Bioactive Compounds

Synthesis and Physical Properties of 2,4,5-Trichlorophenoxyacetylated Amino Acids

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AMINO ACID derivatives of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) were prepared for evaluation as growth regulators, herbicides, nematocides, insecticides, fungicides, and anticancer agents. Reports on the biological evaluations by other investigators have not been completed.

2,4,5-T, chiefly in the form of either an alkanolamine salt or an ester, is one of the most widely used of the herbicidal phenoxy compounds; its toxicant action on woody plants is well known. Pokorny (*11*) in 1941 was the first to report its preparation and Zimmerman and Hitchcock (*14*) were among the first to use halogen substituted phenoxy compounds as growth-regulating substances. They reported (*4*) on the comparative root-inducing activity of 2,4,5-T in 1944, the same year Hamner and Tukey (*3*) announced its herbicidal action on bindweed. Recently, Linden (*10*) has stated that large-scale developments in forestry, such as the clearing and replanting of scrub land, have become practicable only by the use of 2,4,5-T to control stump sprouting. Fisher and Young (*2*) stated that aerial application of 2,4,5-T offers much promise of effectively and economically controlling mesquite on rangeland. Recently, Behrens, Fisher, and Meadors (*1*) reported on the ability of 12 of these amino acid derivatives of 2,4,5-T to kill mesquite seedlings. In all cases the L-form of a particular amino acid derivative of 2,4,5-T was more toxic than the DL-form, which in turn was more toxic than the D-form. There have been reports on the herbicidal activity of double phenoxy (2,4,5-T included) esters (*5*) on mesquite. Thompson, Swanson, and Norman (*13*) have presented one of the most extensive articles on 2,4,5-T derivatives. A comprehensive review of the literature on 2,4,5-T and its derivatives would involve more than 300 publications.

Continued interest in elucidating the mode of action of plant growth modifiers, and in producing compounds possessing good selectivity, has led to the preparation of a number of amino acid series (*6–9*). There are marked differences in the growth-modifying properties of these

Table I. Yields, Physical Properties, and Analyses of 2,4,5-Trichlorophenoxyacetylated Amino Acids

N-(2,4,5-Trichlorophenoxyacetyl)-[a]	Melting Points, °C., [b] Corr.	Yield, % Crude	Yield, % Refined	Formula	Analyses[c] Chlorine, % Calcd.	Chlorine, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Optical Rotation[d] $[\alpha]_D^{25°}$	Optical Rotation[d] C(g./100 ml.) in pyridine
L-alanine	197.0–198.5[e]	69.6	22.9	$C_{11}H_{10}Cl_3NO_4$	32.57	32.34	4.29	3.96	−7.0 ± 0.5	2.74
D-alanine	199.0–201.0[f]	43.0	29.7		32.57	32.31	4.29	4.25	+7.1 ± 0.4	3.15
DL-alanine	208.0–210.0[g]	64.7	29.0		32.57	32.72	4.29	3.92		
L-aspartic acid	166.0–174.0[h]	79.6	18.5	$C_{12}H_{11}Cl_3NO_6$	28.70	28.24	3.78	3.83	+26.4 ± 0.4	2.16
D-asparatic acid	169.0–173.0[i]	53.8	25.6		28.70	28.59	3.78	3.70	−26.1 ± 0.6	2.35
DL-aspartic acid	186.0–187.0[j]	46.9	20.2		28.70	28.57	3.78	3.83		
L-leucine	163.5–165.0[k]	78.6	64.4	$C_{14}H_{16}Cl_3NO_4$	28.85	28.35	3.79	3.99	−9.3 ± 0.5	2.50
D-leucine	164.0–165.5[l]	48.8	47.6		28.85	28.65	3.79	3.82	+9.1 ± 0.5	2.61
DL-leucine	167.5–168.5[m]	38.1	20.9		28.85	28.87	3.79	3.79		
L-methionine	135.5–137.0[n]	82.8	68.5	$C_{13}H_{14}Cl_3NO_4S$	27.50	26.97	3.62	3.76	+1.9 ± 0.4	3.10
D-methionine	134.5–136.0[o]	47.6	27.5		27.50	27.45	3.62	3.57	−1.7 ± 0.3	3.56
DL-methionine	126.5–128.0[p]	81.2	50.8		27.50	27.43	3.62	3.57		
L-phenylalanine	186.0–188.0[q]	75.0	44.1	$C_{17}H_{14}Cl_3NO_4$	26.41	25.98	3.47	3.18	+3.2 ± 1.3	1.05
D-phenylalanine	191.0–192.5[r]	66.1	24.3		26.41	26.78	3.47	3.45	−4.6 ± 1.0	1.34
DL-phenylalanine	204.0–206.0[s]	88.6	53.1		26.41	26.25	3.47	3.50		
L-threonine	182.5–184.0[t]	64.8	28.1	$C_{12}H_{12}Cl_3NO_5$	29.82	29.80	3.92	3.77	−1.2 ± 0.4	3.00
D-threonine	183.0–184.5[t]	49.5	44.7		29.82	29.78	3.92	3.94	+1.1 ± 0.4	3.01
DL-threonine	156.5–158.0[u]	65.0	24.5		29.82	29.59	3.92	4.09		

[a] Prepared by Schotten-Baumann reaction. Exceptions L- and DL-asparatic acid and DL-leucine derivatives prepared by direct azeotropic esterification, D-asparatic acid derivative by Ronwin method (*12*).
[b] Recrystallized one or more times from the solvent or solvent combinations as indicated.
[c] Analyses by D.A. McClelland and K.M. Zbinden.
[d] Optical rotations by J.S. Ard and J.F. Carmichael.

Recrystallizations

[e] Three times from ethyl alcohol-water (EW), three times from acetone–ethyl alcohol-water, twice from acetic acid-water (AW).
[f] Twice from EW, once from AW.
[g] Twice from anhydrous ethyl acetate–petroleum ether (EP), twice from EW, twice from AW.
[h] Once from EW, three times from EP. Melts with decomposition.
[i] Twice from EP.
[j] Twice from EP, once from EW.
[k] Once from EW.
[l] Three times from EW, once from EP.
[m] Once from EP, once from EW.
[n] Once from EP.
[o] Once from AW, three times from EW.
[p] Three times from EP, two from EW.
[q] Once from EP, once from AW.
[r] Twice from EW, twice from EP.
[s] Twice from EW.
Three times from EW.
[u] Once from acetone-water, twice from EW, once from methanol-water, twice from EP.

compounds, espically among the L-, D-, and DL- forms of amino acid derivatives of the chlorine substituted phenoxy acids. The synthesis of the amino acid derivatives of 2,4,5-T reported here is an extension of this work.

EXPERIMENTAL

The amino acids used in this work were the best obtainable from commercial sources and were not further purified. The 2,4,5-T (Amchem Products, Inc., Dow Chemical Co., and Monsanto Chemical Co.) was used as received. 2,4,5-Trichlorophenoxyacetyl chloride was prepared in 74.2% yield by the reaction of 2,4,5-T with thionyl chloride as previously described (*9*).

The following descriptions illustrate the three general procedures (*6-8*, *12*) used in the synthesis of the amino acid derivatives:

N-(2,4,5-Trichlorophenoxyacetyl)-L-methionine (I). L-Methionine (4.48 grams, 0.03*M*) was dissolved in 90 ml. of 1*N* sodium hydroxide (0.09*M*) and the mixture was chilled in an ice bath to 5° C. 2,4,5-Trichlorophenoxyacetyl chloride (II) (8.22 grams, 0.03*M*) was dissolved in 45 ml. of benzene. The solution was chilled, and added dropwise with mechanical stirring, over a 5- to 10-minute period, to the alkaline L- methionine solution. The temperature was maintained at 5° C. The reaction mixture was continuously stirred as it warmed up to room temperature and then was extracted three times with 50-ml. portions of ethyl ether. The ether extracts were combined, washed with a 50-ml. portion of distilled water, and the ether was discarded. The water washing was added to the alkaline aqueous reaction mixture which was then acidified to a pH of 3 with 1*N* hydrochloric acid. Acidification precipitated a white crystalline product which, after standing several hours at 5° C., was filtered off from the solution and washed repeatedly with water. The crude product was vacuum dried to a constant weight of 9.61 grams (82.8%), melting point 122° to 134° C. (Kofler micro melting point apparatus). The crude product, recrystallized from ethyl alcohol-water, gave 7.95 grams (68.5%) of pure I, melting point 135.5-7.0° C. Table I presents data on I and on the other amino acid derivatives prepared.

N-(2,4,5-Trichlorophenoxyacetyl)-D-aspartic Acid (III). D-Aspartic acid (2.66 grams, 0.02*M*) was suspended in 200 ml. of anhydrous ethyl acetate contained in a round-bottomed flask. To this was added II (5.47 grams, 0.02*M*). A water-cooled condenser was attached and the mechanically stirred mixture refluxed for 19 hours. The reaction mixture was then filtered and the filtrate evaporated to dryness, leaving a light-yellow oil which was washed three times with petroleum ether (boiling point 63° to 70° C.). The oil solidified during this treatment. The solid was filtered from the petroleum ether and vacuum dried to a constant weight of 3.99 grams (53.8%), melting point 168° to 172° C. The crude product was recrystallized twice from anhydrous ethyl acetate-petroleum ether with little change in melting point, 169° to 173° C.; yield of III was 1.90 grams (25.6%).

N-(2,4,5-Trichlorophenoxyacetyl)-DL-leucine (IV). DL-Leucine (3.93 grams, 0.03*M*) was added along with 2,4,5-T (7.66 grams, 0.003*M*) to 75 ml. of xylene contained in a round-bottomed flask; 3 drops of concentrated sulfuric acid were added and the apparatus was arranged for azeotropic distillation. The reaction was stirred mechanically at reflux temperature until the theoretical amount of water was collected; this required about 13 hours. The reaction mixture was filtered and the filtrate placed in the refrigerator for several hours. The crystals which formed were filtered off and purified by dissolving in 1*N* sodium hydroxide solution and reprecipitating with hydrochloric acid (1:2) at pH 3, a process which was repeated. The yield of dried crude product was 4.22 grams (38.1%), melting point 160° to 165° C. One recrystallization from hot, anhydrous ethyl acetate-petroleum ether and one from ethyl alcohol-water gave a final yield of IV of 2.32 grams (20.9%), melting point 167.5-8.5° C.

DISCUSSION OF CHEMISTRY

Most of the amino acid derivatives of 2,4,5-T separated from reaction mixtures either as crystalline white solids or as colorless, oily liquids. Often these oils crystallized on prolonged standing at about 5° C. When this did not occur, the oils were throughly washed with water and then evacuated at room temperatures to constant weight, resulting in the production of amorphous solids. Solids were then crystallized from various solvent systems; solvent combinations have been indicated in Table I. In the refinement of crude amino acid derivatives, no attempts were made to improve yields either by modifying procedures or by working up the mother liquors.

The melting point behavior of the L- and D-aspartic acid derivatives of 2,4,5-T cannot be explained. It does not seem to be due to racemization during acylations, because equal and opposite sign rotations were obtained. However, these compounds were difficult to prepare and purify. The L- and D-aspartic acid derivatives of 2-(2,4,5-trichlorophenoxy)-propionic and 4-(2,4-dichlorophenoxy)- and 4-(2-methyl-4-chlorophenoxy)butyric acids could not be prepared.

SUMMARY

Eighteen new amino acid derivatives of 2,4,5-T have been prepared and some of their properties studied.

L-, D-, and DL- optical isomers of alanine, aspartic acid, leucine, methionine, phenylalanine, and threonine were made, but synthesis for all compounds could not be achieved by a single procedure.

Most of the amino acid derivatives of 2,4,5-T were readily prepared and analyzed and had sharp melting points; hence they may be useful in the characterization of amino acids.

Biological evaluation of these compounds as growth regulators and selective herbicides is being conducted by other investigators and will be reported later. Some of the compounds are also being evaluated as nematocides, insecticides, fungicides, and cancer chemotherapeutic agents.

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