(2,4,6-trichlorophenoxyacetyl)-D-leucine could not be further improved by repeated recrystallizations from ethyl acetate–petroleum ether or from methanol-water combinations. Washing the products thoroughly with warm petroleum ether usually improved their quality considerably, although losses were sometimes heavy during the process.

As is true for many substances containing an amino acid unit in alkaline solution (*10*), the fact that 11 of the 13 com-

pounds prepared for D-leucine were *dextro*-rotatory and that 8 of the 13 prepared for D-leucine were *levo*-rotatory was not unusual. In 76 pairs of D- and L-amino acid derivatives of various phenoxyalkylcarboxylic acids synthesized (*2, 4–6 8, 9, 11*), this reversal occurred frequently. In the 4-chloro- (*5*) and the 2-methyl-4-chlorophenoxyacetic acid (*6*) series, reversal occurred with all the amino acids used. No satisfactory explanation can be given for this behavior since it follows no regular pattern.

Other chlorine-substituted phenoxyacetic acids, such as the 2,6-dichloro-, 2,3,4-trichloro-, and 2,3,4,5-tetrachlorophenoxyacetic acids, were not available for this work.

Table II. Effects of 2,4-D and its *N*-Acetyl Leucine Derivatives on Growth of Potato Tuber Explants

Treatment	Average Final Fresh Weight A, mg.	B, mg.
Basal medium with 5% coconut milk	6.8	5.9
plus 2,4-D	59.4	57.8
plus *N*-(2,4-D acetyl)-L-leucine	62.9	48.6
plus *N*-(2,4-D acetyl)-D-leucine	7.2	5.8
plus *N*-(2,4-D acetyl)-DL-leucine	25.4	25.7

Table III. Effects of 2,4-D and its *N*-Acetyl Isoleucine Derivatives on Growth of Potato Tuber Explants

Treatment	Average Final Fresh Weight A, mg.	B, mg.	C, mg.
Basal medium	12.1	12.3	13.9
plus 2,4-D	58.5	51.7	71.6
plus *N*-(2,4-D acetyl)-L-isoleucine	43.6	42.7	74.9
plus *N*-(2,4-D acetyl)-D-isoleucine	11.8	12.0	13.9
plus *N*-(2,4-D acetyl)-DL-isoleucine	17.3	22.8	42.3

Preliminary Growth Regulator Tests

In one experiment, the free acids and D-, L-, and DL-leucine derivatives of both 2,4-dichloro- (*9, 14*) and 2,3-dichlorophenoxyacetic acid were tested at concentrations of $10^{-5}M$ in explants of potato tuber tissue growing in a modified White's basal medium containing 5% coconut milk as previously described (*12, 13*). Although no stimulatory effects of 2,3-D or its amino acid derivatives were observed, the 2,4-D was effective in stimulating the growth of potato tuber tissue cultures as expected (*13*). The responses obtained with the 2,4-D and its *N*-acetyl leucine derivatives are shown in Table II. Each figure represents average final fresh weight in milligrams of nine replicate cultures after a 25-day growth period. *A* and *B* are explants from two different potato tubers. Original weight of each explant was 3.0 mg.

In a later experiment, similar tests were done using the analogous derivatives of isoleucine which had been prepared previously (*9, 14*). The tissue explants were taken from a different batch of potato tubers, which were atypical in that the growth responses to 2,4-D and its derivatives were greater in the medium without coconut milk than in the medium to which coconut milk had been added. Usually, little or no growth takes place in potato tuber tissue explants in the absence of coconut milk, even if 2,4-D is present. However, as Table III shows, similar responses were obtained in tissue explants from three different tubers (*A*, *B*, and *C*), so that this ability to respond to 2,4-D in the absence of a coconut milk supplement was apparently characteristic of the batch of tubers used in this experiment. Conditions are the same as in Table II except that coconut milk was omitted from the medium. *A*, *B*, and *C* are explants from three different tubers.

The data from both tables are quite similar, showing 2,4-D to be effective in stimulating the growth of potato tuber tissue cultures. With both the leucine and the isoleucine derivatives of 2,4-D, the L- compounds were approximately fully as active as the free acid, the D-compounds were completely inactive, and in all cases the DL-compounds showed some activity which was, however, considerably less than that of the L-compounds.

In another experiment, 2-methyl-4-chlorophenoxyacetic acid and its D- and L-leucine *N*-acetyl derivatives (*6*) were tested in similar fashion. As with the 2,4-D derivatives, the free acid and the L-amino acid compound gave a positive response while the D-form was ineffective. In the case of the 2,3-dichloro-derivatives which were also tested, none of the compounds were active.

All these results confirmed some earlier work, which also showed that positive growth responses were obtained when active compounds of this type were supplied as L-amino acid acetyl derivatives but were ineffective in the D-form. These results indicate that the *N*-acetyl L-amino acid derivatives are acting per se and not through any hydrolytic breakdown products. Although a strict control treatment would require the addition of the free phenoxy acid plus the free D-amino acid, it is difficult to believe that this amount of D-amino acid alone could be inhibitory.

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Literature Cited

(1) Freed, V. H., *J. Am. Chem. Soc.* **68,** 2112 (1946).
(2) Krewson, C. F., Carmichael, J. F., Drake, T. F., Mitchell, J. W., Smale, B. C., J. AGR. FOOD CHEM. **8,** 104 (1960).
(3) Krewson, C. F., Carmichael, J. F., Schaffer, P. S., Smale, B. C., Mitchell, J. W., *Ibid.*, **7,** 837 (1959).
(4) Krewson, C. F., Drake, T. F., Mitchell, J. W., Preston, W. H., Jr., *Ibid.*, **4,** 690 (1956).
(5) Krewson, C. F., Drake, T. F., Neufeld, C. H. H., Fontaine, T. D., Mitchell, J. W., Preston, W. H., Jr., *Ibid.*, p. 140.
(6) Krewson, C. F., Neufeld, C. H. H., Drake, T. F., Fontaine, T. D., Mitchell J. W., Preston, W. H., Jr., *Weeds* **3,** 28 (1954).
(7) Krewson, C. F., Saggese, E. J., Carmichael, J. F., Ard, J. S., Drake., T. F., Mitchell, J. W., Smale, B. C., J. AGR. FOOD CHEM. **7,** 118 (1959).
(8) Krewson, C. F., Saggese, E. J., Drake, T. F., *Weeds* **8,** 107 (1960).
(9) Krewson, C. F., Wood, J. W., *J. Org. Chem.* **25,** 143 (1960).
(10) Rao, K. R., Sober, H. A., *J. Am. Chem. Soc.* **76,** 1328 (1954).
(11) Saggese, E. J., Krewson, C. F., *J. Chem. Eng. Data* **5,** 476 (1960).
(12) Shantz, E. M., Steward, F. C., Smith, M. S., Wain, R. L., *Ann. Botany* **19,** 49 (1955).
(13) Steward, F. C., Caplin, S. M., *Science* **113,** 518 (1951).
(14) Wood, J. W., Fontaine, T. D., *J. Org. Chem.* **17,** 891 (1952).

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