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SYNTHETIC PLANT-GROWTH REGULATORS. III. 2,4-DICHLOROPHENOXYACETYL DERIVATIVES OF AMINO ACIDS

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The mode of action of plant-growth regulators is not yet understood. It has been postulated for the halogenated phenoxyacetic acids, however, that a free carboxyl group or a group convertible to a carboxyl group is essential for activity. This and other requirements for plant-growth regulators have been discussed in several recent publications (1–3). Thus salts, esters, amides, and substituted amides of these acids are assumed to be converted to the free acids in the plants affected by these regulators. This implies that the plants have enzyme systems capable of splitting ester and amide linkages. Thus far, to our knowledge, substituted amides of halogenated phenoxyacetic acids reported in the literature have been found to be active if the parent acids were active. It seemed to be a reasonable assumption that, if substituted amides were made by reacting the halogenated phenoxyacetyl chlorides with the various D-amino acids, the plants that are affected by this type regulator might be incapable of splitting the amide linkage, or that splitting would be at a much slower rate, and their effect would be less pronounced. In order to test this hypothesis, a number of halogenated phenoxyacetyl derivatives of DL-, L-, and D-amino acids listed in Table I were prepared and tested[1] for plant-growth regulating activity.

All of the halogenated phenoxyacetyl derivatives of DL- and L-amino acids listed in Table I were found to produce formative changes in tomato plants. N-(2,4-Dichlorophenoxyacetyl)-D-aspartic acid, -D-methionine, and -D-phenylalanine[2] failed to produce formative changes in tomato plants when tested at comparable low dosage levels (30–50 ppm); however, they caused fruit to set and produced parthenocarpic fruit of increased size and of excellent quality. Although only three N-(2,4-dichlorophenoxyacetyl)-D-amino acids have been fully tested, the results suggest that plants affected by 2,4-dichlorophenoxyacetic acid are unable to split readily the amide linkage of these compounds.

The Schotten-Baumann reaction was used to prepare the compounds listed in Table I. A few halogenated phenoxyacetyl derivatives of DL-valine have been previously reported (4), but they were tested as penicillin precursors. These compounds have been kindly supplied to us by Dr. Otto K. Behrens for testing as plant-growth regulators.

[1] We are indebted to Drs. J. W. Mitchell and Paul Marth, Bureau of Plant Industry, Soils and Agricultural Engineering, Beltsville, Maryland, for these plant tests. Details of these tests will be published elsewhere.

[2] We have recently obtained generous samples of pure D-amino acids through the courtesy of Dr. Jesse P. Greenstein, National Institutes of Health, Bethesda, Maryland, for the purpose of extending this investigation.

TABLE I

PHYSICAL AND ANALYTICAL DATA OF HALOPHENOXYACETYLATED AMINO ACIDS

N[a]-(2,4-DICHLOROPHENOXYACETYL)-	M.P., °C.[a] (Corr.)	YIELD, % (Crude)	FORMULA	ANALYSES[b]				$[\alpha]_D^{20°C}$
				Cl		N		
				Calc'd	Found	Calc'd	Found	
L-alanine	197.2–199.2	81.6	$C_{11}H_{11}Cl_2NO_4$	24.28	24.41	4.80	4.84	+11.4°
DL-alanine	212.8–213.8	89.6		24.28	24.21	4.80	4.78	
L-aspartic acid	201.8–202.6[d]	62.7	$C_{12}H_{11}Cl_2NO_6$	21.10	20.81	4.17	4.18	+19.9°
D-aspartic acid	200.8–201.8[d]	66.4		21.10	20.87	4.17	4.18	−20.0°
DL-aspartic acid	217.0–217.5	65.0		21.10	21.10	4.17	4.18	
L-glutamic acid	178.2–179.2[e]	52.2	$C_{13}H_{13}Cl_2NO_6$	20.25	20.24	4.00	3.92	+14.1°
DL-glutamic acid	191.5–192.5	30.5		20.25	20.33	4.00	3.92	
glycine	234–235[f, g, h]	58.0	$C_{10}H_9Cl_2NO_4$	25.50	25.45	5.04	5.05	
DL-histidine, methyl ester, HCl	128.6–129.7[f, i]	48.9	$C_{15}H_{16}Cl_3N_3O_4$	26.03	25.33	10.28	10.43	
DL-isoleucine	142.0–143.4	89.8	$C_{14}H_{17}Cl_2NO_4$	21.22	21.58	4.19	4.20	
L-leucine	149.9–150.4[j]	88.1		21.22	21.27	4.19	4.22	−17.4°
DL-leucine	137.0–138.0[j]	91.2		21.22	21.26	4.19	4.19	
L-methionine	133.8–134.8	90.9	$C_{13}H_{15}Cl_2NO_4S$	20.13	20.22	3.98	3.75	−1.2°
D-methionine	129.7–131.8	71.6		20.13	20.31	3.98	3.77	+1.1°
DL-methionine	143.9–145.1	63.5		20.13	20.11	3.98	3.90	
L-phenylalanine	178.2–179.2[k, l]	87.2	$C_{17}H_{15}Cl_2NO_4$	19.26	19.13	3.80	3.75	−7.7°
D-phenylalanine	154.6–155.7[k, m]	84.5		19.26	19.31	3.80	3.84	+7.4°
DL-phenylalanine	179.2–180.2[k]	84.7		19.26	19.18	3.80	3.83	
L-proline	104.8–106.3[n, o]	82.7	$C_{13}H_{13}Cl_2NO_4$	22.29	22.22	4.40	4.40	−62.4°
DL-proline	143.9–145.1[p]	87.2		22.29	22.15	4.40	4.40	
DL-serine	192–195[f, q, r]	64.9	$C_{11}H_{11}Cl_2NO_5$	23.01	23.00	4.55	4.50	
DL-threonine	137.9–139.0[s]	73.6	$C_{12}H_{13}Cl_2NO_5$	22.01	21.77	4.35	4.33	

N^{α}-(2,4-DICHLOROPHENOXYACETYL)-	M.P., °C.[a] (Corr.)	YIELD, % (Crude)	FORMULA	ANALYSES[b]				
				Cl		N		$[\alpha]_D^{20}$[c]
				Calc'd	Found	Calc'd	Found	
DL-tryptophan	147.0–147.9[t, u]	67.7	$C_{19}H_{16}Cl_2N_2O_4$	17.40	17.45	6.88	6.70	
DL-valine	159.3–159.9	58.9 av.	$C_{13}H_{15}Cl_2NO_4$	22.15	22.27	4.38	4.40	
N,N′-BIS-(2,4-DICHLOROPHENOXYACETYL)-L-cystine	215–216[f, v, w]	77.3	$C_{22}H_{20}Cl_4N_2O_8S_2$	21.94	21.50	4.33	4.39	−142°
N^{α},N^{ϵ}-BIS-(2,4-DICHLOROPHENOXYACETYL)-								
L-lysine	86.0–87.0[x]	93.0	$C_{22}H_{22}Cl_4N_2O_6$	25.68	25.99	5.07	4.67	−6.0°
DL-lysine	175.0–176.1[y]	87.8		25.68	25.56	5.07	5.04	
N,O-BIS-(2,4-DICHLOROPHENOXYACETYL)-								
DL-tyrosine methyl ester	119.7–121.6[z]	54[aa]	$C_{26}H_{21}Cl_4NO_7$	23.59	23.49	2.33	2.37	
N-(4-CHLOROPHENOXYACETYL)-glycine	148.9–150.1	78	$C_{10}H_{10}ClNO_4$	14.55	14.68	5.75	5.61	
N-(2,4,5-TRICHLOROPHENOXYACETYL)-glycine	195.6–196.7	87.5	$C_{10}H_8Cl_3NO_4$	34.03	33.89	4.48	4.51	
DL-valine	194.6–195.6[y]	95.6	$C_{13}H_{14}Cl_3NO_4$	30.00	29.88	3.95	3.95	

[a] Unless indicated otherwise the compounds were recrystallized once from 50% ethanol. [b] The authors are indebted to Mr. J. S. Ard of this Laboratory for the analyses. Position of the acyl group in some of the derivatives was verified by infrared analysis. [c] The compounds were dissolved in a 10% molar excess of sodium hydroxide. [d] Recrystallized successively from 25% ethanol and dioxane-benzene. [e] Recrystallized successively from 50% ethanol, 25% ethanol, and dioxane-benzene. [f] Melts with decomposition. [g] Sinters at 228°. [h] Recrystallized from 70% ethanol. [i] Reprecipitated from ethanol-ether. [j] Recrystallized twice from 50% ethanol. [k] Recrystallized from 60–70% ethanol. [l] Sinters at 150°. [m] Sinters at 153.9°. [n] Reprecipitated (as an oil) by acidification of sodium salt solution. Recrystallized successively from chloroform-hexane, and from acetone-hexane. [o] Sinters at 103°. [p] Recrystallized successively from a 25% methanol, and acetone-hexane. [q] Darkens at 187°. [r] Recrystallized from 50% methanol. [s] Recrystallized from ethyl acetate-hexane. [t] Sinters at 143.9°. [u] Recrystallized from ether-petroleum ether. [v] Bath preheated to 195°; softens at 213°. [w] Recrystallized from 80% ethanol. [x] Could not be recrystallized from organic solvents; twice precipitated (acid) from aqueous solution of sodium salt as a buff-colored resin. [y] Recrystallized from 95% ethanol. [z] Recrystallized twice from 90% methanol. [aa] Yield based on once recrystallized material.

EXPERIMENTAL

Intermediates. The amino acids[2] used in this work were purchased from several commercial sources and were used without further purification. The halogenated phenoxyacetic acids were also obtained from commercial sources, but were recrystallized.

Acid chlorides. 2,4-Dichlorophenoxyacetyl chloride was prepared in 84–90% yield, in a manner analogous to that described by Freed (5), by reacting 1 mole of 2,4-dichlorophenoxyacetic acid, recrystallized from benzene, with 3 moles of thionyl chloride; the boiling point of the acid chloride was 155–157°/22–23 mm. The preparation consisted of a supercooled liquid, as reported (5), which occasionally crystallized at room temperature. By the same reaction, 4-chlorophenoxyacetyl chloride was obtained in a 76% yield, b.p. 167–174°/59–61 mm.; reported 142°/17 mm. m.p. 18.8° (6).

The preparation of 2,4,5-trichlorophenoxyacetyl chloride by the method of Hill, *et al.* (7) was found to be very time-consuming; it involved the dropwise addition of 1 mole of thionyl chloride to 0.25 mole of the acid over a period of 24 hours and refluxing the reaction mixture on a steam-bath for two hours, yield 80%. By modifying their procedure, 2,4,5-trichlorophenoxyacetyl chloride was obtained in 87% yield. The modification consisted of adding 1.5 moles of thionyl chloride immediately to 0.5 mole of the corresponding purified acid (recrystallized from benzene and decolorized), refluxing for two hours, and recovering the acid chloride in the usual manner, b.p. 142–144°/2–3 mm., m.p. 70–71° (uncorr.); reported (7) b.p. 165–167°/6 mm., m.p. 78–79° (uncorr.).

Amides. In general, the halogenated phenoxyacetyl derivatives of the monoamino, monocarboxylic acids were prepared by dissolving the amino acids, varying in amount from 0.005 to 0.1 mole, in three mole-equivalents of 1 *N* sodium hydroxide solution, chilling the resulting solutions of the sodium salts to about 5° in an ice-bath, and adding with rapid stirring a solution of one mole-equivalent of the acid chloride in benzene over a period of five to 10 minutes, or at such a rate that the temperature of the reaction mixture did not exceed 15°. In the cases of the aspartic acids, L- and DL-proline, the phenylalanines, L-cystine, DL-serine, and DL-threonine, heavy emulsions or gels formed shortly after the addition of the acid chloride. In these instances, additional amounts of water were added to thin the reaction mixtures. After the addition of the acid chloride was completed, the ice-bath was removed, and stirring was continued for 2 to 3 hours. The reaction mixture was extracted with ether, and the aqueous phase was separated and acidified with 1:2 hydrochloric acid solution until blue to Congo Red.

In most cases the crude acylated derivative separated as a pure white solid. This was filtered off, washed with water, and recrystallized from 50% ethanol, with the exceptions noted in Table I. Usually one crystallization from ethanol was sufficient to give a product having a satisfactory analysis and a constant melting point. The 2,4-dichlorophenoxyacetyl derivatives of the following amino acids were obtained as oils, following acidification of solutions of their sodium salts, but they solidified readily upon being chilled and scratched: L-aspartic acid, DL-aspartic acid, DL-glutamic acid, DL-leucine, DL-lysine, D-methionine, DL-proline, and DL-valine. The DL-lysine derivative precipitated as an oil which solidified only after being stored in the refrigerator overnight. The DL-threonine derivative solidified only after remaining several days in the refrigerator. The L-proline and DL-tryptophan derivatives and the bis-derivative of DL-tyrosine methyl ester were much more refractory and were obtained only as amorphous or granular solids after repeated attempts at crystallization with the solvents shown in Table I. All attempts to form the amides of histidine and tyrosine by reacting 2,4-dichlorophenoxyacetyl chloride with aqueous solutions of their sodium salts, as described above, resulted in the formation of unidentifiable oils or products. Histidine and tyrosine could be acylated only in the form of their methyl esters as described below. Several unsuccessful attempts were made to prepare either the mono- or di-acyl derivatives of L-arginine with 2,4-dichlorophenoxyacetyl chloride; products having fairly constant chlorine and nitrogen contents between those of the two derivatives were obtained.

The following directions are given to illustrate the general and the specialized procedures employed in the synthesis of the afore-mentioned amino acid derivatives:

N-(2,4-Dichlorophenoxyacetyl)-D-*aspartic acid.* D-Aspartic acid (1.9 g.; 0.014 mole) was dissolved in 43 ml. of 1 *N* sodium hydroxide (0.043 mole), and 75 ml. of water was added. To the resulting solution, which was maintained at 5–10° (ice-bath), was added dropwise with rapid stirring a solution of 3.4 g. (0.014 mole) of 2,4-dichlorophenoxyacetyl chloride in 15 ml. of benzene. The ice-bath was removed about 15 minutes after the addition was complete, and the stirring was continued for a total period of 3 hours. The reaction mixture was transferred to a separatory-funnel and was extracted with ether. The aqueous layer was separated and was acidified with dilute HCl (1:2) until acid to Congo Red. The derivative, which precipitated immediately as a white solid, was filtered, washed several times with water, and dried overnight in a vacuum oven at 50°. The yield was 3.18 g. (66.4%), m.p. 190.5–192.5° (corr.). After recrystallization from 25% ethanol, the product consisted of fine crystalline needles melting at 198.7–200.8° (corr.). Because of a slightly high chlorine content the amide was recrystallized a second time from a hot dioxane solution to which benzene was added; the melting point of this material was 200.8–201.8° (corr.).

N^{α},N^{ϵ}-*bis-(2,4-Dichlorophenoxyacetyl)*-DL-*lysine.* DL-lysine monohydrochloride (6.04 g., 0.033 mole) was dissolved in 165 ml. of 1 *N* NaOH (0.165 mole). To the resulting solution cooled in an ice-bath, a solution of 15.8 g. (0.066 mole) of the acid chloride in 60 ml. of benzene was added. The temperature of the reaction mixture remained at 10–15°. After the ice-bath was removed, the reaction mixture was stirred for 4 hours, during which it became a very stable, thin, white emulsion which was very difficult to extract with ether. After breaking the emulsion with solid anhydrous sodium sulfate, the extraction of the reaction mixture with ether was completed. Acidification (to Congo Red) of the aqueous fraction with dilute HCl (1:2) gave a colorless oil which solidified and grew into a voluminous porous mass when kept in the refrigerator for 5 minutes. After being washed with water and dried (vac. 50°), the yield of crude product was 16 g. (87.9%), m.p. 172–174° (corr.). Recrystallization of the entire lot from 200 ml. of 95% ethanol gave 14.4 g. of a granular product which was slightly "off white" in color and melted at 175.0–176.1° (corr.). The corresponding L-lysine derivative was obtained as an oil which solidified to a hard, granular solid on being kept in the refrigerator overnight. Attempts to recrystallize the corresponding L-lysine derivative from numerous organic solvents failed. It was finally purified through solution of the sodium salt in a large volume of water and reprecipitation with dilute hydrochloric acid.

N^{α}-*(2,4-Dichlorophenoxyacetyl)*-DL-*histidine methyl ester hydrochloride.* The methyl ester of DL-histidine dihydrochloride was obtained in 88.9% yield, m.p. 189.5° (corr., dec.), by the method adapted partly from Pauly's (8) original directions and partly from Fischer's (9) later modification of Pauly's directions for the preparation of the corresponding L-histidine compound. Histidine methyl ester dihydrochloride (1 g., 0.004 mole) was dissolved in 10 ml. of boiling absolute methanol (dried with magnesium). The solution was cooled, and to it was added gradually a cooled solution of 0.18 g. (0.008 mole) of sodium in 4 ml. of dry methanol. The precipitation of the sodium chloride was completed by the addition of 10 ml. of dry ether. After filtering off the salt, the filtrate was evaporated to dryness on the steam-bath under reduced pressure. Freshly washed and dried chloroform was added to the residue which was evaporated under the same conditions to flush out the last traces of methanol. The semisolid white residue, DL-histidine methyl ester, was dissolved in 16 ml. of purified chloroform, and to it was added a solution of 0.79 g. (0.0033 mole) of 2,4-dichlorophenoxyacetyl chloride dissolved in 5 ml. of chloroform. A flocculent precipitate, which became gummy, formed immediately and was discarded. The supernatant solution was evaporated under a vacuum on the steam-bath to an oil which later partly solidified to a paste. The product remained as a tacky resin after being triturated with ether and stored in the refrigerator overnight, yield 0.6 g. (44%). The product was soluble in water and 95% ethanol, and insoluble in ether and ethyl acetate. The crude derivative was purified by dissolving it in 95% ethanol and adding ether. The oil that precipitated changed to a white solid on being stored in the refrigerator for several hours. After being dried for several days in a vacuum-oven at 50°, the derivative melted at 128.6–129.7° (corr.), yield 0.4 g. (30%).

N,O-bis-(2,4-Dichlorophenoxyacetyl)-DL-*tyrosine methyl ester.* The methyl ester of DL-

tyrosine was prepared in 70% yield, m.p. 110–111°, by a method identical with the one given by Fischer (10) for the preparation of the L-isomer. DL-Tyrosine methyl ester (0.45 g., 0.0023 mole) was dissolved in a mixture of 10 ml. of freshly washed and dried chloroform and 2.5 ml. of dry pyridine. To the resulting solution was added, with cooling, a solution of 1.11 g. (0.0046 mole) of 2,4-dichlorophenoxyacetyl chloride in 5 ml. of dry chloroform. After standing 24 hours, the yellow-colored homogenous mixture was transferred to a separatory-funnel and was washed with four portions of 10% HCl, totaling 100 ml., in order to remove excess pyridine and its hydrochloride. The chloroform solution was then washed successively with water, dilute sodium bicarbonate solution, and water, and then dried with sodium sulfate. After removing the solvent under a vacuum on the steam-bath, the residue was dried (vac., 50°) to a light colored tacky resin, yield 1.46 g. The resin was crystallized from 90% methanol to give 0.75 g. (54%) of a white granular solid, m.p. 116.6–119.7° (corr.), sinter 115° (corr.). A second recrystallization from 90% methanol gave a product melting at 119.7–121.6° (corr.), yield 0.6 g. (43%).

SUMMARY

A series of new halogenated phenoxyacetyl derivatives of DL-, L-, and D-amino acids have been prepared and tested for plant-growth regulating activity. The derivatives of DL- and L-amino acids were found to be active plant-growth regulators, whereas derivatives of D-amino acids were relatively inactive. The results suggest that plants affected by 2,4-dichlorophenoxyacetic acid are unable to split readily the amide linkage of 2,4-dichlorophenoxyacetyl-D-amino acids. These and other derivatives of D-amino acids should prove to be very valuable to investigators studying the mode of action and various responses of plant-growth regulators. These derivatives may also be useful in the characterization of amino acids because of their relative ease of preparation, good yields, fairly sharp melting points, and high chlorine content.

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