

## SYNTHETIC PLANT-GROWTH REGULATORS

### I. 2,4-DICHLORO-5-IODOPHENOXYACETIC ACID AND DERIVATIVES

WINTHROP C. WOLFE, JOHN W. WOOD, LORAIN W. KLIPP, THOMAS D.  
FONTAINE, AND J. W. MITCHELL

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The application of plant-growth regulators to specific agricultural problems has created considerable interest in this field of research. Many compounds have been tested and some have been found active, but their mechanism of action is not understood. Our earlier work (1, 2) on the translocation of a radioactive labeled plant-growth regulator, 2-iodo<sup>131</sup>-3-nitrobenzoic acid, gave very promising results. However since certain of the phenoxyacetic acids are the most powerful regulators discovered to date, an investigation of the mechanism of action of a typical phenoxyacetic acid would contribute much to our knowledge. The synthesis and properties of new plant-growth regulators, 2,4-dichloro-5-iodophenoxyacetic acid and 15 of its derivatives, are the subject of this paper. The synthesis of 2,4-dichloro-5-iodophenoxyacetic acid and its derivatives containing radioactive iodine, used in translocation and mechanism of action investigations, is the subject of Part II of this paper.

#### EXPERIMENTAL

*2,4-Dichloro-5-nitrophenoxyacetic acid.* The synthesis of this compound was conducted, in part, in accordance with the instructions of Newman and Wotiz (3). Twenty-two g. of finely ground 2,4-dichlorophenoxyacetic acid (4), recrystallized from benzene, and of m.p. 140–141°, was added, with shaking, to 80 ml. of C.P. fuming nitric acid (*sp. gr.* 1.50) and allowed to react for 1½ to 2 minutes at 30–40°. To stop the reaction the clear, orange solution was poured on to 400 g. of crushed ice. The crude product was recrystallized first from 50% ethanol, m.p. 155–157° (yield 70%), and finally from a mixture of 85% benzene and 15% absolute ethanol to yield 2,4-dichloro-5-nitrophenoxyacetic acid, m.p. 158.5–159.5° (*cor.*) (yield 50%).

*Anal.* Calc'd for C<sub>8</sub>H<sub>5</sub>Cl<sub>2</sub>NO<sub>3</sub>: C, 36.16; H, 1.88; Cl, 26.65; N, 5.93; Neut. equiv., 266.0.  
Found: C, 36.24; H, 1.99; Cl, 26.61; N, 5.90; Neut. equiv., 265.5.

*5-Amino-2,4-dichlorophenoxyacetic acid.* This product, m.p. 173.5–174.5° (*cor.*), was prepared in 95% yield by the reduction of 100 g. of 2,4-dichloro-5-nitrophenoxyacetic acid with ferrous sulfate and ammonia by the method of Jacobs and Heidelberg (5).

*Anal.* Calc'd for C<sub>8</sub>H<sub>7</sub>Cl<sub>2</sub>NO<sub>2</sub>: C, 40.70; H, 2.99; Cl, 30.04, N, 5.26.  
Found: C, 41.1; H, 3.1; Cl, 29.9; N, 5.25.

*2,4-Dichloro-5-iodophenoxyacetic acid.* The sodium salt of 5-amino-2,4-dichlorophenoxyacetic acid (11.81 g.) was diazotized in sulfuric acid at 0–5°. Urea was added to destroy excess nitrous acid. Twenty ml. (8.72 g.) of potassium iodide was then added rapidly, with stirring, and the reaction mixture warmed to 70–80° for thirty minutes, then cooled, filtered with suction and the product washed with 5% sodium bisulfite and water. The product, recrystallized from 50% ethanol, was obtained in a 65% yield, m.p. 151.5–152.5° (*cor.*).

*Anal.* Calc'd for C<sub>8</sub>H<sub>5</sub>Cl<sub>2</sub>IO<sub>3</sub>: C, 27.69; H, 1.45; I, 36.58.  
Found: C, 27.71; H, 1.72; I, 36.83 (assuming theor. % Cl).

*5-Acetamino-2,4-dichlorophenoxyacetic acid.* This product was prepared from 5-acetamino-2,4-dichlorophenol by the method of Synerholm and Zimmerman (5). The crude

product, m.p. 199–209°, when recrystallized from 95% ethanol melted at 213.5–214.5°, cor., (yield 49%). The structure of 5-amino-2,4-dichlorophenoxyacetic acid was established by acetylation to the 5-acetamino derivative. The product melted at 213.5–214.5° (cor.) and the melting point was not depressed by mixing with a sample of the compound prepared from 5-acetamino-2,4-dichlorophenol.

*Anal.* Calc'd for  $C_{10}H_9Cl_2NO_4$ : C, 43.19; H, 3.27; Cl, 25.49; N, 5.03.

Found: C, 43.18; H, 3.23; Cl, 25.60; N, 4.97.

#### PREPARATION AND PLANT-GROWTH REGULATING ACTIVITY OF DERIVATIVES OF 2,4-DICHLORO-5-IODOPHENOXYACETIC ACID

The derivatives of 2,4-dichloro-5-iodophenoxyacetic acid prepared by chemists of the Bureau of Agricultural and Industrial Chemistry, and tested for plant-growth regulating activity by Mitchell and Linder, Bureau of Plant Industry, Soils, and Agricultural Engineering, are listed in Table I. The inhibition of growth in the terminal bud of test kidney bean seedlings was used to compare the regulating activity of 2,4-dichloro-5-iodophenoxyacetic acid and its derivatives with the activity of 2,4-dichlorophenoxyacetic acid. The activity of 2,4-dichloro-5-iodophenoxyacetic acid was found to be only about 60% that of 2,4-dichlorophenoxyacetic acid, showing the marked influence of iodine in the 5-position. On the other hand, the esters and amide were found to have a greater activity than their parent acid, 2,4-dichloro-5-iodophenoxyacetic acid, when tested on an equivalent weight basis, whereas, the salts had lower or no better activity than their parent acid. The activity of some esters approached, but in no case reached, the activity of 2,4-dichlorophenoxyacetic acid. Detailed results of the plant-growth regulating activity of 2,4-dichloro-5-iodophenoxyacetic acid and its derivatives will be reported elsewhere.

#### II. RADIOACTIVE LABELED 2,4-DICHLORO-5-iodo<sup>131</sup>-PHENOXYACETIC ACID AND DERIVATIVES

JOHN W. WOOD, WINTHROP C. WOLFE, H. M. DOUKAS, L. W.  
KLIPP, THOMAS D. FONTAINE, AND J. W. MITCHELL

In Part I of this paper the synthesis of stable 2,4-dichloro-5-iodophenoxyacetic acid (2,4-D-5-I) has been reported. Part II will describe the apparatus used in the synthesis of 2,4-D-5-I and its derivatives containing radioiodine, the distribution of radioiodine in product and by-products during the synthesis, and the use of this compound for investigating the mechanism of action of plant-growth regulators.

#### EXPERIMENTAL

*2,4-Dichloro-5-iodo<sup>131</sup>-phenoxyacetic acid.* The reaction apparatus shown in Figure 1 was mounted on a stainless steel pan behind an L-shaped lead shield, 2 inches thick with a front vertical section 20 inches wide and 24 inches high, and in a well-ventilated hood. A control panel was mounted on the front vertical section of the lead shield. Other shielding was provided by portable lead bricks, 2 × 4 × 8 inches in size. Manipulative work was conducted with extension tongs.

*Diazotization of 5-amino-2,4-dichlorophenoxyacetic acid* (11.81 g.) and reaction with

potassium iodide containing 20 mc.I<sup>131</sup> were conducted in beaker A by procedures given in Part I of this paper. The crude product was cooled to 15–20° and then transferred with vacuum to funnel B, the filtrate being allowed to run into flask C and then into flask D. The crude product was washed with 5% sodium bisulfite solution, then with water, and the

TABLE I  
DERIVATIVES OF 2,4-DICHLORO-5-IODOPHENOXYACETIC ACID

DERIVATIVES	M.P., °C (COR.)	EMPIRICAL FORMULA	ANALYSIS		
			Element	Calc'd, %	Found, %
Ammonium salt <sup>a</sup>	234–235	C <sub>8</sub> H <sub>8</sub> Cl <sub>2</sub> INO <sub>3</sub>	Nitrogen	3.85	3.71
Ethylammonium salt <sup>a</sup>	194–195	C <sub>10</sub> H <sub>12</sub> Cl <sub>2</sub> INO <sub>3</sub>	Nitrogen	3.57	3.56
Diethylammonium salt <sup>b</sup>	145.5–146.5	C <sub>12</sub> H <sub>16</sub> Cl <sub>2</sub> INO <sub>3</sub>	Nitrogen	3.33	3.32
Morpholine salt <sup>b</sup>	193.5–197.5	C <sub>12</sub> H <sub>14</sub> Cl <sub>2</sub> INO <sub>4</sub>	Nitrogen	3.20	3.22
Triethanolamine salt <sup>a</sup>	113–114	C <sub>14</sub> H <sub>20</sub> Cl <sub>2</sub> INO <sub>6</sub>	Nitrogen	2.82	2.97
Sodium salt <sup>c</sup>		C <sub>8</sub> H <sub>4</sub> Cl <sub>2</sub> INaO <sub>3</sub>	Sodium	6.23	6.22
Potassium salt <sup>c</sup>		C <sub>8</sub> H <sub>4</sub> Cl <sub>2</sub> IKO <sub>3</sub>	Potassium	10.15	9.94
Calcium salt <sup>d</sup>		C <sub>16</sub> H <sub>8</sub> CaCl <sub>4</sub> I <sub>2</sub> O <sub>8</sub>	Calcium	5.48	5.38
Cupric salt <sup>d</sup>		C <sub>16</sub> H <sub>8</sub> Cl <sub>4</sub> CuI <sub>2</sub> O <sub>8</sub>	Copper	8.41	8.41
Acid chloride <sup>e</sup>		C <sub>8</sub> H <sub>4</sub> Cl <sub>3</sub> IO <sub>2</sub>	—	—	—
Methyl ester <sup>f</sup>	109.5–111.5	C <sub>9</sub> H <sub>7</sub> Cl <sub>2</sub> IO <sub>3</sub>	Carbon	29.94	30.02
			Hydrogen	1.95	2.10
			Iodine	35.16	35.01
Ethyl ester <sup>f</sup>	83.5–84.5	C <sub>10</sub> H <sub>9</sub> Cl <sub>2</sub> IO <sub>3</sub>	Iodine	33.84	33.82
Isopropyl ester <sup>f</sup>	88–89	C <sub>11</sub> H <sub>11</sub> Cl <sub>2</sub> IO <sub>3</sub>	Carbon	33.87	34.04
			Hydrogen	3.10	3.14
<i>n</i> -Butyl ester <sup>f</sup>	70.5–71.0	C <sub>12</sub> H <sub>13</sub> Cl <sub>2</sub> IO <sub>3</sub>	Carbon	35.75	35.77
			Hydrogen	3.25	3.48
Amide <sup>g</sup>	160–161	C <sub>8</sub> H <sub>6</sub> Cl <sub>2</sub> INO <sub>2</sub>	Nitrogen	4.05	4.05

<sup>a</sup> An ethanol solution of dry ammonia or amine was added to an ethanol solution of the acid. The ethylammonium and triethanolamine salts were then recrystallized from ethanol.

<sup>b</sup> The acid was reacted with excess amine at room temperature and the reaction mixture diluted with ethanol. The diethylammonium salt was recrystallized from benzene and the morpholine salt from ethanol.

<sup>c</sup> The calculated amount of one-normal alkali solution was added to an ethanol solution of the acid. The salt was then recrystallized from 50% ethanol.

<sup>d</sup> An aqueous ethanol solution of metal acetate was added to an ethanol solution of the acid. The calcium salt was recrystallized from 50% ethanol.

<sup>e</sup> The acid was refluxed 1½ hours with excess thionyl chloride; the product was vacuum distilled, boiling at 180°/5 mm.

<sup>f</sup> The acid chloride was refluxed 1½ hours with excess absolute alcohol and the ester was recrystallized from 50% ethanol.

<sup>g</sup> The acid chloride was treated with ice cold conc'd ammonia and the amide recrystallized from 50% ethanol.

combined filtrates collected in flask D. Absolute ethanol (150 ml.) was added through E to flask C and the crude 2,4-D-5-I was dissolved by refluxing with hot ethanol. The hot ethanolic solution of 2,4-D-5-I was drawn from funnel B into flask C by applying vacuum at point 1, and then forced from flask C, with a slight pressure applied at point 1, into a 500-ml. round-bottomed flask (position—beaker A) and heated with 0.5 g. of activated carbon.

The hot solution was transferred to funnel B, filtered to remove the carbon, and the filtrate collected in flask C. Pressure was then applied at point 1, and the hot filtrate was forced out of flask C into flask G. Hot water (150 ml.) was then added to flask G and the contents thoroughly mixed, then cooled and filtered. Ten g. of nonradioactive 2,4-dichloro-5-iodo-phenoxyacetic acid, as carrier, was added to the moist radioactive product. The final product, recrystallized from 50% ethanol, melted at 151.5–152.5° (yield 18.1 g.).

If it is assumed that a complete recovery of the nonradioactive acid (10.0 g.) was achieved, then 8.1 g. of radioactive material or 47% of theory was obtained on the basis of the radioactive synthesis. The specific activity of the acid (18.1 g.) was  $5.79 (\pm) 0.06 \times 10^8$  counts per second per milligram, which accounted for approximately 47% of the radioactivity intro-

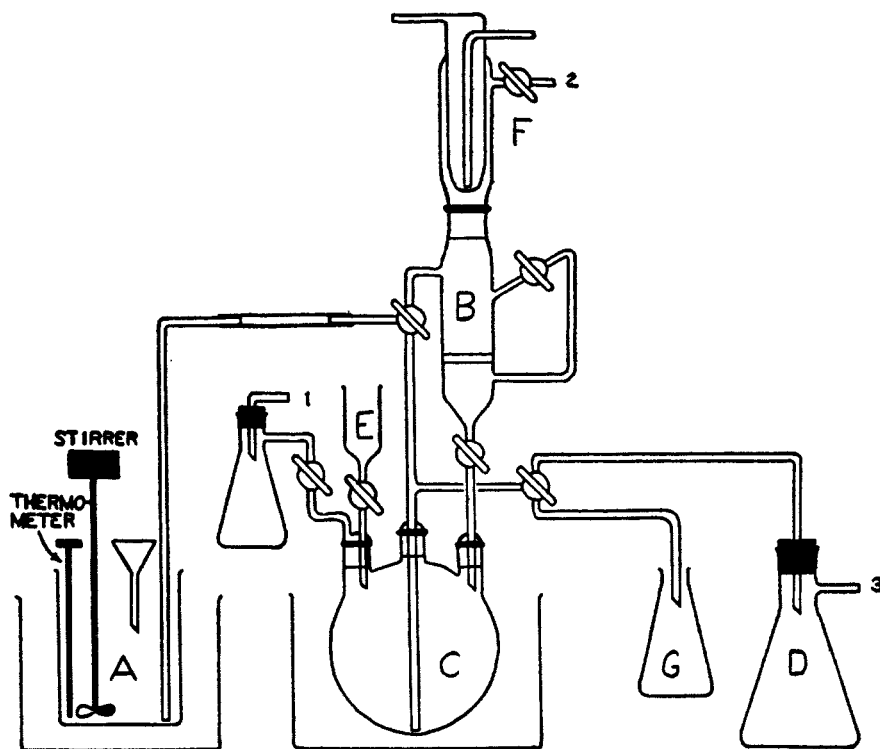


FIGURE 1. REACTION APPARATUS

duced in the synthesis. The distribution of radioactivity in this synthesis is given in Table II.

*Derivatives of 2,4-dichloro-5-iodo<sup>131</sup>-phenoxyacetic acid.* The following eleven derivatives were prepared, essentially as stated in Part I of this paper: ammonium, ethylammonium, diethylammonium, triethanolamine, sodium, and calcium salts; acid chloride; amide; and methyl, isopropyl, and *n*-butyl esters.

#### RADIOACTIVITY MEASUREMENTS

Radioactivity assays were made on aliquots of the original radioiodide<sup>131</sup> solution,<sup>1</sup> an alcoholic solution of recrystallized 2,4-dichloro-5-iodo<sup>131</sup>-phenoxyacetic acid, the re-

<sup>1</sup> Radioiodine was obtained through the Isotopes Branch, Manhattan District, Oak Ridge, Tennessee.

TABLE II  
DISTRIBUTION OF RADIOACTIVITY IN SYNTHESIS OF 2,4-DICHLORO-5-iodo<sup>131</sup>-PHENOXYACETIC ACID

MATERIAL	DILUTION RATIO OF ASSAY SOLUTION	COUNTING RATE <sup>a</sup> OF ASSAY SOLUTION COUNTS/SEC./0.1 ML.	SPECIFIC ACTIVITY OF MATERIAL COUNTS/SEC./ML.	AMOUNT OF MATERIAL	TOTAL RADIOACTIVITY PRESENT IN MATERIAL		TOTAL RADIOACTIVITY COUNTED FOR PER CENT
					Counts/sec.	Millicuries <sup>b</sup>	
Radioiodide <sup>131</sup> used in synthesis.	1:25,000	37.5 ± 0.4	9.38 ± 0.10 × 10 <sup>6</sup>	24 ml.	2.25 ± 0.03 × 10 <sup>8</sup>	20.1 ± 0.7	100.0
Recrystallized 2,4-Dichloro-5-iodo <sup>131</sup> phenoxyacetic acid.	—	29.8 ± 0.33 <sup>c</sup>	5.79 ± 0.06 × 10 <sup>6d</sup>	18.1 g.	1.05 ± 0.01 × 10 <sup>8</sup>	9.5 ± 0.3	47.3
Reaction mixture + washings.	1:25	100.5 ± 1.11	2.51 ± 0.03 × 10 <sup>4</sup>	1120 ml.	2.81 ± 0.03 × 10 <sup>7</sup>	2.5 ± 0.1	12.4
Mother liquor from 1st recrystallization.	1:58.8	122.0 ± 1.34	4.88 ± 0.05 × 10 <sup>4</sup>	340 ml.	2.44 ± 0.03 × 10 <sup>7</sup>	2.2 ± 0.1	10.9
Mother liquor from 2nd recrystallization.	1:10	330.0 ± 3.63	3.30 ± 0.04 × 10 <sup>4</sup>	485 ml.	1.60 ± 0.02 × 10 <sup>7</sup>	1.4 ± 0.1	7.0
Radioactivity unaccounted for (possibly present in carbon black and in residues on walls of reaction vessels and filtration apparatus)							22.4
Total							100.0

<sup>a</sup> All values are at zero absorber and have been corrected for decay, coincidence, etc.

<sup>b</sup> Total disintegration rate, milllicuries (mc.) =  $\frac{\text{total radioactivity (counts/sec.)} \times \text{geometry (3.34 disintegrations/count)}}{3.7 \times 10^7}$

<sup>c</sup> 0.1 ml. = 0.00515 mg. acid

<sup>d</sup> counts/sec./mg.

action mixture plus washings, and the mother liquors from the first and second recrystallizations of the acid. The aliquots were diluted with a special dilution medium<sup>2</sup> composed of 0.02 *M* sodium hydroxide, 0.0015 *M* potassium iodide, and 0.005 *M* sodium bisulfite.

Samples were prepared, for counting, by transferring 0.1-ml. aliquots of the various solutions by means of calibrated micro-pipettes to the surfaces of palladium-faced silver disks,<sup>3</sup>  $\frac{1}{16}$  inch thick by 1 inch in diameter. One drop of a dilute aqueous silver nitrate solution (1 mg./ml.) was then added to the deposit, and the latter was evaporated to dryness under an infra-red heat lamp. Measurements were carried out by means of a Victoreen counter, Model VG-10<sup>4</sup> with mica window 2.6 mg./cm<sup>2</sup>, and an Autoscaler<sup>5</sup> having a scaling ratio of 4096.

The counting rates of the samples were determined by measuring the samples with calibrated aluminum absorbers of various thicknesses interposed before the window of the counter. The counting rates, after being corrected for background (13-14 counts/minute with the counter in a lead shield having a wall thickness of two inches) and decay, were plotted on triple cycle semi-logarithmic graph paper as a function of activity (ordinate) versus absorber thickness (abscissa), taking into account the thickness of the counter window and the absorption of the beta particles in the air above the sample. The specific activities at zero absorber were then obtained by extrapolation. The values had an overall accuracy of ( $\pm$ ) 1.1% (probable error). The absolute disintegration rates of the various radioiodine samples were then calculated from the values for the specific activities at zero absorber and the geometry and efficiency of the counter. The latter value was found to be 3.34 disintegrations per count as determined from the ratio of the absolute disintegrations rate of a calibrated Radium D + E standard<sup>6</sup> to its counting rate at zero absorber, obtained in the same manner as that of the radioiodine solutions.

#### TRACER EXPERIMENTS

These compounds were applied to a considerable number of different plants, and they were found to accumulate primarily in the young meristematic tissues. For prolonged investigations compounds of higher specific activity are required and they can be synthesized using the procedures and apparatus described. The detailed results on the translocation and mechanism-of-action investigations on plants will be published elsewhere.

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#### SUMMARY

A new synthetic plant-growth regulator, 2,4-dichloro-5-iodophenoxyacetic acid and 15 of its derivatives have been synthesized and their biological prop-

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<sup>2</sup> Recommended by the Monsanto Chemical Company, formerly of the Clinton Laboratories, Oak Ridge, Tennessee, and the Radioactivity Section of the National Bureau of Standards, Washington, D. C.

<sup>3</sup> Obtained through the courtesy of Dr. L. F. Curtiss, Radioactivity Section, National Bureau of Standards, Washington, D. C.

<sup>4</sup> Victoreen Instrument Company, Cleveland, Ohio.

<sup>5</sup> Tracerlab Inc., Boston, Massachusetts.

<sup>6</sup> This standard, calibrated and furnished by the Radioactivity Section, National Bureau of Standards, Washington, D. C., was found to have an absolute activity of 108.9 ( $\pm$  2%) disintegrations/sec. on December 29, 1948, after correcting for its decay following the calibration date.

erties studied. The new plant-growth regulator and eleven derivatives containing radioiodine have been synthesized. The apparatus used in these syntheses is described, and the distribution of radioactivity in the synthesis of 2,4-dichloro-5-iodo<sup>131</sup>-phenoxyacetic acid is recorded. The use of these compounds in investigations on the mechanism of action of phenoxyacetic acid type plant-growth regulators is indicated.

BELTSVILLE, MARYLAND

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