Synthesis of Carbon-14–Labeled Dalapon and Trial Applications to Soybean and Corn Plants

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Sodium 2,2-dichloropropionate-2-C^{14} (sodium dalapon) at 1 mc. per mmole has been prepared by direct chlorination of propionic acid. Separation from other chlorinated acids was achieved by gradient elution from a phosphate-buffered silica gel column. A yield of 32 mole % based on the propionic acid was obtained. Radiochemical purity was 96%. This material was applied to corn and soybean plants. The distribution of radioactivity was measured. Radioactivity recovered by extractions was identified as dalapon by paper chromatography. No breakdown products were found.

DALAPON (2,2-DICHLOROPROPIONIC ACID) is a useful herbicide for the selective control of grasses. It is therefore of considerable importance to study the residues in crop plants and to learn something about the uptake, distribution, and stability of the compound in various plants. In order to provide a radiotracer for such studies sodium dalapon-2-C^{14} has been prepared. Brief tracer studies with corn and soybean plants are included in this paper.

Synthesis

The measurement of potentially small residues and even smaller amounts of possible breakdown products necessitated a specific activity of 1 mc. per mmole. This required a microscale synthesis.

Two procedures were investigated. In the first of these pyruvamide was hydrolyzed (*5*, *6*) in an acid medium and the pyruvic acid recovered by continuous ether extraction. After ether was removed, the pyruvic acid was treated with phosphorus pentachloride (*12*). Under optimum reaction conditions the maximum yield of dalapon was 20%. By-products included unreacted pyruvic acid, acetic acid, 2-chloroacrylic acid, and 2,2,3-trichloropropionic acid.

A second method involving the direct chlorination of propionic acid was investigated and was used in preparing the tracer material. The procedure is an adaptation of a microscale method described by Bass (*1*), Bass and Burlew (*2*), and Brust and Senkbeil (*3*).

Chlorination. The chlorination is carried out in two steps. One mole of chlorine reacts at 121° C. to yield principally 2-chloropropionic acid. The second mole of chlorine is introduced at 173° C. to yield predominantly 2,2-dichloropropionic acid. Phosphorus trichloride is employed as a catalyst.

Special equipment (Figure 1) had to be designed to maintain a flow of a small volume of chlorine gas through an exceedingly small quantity of liquid at controlled, elevated temperatures.

Chlorine (Dow) was forced under a head of 85% phosphoric acid from a graduated reservoir, through a drying tube containing phosphoric acid anhydride, into a reactor through a small capillary supply tube. The reactor was fabricated from glass tubing (2-mm. inside diameter), with a slight enlargement at the closed end. The chlorine supply tube was made from a 1-mm. bore capillary tube by drawing it down to a terminal cross section of about 50 microns inside diameter. Proper reaction temperatures were maintained by refluxing solvents of suitable boiling points. A dry ice trap was employed to catch volatile by-products, while a calcium chloride tube protected the system from atmospheric moisture.

The optimum conditions of chlorine supply, catalyst, time, and temperature of reaction were determined by analysis of the products obtained from 25 cold runs. The manner in which the phosphorus trichloride was supplied to the reactor was very important. When it was distilled into the reactor by vacuum transfer, frequent stoppages of the chlorine flow occurred from plugging of the capillary tube with resinous by-products. When the phosphorus trichloride was added as two or three drops from a micropipet with attendant exposure to atmospheric moisture, satisfactory flow was obtained throughout the chlorination.

The variation in composition of the crude reaction product as a function of chlorination time is shown in Figure 2. The reaction was not stepwise as at no time was there a single product found.

The composition of these crude chlorination products was determined by infrared analysis. A typical spectrum scanned on a double-beam infrared-spectrometer with sodium chloride optics is shown in Figure 3. This particular sample was prepared by dissolving 89 mg. of reaction product in 1 ml. of carbon disulfide. It was scanned in a 0.1-mm. thick cell. Absorption bands for 2,2-dichloropropionic acid (DCP), 2,2,3-trichloropropionic acid (TCP), 2-chloropropionic acid (MCP), and several unidentified materials (x) can be seen. Absorptions at 4.34 and 4.64 microns and in the 6-micron region are due to the solvent.

Purification. Isolation of 2,2-dichloropropionic acid from the mixture of acidic materials in the crude reaction product was achieved with a phosphate-buffered silica gel chromatographic column. The column was prepared as follows: To 60 grams of silica gel (Davison) were added 38 ml. of a saturated aqueous solution of sodium dihydrogen orthophosphate adjusted to a pH of 4. (A lower pH gives a faster column, but somewhat poorer resolution.) The resulting mixture was stirred well; then chloroform was added. Two-milliliter portions of this suspension were added successively to a 100-ml. buret filled with chloroform. As the silica gel settled, it was packed with a glass rod enlarged at the end to nearly the diameter of the buret. After the silica gel was added, the column was rinsed with 200 ml. of water-washed chloroform.

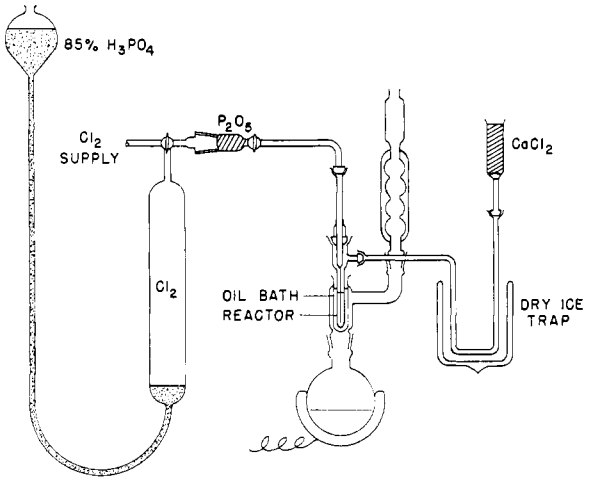

Figure 1. Propionic acid chlorination apparatus

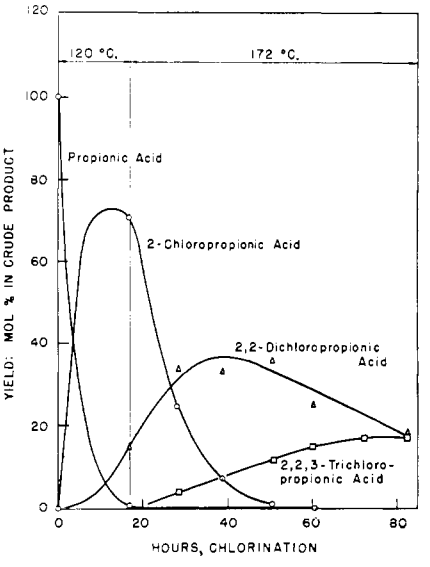

Figure 2. Composition of reaction products obtained during chlorination of propionic acid

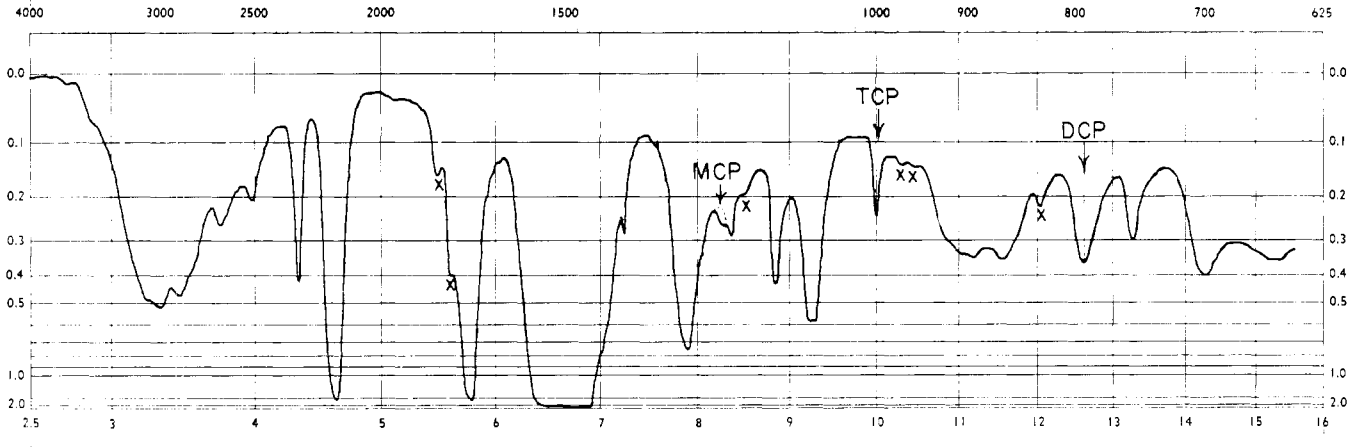

Figure 3. Infrared spectrum of propionic acid chlorination product

After the sample to be separated had been placed on the column, elution was effected with chloroform containing progressively increasing amounts of 1-pentanol. This gradient elution system was fashioned after the one described by Donaldson, Tulane, and Marshall (*9*). Chloroform, water washed, was added to the column and to a 500-ml. Erlenmeyer mixing flask which had a top outlet connected to the column. An inlet line from an elevated reservoir passed down into the mixing flask and emptied near the bottom. A magnetic stirrer was used to assure good mixing. Six hundred twenty milliliters of 2% 1-pentanol in chloroform were added to the reservoir. Fractions of 20 to 40 ml. were collected. The 2% 1-pentanol was followed by 60 ml. of 8% 1-pentanol in chloroform, 160 ml. of 16%, 260 ml. of 20%, and 300 ml of 40%. A total of 1400 ml. of solvent were used. Each fraction was titrated.

An elution curve for a mixture of 0.19 meq. of MCP, 1.84 meq. of DCP, and 0.70 meq. of TCP is given in Figure 4. The respective recoveries were 0.26, 1.67, and 0.71 meq.

Radioactive Preparation. Propionic 2-C^{14} acid (Nuclear-Chicago Corp.) (170 mg.) with a specific activity of 2.06 mc. per mmole was transferred via a vacuum manifold from the break-seal shipping vial into the reaction vessel submerged in a liquid nitrogen bath. The vessel was removed from the vacuum line and 21.5 mg. of phosphorus trichloride were added from a micropipet followed by 186 mg. of inactive propionic acid. The chlorine

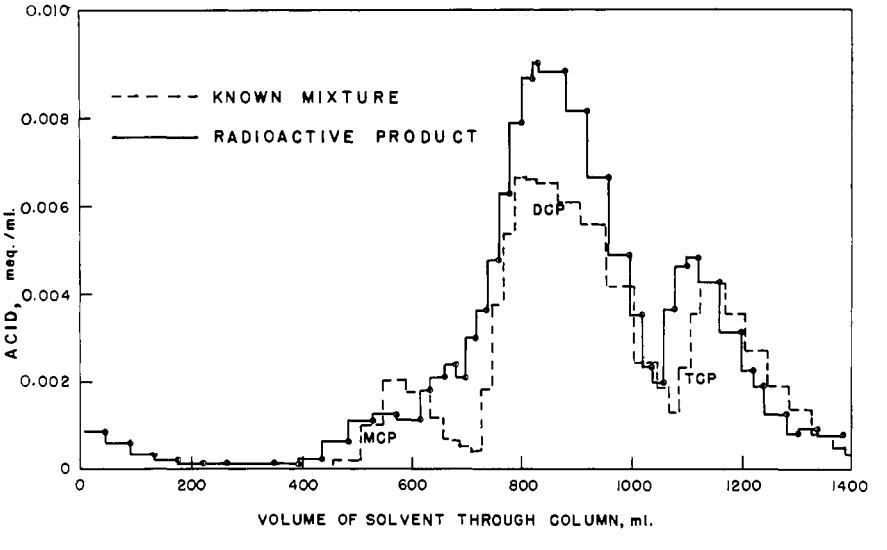

Figure 4. Chromatographic separation of chloropropionic acids

MCP 2-Chloropropionic acid. DCP 2,2-Dichloropropionic acid. TCP 2,2,3-Trichloropropionic acid

Table I. C^{14} in a Soybean Plant Treated with Dalapon-C^{14} by Root[a]

Plant Sample	Plant Tissue Wet Weight, G.	Activity in Gas Counting Aliquot, Net C.P.M.[b]	Total, D.P.M.	Dalapon, as Acid γ	Dalapon, as Acid P.p.m.
Root	1	2455 ± 9[c]	9,270	31	31
Stem, below 1st node	1.193	1865 ± 31	7,530	26	22
1st internode	0.291	1332 ± 14	3,220	11	38
2nd internode	0.236	3678 ± 61	15,000	51	216
Leaf, primary	0.913	263 ± 6	535	1.8	2.0
opposite primary	1.286	249 ± 5	592	2.0	1.6
1st trifoliolate	0.475	40 ± 2	350	1.2	2.5
2nd trifoliolate	0.285	620 ± 11	1,330	4.4	15.5
			Total recovered	128.4	

[a] Total activity applied was 1.45×10^6 d.p.m. (disintegrations per minute). Concentration of dalapon in nutrient was 32 p.p.m.
[b] Above background of 43–48 c.p.m.
[c] Standard deviation of net count rate, std. dev. $= \sqrt{\frac{\text{c.p.m.}}{\text{time counted, min.}}}$

Table II. C^{14} in Corn Plants Treated with Dalapon-C^{14} by Root[a]

Position	No. of Plants	Extract, Ml.	C.P.M./ Ml.	Total, C.P.M.	C.P.M./ Plant	D.P.M.	% of Applied
Tops	7	Water, 172	1800	310,000	44,200		
	1	Alcohol, 44	1000	44,000	44,000		
					Av. 44,100	184,000	9
Roots	7	Water, 118	170	20,100	2,870		
	1	Alcohol, 33	60	1,980	1,980		
					Av. 2,425	10,100	0.5

[a] Supplied per plant: 1.95×10^6 d.p.m.

Table III. C^{14} in Soybean Plants Treated by Spotting One Leaf with Dalapon-C^{14}

Plant Sample	Plant Tissue Wet Weight, G.	Net C.P.M. in Aliquot	Total D.P.M.	Dalapon, as Acid γ	Dalapon, as Acid % of applied	Dalapon, as Acid P.p.m.
		Treated with 6.3 γ				
Root	1	30 ± 3[a]	60	0.00390	0.06	0.004
Stem, below 1st node	1.226	102 ± 3	410	0.02670	0.42	0.022
1st internode	0.546	177 ± 4	355	0.02310	0.37	0.042
2nd internode	0.171	97 ± 3	193	0.01256	0.20	0.074
Leaf, primary, treated part	0.12	281 ± 6	91800	5.96000	94.80	50
primary, adjacent part	0.769	1001 ± 10	2300	0.14970	2.38	0.195
primary, distal part	0.864	...	...	...	...	...
primary, petiole	0.209	74 ± 4	150	0.00975	0.16	0.046
untreated primary	1.418	23 ± 5	90	0.00585	0.10	0.004
1st trifoliolate	0.564	69 ± 3	137	0.00891	0.14	0.016
2nd trifoliolate	0.279	152 ± 4	305	0.01985	0.32	0.071
3rd trifoliolate	0.079	74 ± 4	150	0.00975	0.16	0.123
			Total recovered		99.11	
		Treated with 464 γ				
Root	2.673	726 ± 6[b]	99833	6.54	1.41	2.45
Stem, below 1st node	1.367	232 ± 4	9576	0.63	0.14	0.458
1st internode	0.422	603 ± 11	20463	1.34	0.29	3.18
2nd internode	0.258	484 ± 7	15960	1.05	0.23	4.05
3rd internode	0.242	798 ± 12	26163	1.71	0.37	7.08
4th internode and growth tip	0.082	1080 ± 10	33340	2.20	0.47	26.6
Leaf, primary, treated portion	0.315	12977 ± 33	5136750	336.60	72.54	1065
primary, untreated portion	1.103	16930 ± 40	1616950	106.96	22.80	96.0
untreated primary	1.300	167 ± 3	6612	0.43	0.09	0.33
1st trifoliolate	0.987	417 ± 2	15675	1.03	0.22	1.04
2nd trifoliolate	0.399	756 ± 6	25650	1.68	0.36	4.22
3rd trifoliolate	0.242	393 ± 2	12454	0.82	0.18	3.37
			Total recovered		99.10	

[a] Above background of 46–48 c.p.m. Counted as carbon dioxide. Error given as standard deviation of net count rate.
[b] Above background of 20–21 c.p.m. Counted as barium carbonate. Error given as standard deviation of net count rate.

arm of the reactor was connected to the dry ice trap.

Monochlorination was achieved by passing 8.77 mmoles of chlorine into the reactor over a 17-hour period. The reactor temperature was controlled at 121° C. with vapors from refluxing perchloroethylene. At this temperature, 2-chloropropionic acid was the predominant reaction product.

Further substitution with chlorine was carried out at the temperature of refluxing *sec*-butylbenzene vapors (ca. 173° C.). Eleven millimoles of chlorine were passed into the reaction mixture during 31 hours.

The crude reaction product was transferred directly from the reactor to the liquid-free surface of the silica gel column. Elution and titration were carried out as described previously. The elution curve for a hot run is given in Figure 4. There is an indication of an unknown material coming through the column between the mono- and dichloropropionic acids and possibly another between the di- and trichloropropionic acids. Therefore, although the elution curves gave a good analysis of the mono-, di-, and trichloropropionic acid composition it was felt desirable to obtain further characterization of radiochemical purity. However, because the products at this point were in the form of aqueous sodium salts they were not amenable to good quantitative infrared analysis. A paper chromatographic system was therefore employed.

Ascending chromatograms were developed on Whatman No. 1 filter paper with a solvent made up of 50 parts of chloroform, 50 parts of *tert*-butyl alcohol, 5 parts of ethyl alcohol, 1 part of diethylamine, and saturated with water (somewhat less than 7 parts). An improved resolution of compounds of about the same R_f as chloropropionic and dichloropropionic acids was obtained by increasing the moisture content of the paper to about 30% of the paper's dry weight by a careful light spraying with water. After development, the residual solvent was removed by hanging the sheets over a steam bath while keeping them dry with a heat lamp (*8*). The dried chromatograms were sprayed with 0.1*N* silver nitrate and air dried. After 12 hours in the dark, the paper was exposed to room light. The chlorinated acids developed as purple spots on a tan background. Typical R_f values were: 0.19 for 2-chloro-, 0.38 for 2,2-dichloro-, and 0.51 for 2,2,3-trichloropropionic acid.

Autoradiograms were made on Kodak Type K industrial x-ray film. These confirmed the presence of additional compounds with R_f values of 0.27, 0.06, and 0.66. The first was eluted from the column between the mono- and dichloropropionic acids; the second along with the dichloropropionic acid; and the

supply tube was inserted, and the side third between the dichloro- and trichloropropionic acids. These have not been identified.

Based on the titration curves and paper chromatography, various groups of the extracts were selected, filtered, pooled, and dried. The dry sodium salts of mono-, di-, and trichloropropionic acids were isolated in this manner. The yield of 96% radiochemically pure sodium-2,2-dichloropropionate was 32 mole % based on propionic acid. The specific activity as determined by combustion to carbon dioxide and internal Geiger-Müller gas counting was 0.982 mc. per mmole.

In addition, 3.8 mole % were recovered as 95% radiochemically pure 2-chloropropionic acid and 13.7 mole % as 97% radiochemically pure 2,2,3-trichloropropionic acid.

Trial Applications of Dalapon-2-C^{14} to Plants

Corn and soybean plants were treated with the sodium dalapon-2-C^{14}; allowed to grow for a period of time estimated to be sufficient for the herbicide to enter the plant and be distributed throughout; and then they were harvested. Radioautographs were prepared to obtain a general indication of the distribution of radioactivity. Other plants were sectioned and analyzed for radioactivity. Similar studies have been reported (*7, 11*). In order to identify radioactive compounds in the plant tissues, extracts were examined by paper chromatography.

The plants were grown in the laboratory under artificial light. Four fluorescent tubes (Sylvania 40-watt cool white) and two flood bulbs (General Electric 150-watt) were operated 14 to 16 hours per day. Plants for foliar application experiments were grown in pots. Others were grown in a mineral culture solution (*13*).

Soybean plants were treated by root with the dalapon-C^{14} by growing them in a nutrient with a dalapon concentration of 38 p.p.m. Specific activity used was 0.02 mc. per mmole. The plants were harvested after 4 days.

Foliar application was made to other soybean plants. An aqueous solution of the dalapon-C^{14} at 1 mc. per mmole was spotted by micropipet to one of the primary leaves of the plants. One dose was 6.3 γ in 10 μl. There was no noticeable surface burn at this low dosage rate. The applied spot had dried on the leaf in about 10 minutes after application. The plant was harvested after 4 days. Another was treated with three 10-μl. spots containing a total of 464 γ. The spots remained wet for 1 hour. After 3 days at 85° C. and 55% relative humidity, this plant was harvested.

Corn plants in the four- to six-leaf stage were treated similarly by root, but at a concentration of 2.5 p.p.m., and a specific activity of 1 mc. per mmole. They were harvested at 11 days.

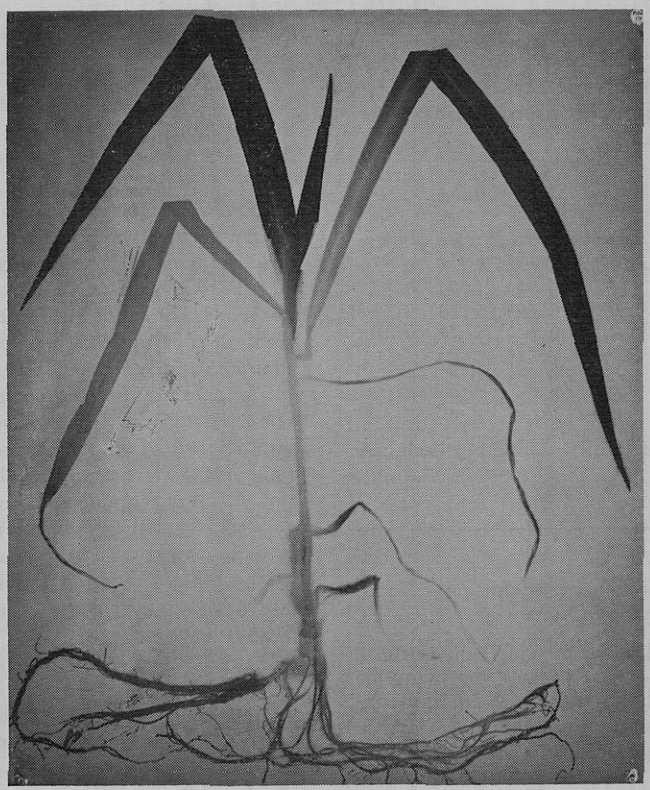

Figure 5. Radioautogram of a corn plant grown in a culture solution containing dalapon-C^{14}

Analyses

Radioautograms of whole plants were made by placing them between sheets of 0.5-mil saran film and pressing them against Eastman Type K industrial x-ray film in a cardboard film holder with weights. The films were developed after about 1 month of exposure.

Radioactivity analyses of extracts were made by drying aliquots on planchets and counting these under an end window Geiger-Müller tube.

For quantitative determination of the distribution of radioactivity, the plants were sectioned for analysis. The wet weight of each section was determined before the sample was combusted using a modification of the Van Slyke-Folch procedure (*14, 15*). The resulting carbon dioxide was collected and counted in a Geiger-Müller tube using the method described by Eidinoff (*10*) or was plated as barium carbonate and counted with an end window Geiger-Müller tube.

Water extracts of the plants were made with a Waring Blendor. The activity was extracted from the acidified water extracts with diethyl ether. Less than 2% remained in the water. The ether extracts were concentrated and chromatogrammed.

Alcohol extracts of a corn plant were obtained by a 24-hour Soxhlet extraction of the tops and roots with 80% aqueous ethyl alcohol. One-milliliter aliquots of each were dried on planchets and counted. The leaf extract was filtered, made slightly alkaline, and washed with diethyl ether. The original ethyl alcohol extract and the final water solution were chromatogrammed.

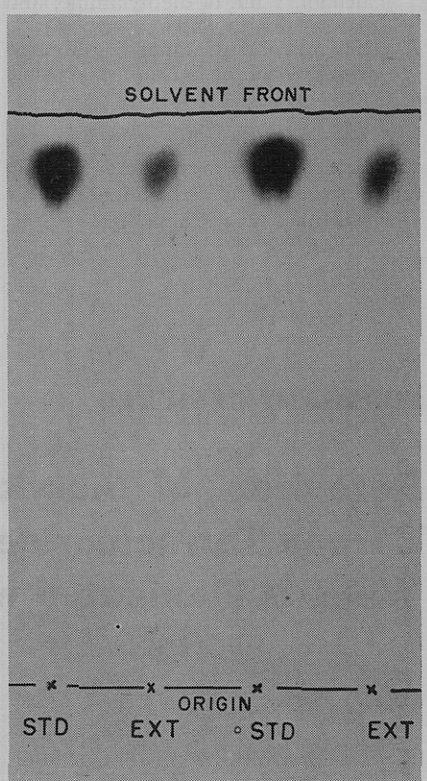

Figure 6. Radioautogram of a chromatogram of an extract from a corn plant grown in a culture solution containing dalapon-C^{14}

Table IV. Identification of C^{14} in Plant Extracts as Dalapon-C^{14} by Paper Chromatography

Plant	Solvent System	R_f Extract[a]	R_f Dalapon reference	R_f Pyruvic acid reference
Soybean	*A*	0.72[b]	0.73	0.26
	B	0.69[b]	0.70	0.56
	C	0.64[b]	0.72	0.18
Corn	*D*	0.27[b]	0.25	
	E	0.9[b]	0.9	0.6
	E	0.9[c]	0.9	0.6
	E	0.9[c]	0.9	0.6

[a] Only one spot was found in each case.
[b] Water.
[c] Alcohol.

Solvent systems used for paper chromatography of extracts were:

A. 100 ml. of *n*-butyl alcohol, 15 ml. of water, and 10 ml. of diethylamine.
B. 250 ml. of methyl alcohol, and 1 ml. of 1*N* sodium ethylate.
C. 100 ml. of *n*-butyl alcohol equilibrated with 100 ml. of 1.5*N* ammonium hydroxide.
D. 50 ml. of chloroform, 50 ml. of *tert*-butyl alcohol, 5 ml. of ethyl alcohol, 1 ml. of diethylamine, and saturated with water.
E. 100 ml. of 1-pentanol equilibrated with 100 ml. of 5*M* aqueous formic acid (*4*).

R_f values of radioactive components on the chromatograms were computed from loci determined by radioautography or Geiger-Müller tube scanning.

Results of Plant Experiments

Radioactivity entered soybean and corn plants whose roots were exposed to dalapon-C^{14}. It was distributed throughout the plants as indicated in Tables I and II and Figure 5. There were higher concentrations in young tissues. The percentages of applied activity taken into the roots and into the tops for soybean were 0.6 and 2, respectively. For corn, under slightly different conditions, percentages were about 0.5 and 9.

Radioactivity entered soybean plants, when spotted on a leaf, and was distributed throughout the plants as shown in Table III. Here again the young tissues had the highest concentrations of activity. The leaf opposite the treated leaf and the roots had the least activity. About 2% of the applied activity moved into parts of the plants other than the treated leaves.

Examination by paper chromatography of extracts prepared from root-treated soybean plants showed the presence of radioactivity which moved with R_f values corresponding to the R_f of known dalapon-C^{14}. No other radioactive spots were observed (Table IV).

From water and alcohol extracts of corn plants, radioactivity was recovered. This activity moved on chromatograms with R_f values corresponding to that of dalapon (Table IV, Figure 6). No other compounds were found. Two plausible breakdown products, lactic and pyruvic acids, move in system *E* with R_f values (0.62 and 0.65, respectively) well below the R_f of dalapon (0.9).

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FUMIGANT RESIDUES

Retention of Acrylonitrile and Carbon Tetrachloride by Shelled Walnuts Fumigated with Acrylon

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IN EXPLORATORY TESTS with four fumigants to control infestations of the Indian-meal moth, *Plodia interpunctella* (Hbn.), in imported shelled walnuts, Acrylon provided 100% control with no undesirable effects on flavor, odor, or appearance (*1*). A feature of the experiment was the successful use of polyethylene bags as fumigation chambers as described below. Acrylon is also known as Acritet (Stauffer Chemical Co., New York) and consists of acrylonitrile and carbon tetrachloride, 34 to 66% by volume, or 20.4 to 79.6% by weight.

Acrylon applied at the rate of 3 ml. per 55 pounds (4.1 grams per 25 kg.) of nut meats for an exposure period of 16 hours, was consistently successful in controlling infestations during a 3-year period in which some 500,000 pounds of shelled walnuts were treated (*13*). However, information was needed on the residues of acrylonitrile and of carbon tetrachloride that remained after aeration and packaging of the treated nuts. This report deals with concentration-time relationships for the Acrylon components over a period of 38 days.

Methods

Fumigation under Atmospheric Pressure. Double-walled polyethylene bags, large enough to accommodate the contents of a 55-pound box of shelled walnuts, served as small and inexpensive