Metabolism of Nellite Nematocide

(Phenyl *N,N'*-Dimethylphosphordiamidate) in Cucumber Plants

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Phenyl *N,N'*-dimethylphosphordiamidate, Nellite nematocide, is rapidly metabolized by the cucumber plant. The degradation products consist of the expected derivatives arising as a result of hydrolysis, conjugates of both the phenyl and the methylamido portions of the molecule, and other metabolites resulting from more extensive degradation. Radioactivity in the live plant at maturity is reduced to a very low level, suggesting that at this stage of plant growth the nematocide has been almost completely metabolized. The fruit contains no detectable radioactivity.

Control of nematodes in ornamentals, turf, and major crops by insecticidal phosphorus compounds has been well demonstrated (Turner, 1963; Youngson and Goring, 1963). Youngson (1959) has disclosed a group of phosphorus compounds that are extraordinarily toxic to root-knot nematodes. One of these, phenyl *N,N'*-dimethylphosphordiamidate (Nellite nematocide), has been shown to give excellent root-knot nematode control when applied in irrigation water.

The role of metabolism in reducing residues of pesticides in plants is very important. This report describes such a study involving Nellite nematocide when applied to cucumbers.

EXPERIMENTAL

The methods described below for synthesis of the labeled compounds were adapted from the work of Audrieth and Toy (1941, 1942).

Phenyl-1-^{14}C *N,N'*-Dimethylphosphordiamidate. Phenol-1-^{14}C (94 mg., 1.0 mmole, specific activity 0.27 mc. per mmole) in 1.3 ml. of chloroform was added dropwise with stirring to a solution of phosphoryl chloride (200 mg., 1.3 mmoles) and pyridine (308 mg., 3.9 mmoles) in 1 ml. of chloroform, all at 0° C. The mixture was allowed to stand at 0° for 50 minutes with occasional stirring, then added slowly with vigorous stirring to 2 ml. of a 40% aqueous methylamine solution (19.82 mmoles) at 0°. After the addition was completed, the solution stood for $^1/_2$ hour at 0°. The chloroform phase was separated, the aqueous phase was extracted three times with chloroform, and the extracts were combined with the original chloroform. This solution was dried over a minimal amount of anhydrous magnesium sulfate and then evaporated to dryness. Traces of pyridine were removed in vacuo over sulfuric acid. The residue was then partitioned four times between water and carbon tetrachloride. The carbon tetrachloride phase contained diphenyl-1,1'-$^{14}C_2$ *N*-methylphosphoramidate as a by-product (5.2% yield based on phenol-1-^{14}C).

The crude product in water was transferred to chloroform and passed through a column (8.5 × $^3/_8$ inch) of alumina containing water (10% by weight) as the stationary phase with chloroform as the developing solvent. The product issued in the first 50 ml. of solvent: 44 mg., 22% based on phenol-1-^{14}C, specific activity 0.27 mc. per mmole. It was 100% radiochemically pure as shown by paper chromatography in three solvent systems listed in Table V. The ultraviolet and infrared spectra of the pure product were identical with those of an authentic sample. This compound is hereafter referred to as phenyl-1-^{14}C.

Phenyl *N,N'*-dimethyl-$^{14}C_2$-phosphordiamidate. The phenyl ester of phosphorodichloridic acid (89.1 mg., 0.423 mmole) in 1.3 ml. of chloroform was added slowly with stirring to a solution of methyl-^{14}C-amine (52.4 mg., 1.69 mmoles, specific activity 0.59 mc. per mmole) in 2.5 ml. of water at 0° C. The mixture was allowed to stand at 0° for 1.5 hours and then at room temperature for $^1/_2$ hour with occasional stirring. The chloroform was then separated and the aqueous phase was extracted five times with chloroform. The chloroform extracts were combined and dried over a minimal amount of anhydrous magnesium sulfate. The crude product in the chloroform phase was purified by column chromatography as described above for the phenyl-labeled material: 52.9 mg., 62.9% based on the dichloridic acid derivative, specific activity 1.19 mc. per mmole. The labeled product was 100% radiochemically pure. This compound is hereafter referred to as dimethyl-^{14}C.

Cucumber Variety and Culture. The cucumber plants (*Cucumis sativus*) employed in these experiments were all of the variety Early Fortune.

Two methods of greenhouse culture were used. For soil culture, seeds were planted about $^1/_2$ inch deep in

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1-gallon glazed earthenware crocks filled with a mixture of a loamy sand soil, pH 5.5, and Sponge Rock soil modifier. Immediately after planting, the seeds in each crock were drenched with a solution of 10 mg. of radioactive Nellite nematocide in 800 ml. of water. For hydroponic culture, seeds were planted in 3-inch-deep plastic pots filled with vermiculite, surface area 6.25 sq. inches, watered with Hoagland's nutrient solution, and maintained without added nematocide while the seeds germinated. As soon as the first terminal leaves had developed, the pots were placed in a suitable container in such a way that the roots were bathed in Hoagland's solution containing 14.3 mg. (85 μc.) of dimethyl-^{14}C per 2 liters of solution. The solution was aerated throughout the experiments.

Rate of Uptake of Dimethyl-^{14}C by Cucumber Plants. This rate study was carried out with plants grown in hydroponic culture (Table I). At appropriate times, all the leaves and petioles were excised from each of three plants selected at random. The excised plant parts were weighed, and the radioactivity was assayed by means of a thin end-window Geiger-Müller tube. Whenever possible, infinitely thick samples were counted; in all cases, the count rates were corrected to infinite thickness.

Measurement of Carbon Dioxide-^{14}C Expiration. Four-week-old cucumber plants (weight about 4 grams) in vermiculite were treated with dimethyl-^{14}C (0.5 mg., specific activity 5.92 μc. per mg.) and with phenyl-1-^{14}C (0.5 mg., specific activity 1.35 μc. per mg.) in separate experiments. The radioactive chemical was applied to a leaf of the plant as an aqueous solution. The plants, but not the pots, were sealed in a closed system (volume 5 gallons) and allowed to respire over a 14-hour period of darkness. The gas in the closed system was slowly aspirated with six volumes of carbon dioxide-free air and passed through a trap containing a solution of 0.25*N* barium hydroxide having 2 grams of barium chloride per 100 ml. of solution. The barium carbonate-^{14}C was collected and counted (Table II).

Table I. Total and Specific Radioactivity in Cucumber Leaves

Time, Hours	Plant Weight, Grams[a]	Total Radioactivity[a] as μg. Equivalents of Nellite Nematocide	Specific Radioactivity[a] as μg. Equivalents of Nellite Nematocide/G. of Plant Material
2	1.4 ± 0.1	0.16 ± 0.02	0.11 ± 0.02
4	1.5 ± 0.1	0.6 ± 0.3	0.4 ± 0.2
8	1.4 ± 0.1	1.5 ± 0.2	1.1 ± 0.2
24	1.87 ± 0.04	7 ± 2	4 ± 1
48	2.4 ± 0.1	38 ± 5	16 ± 2
96	3.9 ± 0.1	73 ± 3	18.7 ± 0.8
192	7.5 ± 0.2	356 ± 77	47 ± 10
384	17 ± 2	1146 ± 155	67 ± 7
720[b]	42 ± 8	1656 ± 57	39 ± 8
1152[c]	127.35	687	5

± standard error; average of three replicates.
At 744 hours, reservoir of nutrient solution containing dimethyl-^{14}C was replaced by one with no radioactive material.
[c] No replication.

Table II. Carbon-^{14}C Dioxide Evolution from Cucumber Plants Treated with Radioactive Nellite Nematocide

Experiment	Total $^{14}CO_2$ Evolved as Mg. of $Ba^{14}CO_3$	Total Radioactivity in $Ba^{14}CO_3$, μc. × 10^2	Total $^{14}CO_2$ Evolved as μg. Equivalents of Nellite Nematocide
Dimethyl-^{14}C	213	1.45	2.45
Phenyl-1-^{14}C	233	0.077	0.57

Radioautographs. Radioautographs were prepared from 49-day-old cucumber plants grown in soil treated with phenyl-1-^{14}C and dimethyl-^{14}C in separate experiments. The plant tissue was freeze-dried, and the total dried plant was subsequently exposed to Eastman No-screen x-ray film with a sheet of Saran Wrap between the plant and the film. Films were processed in the usual manner after a 31-day exposure (Figure 1). The radioautograph from phenyl-1-^{14}C showed exactly the same pattern of radioactivity distribution.

Radioactivity in Cucumber Fruit. A slice of the cucumber fruit from a plant grown in soil treated with dimethyl-^{14}C was assayed for radioactivity using infinite thickness counting. The plant was 86 days old at the time of harvest. There was no significant radioactivity present in the fruit; the minimum detectable residue was 0.03 p.p.m. as the equivalent of Nellite nematocide.

General Metabolism Studies. Metabolism studies were carried out with 1-month-old soil-grown cucumber plants. The total plant was harvested at 30 days and freeze-dried. The dried tissue was continuously extracted in a Soxhlet extractor for 17 hours each with chloroform, 80% ethanol, and 10% acetic acid, in that order. The residue remaining

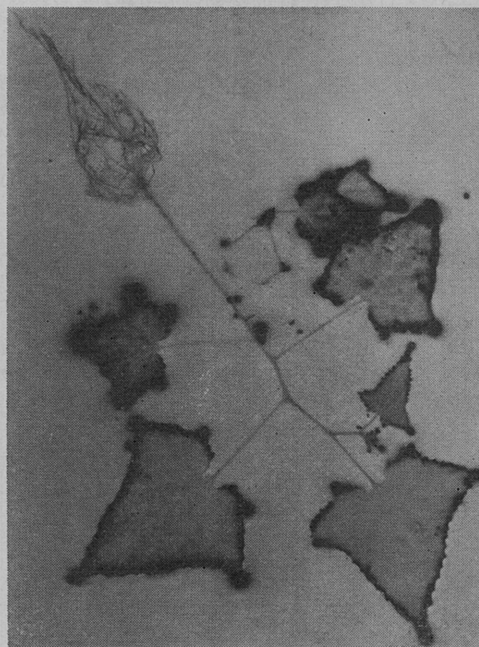

Figure 1. Radioautograph of 49-day-old cucumber plant grown in soil treated with dimethyl-^{14}C

was considered to be crude cellulose—that is, α-cellulose, hemicellulose, and lignin. Each extract was submitted to further examination as shown in Figure 2. Parallel experiments were carried out using phenyl-1-^{14}C and dimethyl-^{14}C. Table III is a compilation of the separated radioactive materials and includes the quantities present in terms of the equivalent of Nellite nematocide.

Metabolites from Phenyl-1-^{14}C Treatment. Four compounds were found in fraction B (Figure 2). The first, unchanged Nellite nematocide, was characterized by cocrystallization with an authentic sample from carbon tetrachloride. The compound was recrystallized to constant specific activity. It was also submitted to chromatography using Whatman No. 1 paper and benzene saturated with water as a developing solvent. The R_f value of the radioactive material was 0.31, and this corresponded exactly with the known compound when run on the same paper.

Phenol was isolated from fraction B by ether extraction of the bicarbonate solution resulting from saturation of fraction B with dry ice. For characterization, the *p*-phenylazobenzoyl ester was prepared from the radioactive sample and this recrystallized (ethanol) to constant specific activity in the presence of the corresponding derivative of phenol. In addition, the ester was cochromatographed with an authentic sample of the phenol derivative and the radioactivity coincided exactly with the yellow spot. The solvent system for this chromatography, along with the R_f value, is shown in Table IV.

Hydrogen phenyl methylphosphoramidate and dihydrogen phenyl phosphate were provisionally identified as present in fraction B by cochromatography with known compounds. The solvent systems and R_f values are given in Table IV. Additional evidence for the identity of these two compounds was acquired by means of paper electrophoresis. For this experiment, 5*N* acetic acid was used as the electrolyte with Whatman No. 1 paper. At ambient temperature, $5^2/_3$ hours, and a current density of 0.16 ma. per cm. of paper width, the apparent mobility was 8.1 cm. (+) and 10.5 cm. (+) for hydrogen phenyl *N*-methylamidophosphoramidate and dihydrogen phenyl phosphate, respectively. The apparatus used was of the closed-strip type described by Block *et al.* (1958).

Fraction C of Figure 2 contained only unchanged nematocide, identified as described above.

Fraction D contained lipophilic materials. By means of paper chromatography using acetone, 25% chloroform in petroleum ether, and 1% 1-propanol in petroleum ether, this fraction was shown to contain at least three radioactive entities, none of which were known compounds. Alkaline hydrolysis of the fraction yielded phenol and dihydrogen phenyl phosphate. These compounds were identified by paper chromatography and paper electrophoresis in the manner described.

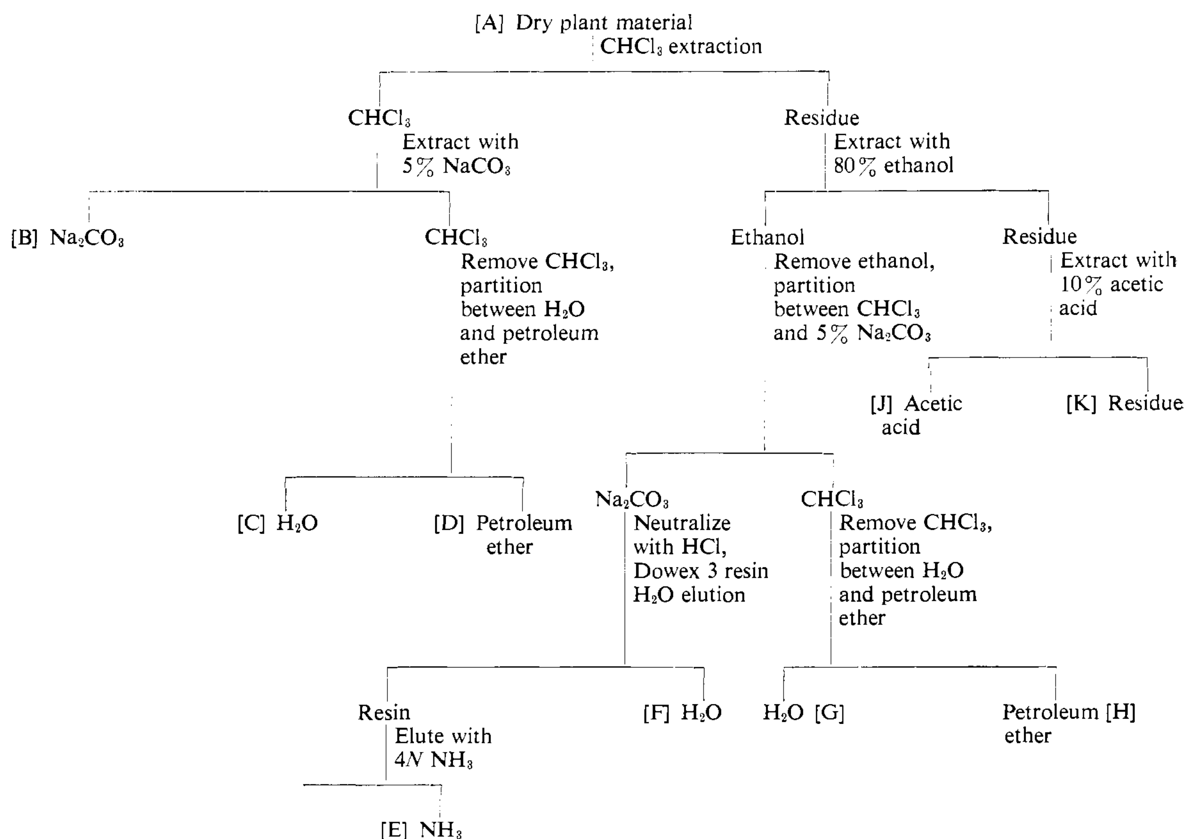

Figure 2. Fractionation procedure applied to cucumber plant tissue

Table III. Distribution and Quantity of Nellite Nematocide and Metabolites in Cucumber Plant Tissue

Radioactive[a] Material	Total Metabolite as μg. Equivalents of Nellite Nematocide	% of Total as Equivalents of Nellite Nematocide
Phenyl-1-^{14}C Experiment		
Dried plant [A][b]	6381	100
Nellite nematocide [B, C]	1806	28
Dihydrogen phenylphosphoric acid [B, E]	1232	19
Hydrogen phenyl-*N*-methyl-phosphoroamidate [B, E]	555	9
Phenol [B]	1921	30
Lipophilic material [D]	198	3
Water-soluble neutral material, Unknown 1 [F]	376	6
Water-soluble polysaccharide and protein [J]	70	1
Crude cellulose [K]	223	4
Dried plant [A][c]	2588	100
Nellite nematocide [C, G]	2034	79
Hydrogen phenyl *N*-methyl phosphoramidate [E] [B?]; Hydrogen *N*,*N*'-dimethyl-phosphordiamidate [B?]; Dihydrogen *N*-methyl-phosphoramidate [B?]	194	7
Lipophilic material [D, H]	106	4
H_2O-soluble neutral material [F]	109	4
Acidic compounds [E]	18	1
Water-soluble polysaccharide and protein [J]	28	1
Crude cellulose [K]	98	4

[a] Letters in [ ] keyed to Figure 2.
[b] Total weight of dried plant 2.12 grams (30.39 grams wet weight) and it contained 8.62 μc.
[c] Total weight of dried plant 1.25 grams (17.86 grams wet weight) and it contained 15.3 μc.

Table IV. R_f Values for Nellite Nematocide and Related Compounds

Compound	R_f Values in Solvent Systems[a] 1	2	3	4		6
Phenyl *p*-phenazo-benzoate	0.51					
Hydrogen phenyl *N*-methyl phosphor-amidate		0.54	0.70			
Dihydrogen phenyl phosphate		0.01	0.23	0.60		
Nellite nematocide		0.95	0.88	0.95		0.31
Unknown 1 (fraction F of phenyl-1-^{14}C experiment)				0.59	0.54	
N,*N*'-Dimethyl hy-drogen phosphordi-amidate[b]		0.50	0.74			
Dihydrogen *N*-methyl-phosphoramidate[b]		0.08	0.41			

[a] 1. Stationary phase, dimethylformamide from 50% solution in acetone; mobile phase, cyclohexane saturated with dimethylform-amide.
2. 1-Butanol saturated with 1.5*N* ammonia.
3. Ethanol–3*N* ammonia (2/1).
4. 1-Butanol–propionic acid–water (10/5/7).
5. 1-Butanol–acetic acid–water (4/1/5).
6. Benzene saturated with water.
[b] Tentatively identified.

Fraction E contained only dihydrogen phenyl phosphate and hydrogen phenyl *N*-methylphosphoramidate, identified by means described above.

Fraction F, containing water-soluble, neutral materials, appeared to contain one radioactive entity as judged by paper chromatography. The mobility of this radioactive compound is given in Table IV (Unknown 1). This compound was hydrolyzed with 0.1*N* hydrochloric acid on the steam bath in a sealed tube for 16 hours. Ether extraction of the hydrolyzate recovered 89.5% of the radioactivity, subsequently identified as phenol by methods described above. The remaining radioactivity was unchanged Unknown 1. Paper chromatography clearly demonstrated that Unknown 1 was not phenyl-β-D-glucopyranoside.

The residue remaining after 80% ethanol extraction was then continuously extracted in 10% acetic acid. The first extraction, 20 hours, removed 94% of the extractable radioactivity. The acetic acid extract (fraction J, Figure 2) nominally consisted of low molecular weight polysaccharides and protein, and contained a relatively small amount of radioactive material (Table III). This material was not investigated further, except to show that it was not dialyzable against running water.

The extractive-free residue, fraction K, was delignified by the procedure of Wise *et al.* (1946). In comparing the weights and specific activities of the crude cellulose and of the holocellulose left over from the delignification, the lignin had a specific activity 7.7 times that of the holocellulose. Since the crude cellulose, fraction K, was 23% lignin by weight, 69% of the radioactive material in fraction K could be attributed to the lignin, the other 31% to the holocellulose.

The holocellulose was hydrolyzed using the procedure described by Monier-Williams (1921). Fifty-seven per cent of the radioactivity was lost in carrying out the hydrolysis. This suggests that the radioactive material released as a result of the holocellulose hydrolysis was relatively volatile material.

Metabolites from Dimethyl-^{14}C Treatment. Fraction B (Figure 2) contained relatively little radioactivity and it could not be transferred to chloroform from acid or alkaline solutions. Attempts to chromatograph the material using ammoniacal solvents after the paper had been loaded from acid solution resulted in complete loss of radioactivity. Attempts to load the paper from ammoniacal solution resulted in a loss of 79% of the radioactivity. In this latter case, paper chromatography using ammoniacal solvents resulted in diffusion of the radioactivity to the point where it was impossible to resolve any compounds with certainty. However, paper electrophoresis of the radioactivity from alkaline solution, using 5*N* acetic acid as electrolyte, Whatman No. 1 paper, $5^2/_3$ hours at ambient temperature, current density 0.16 ma. per cm. of paper width, revealed that 78% of the activity was nonmobile and the other 13% moved 5 to 6 cm. (−). These compounds are not the expected methylamidophosphoric acids.

Fraction C contained only unchanged Nellite nematocide, identified using methods described above.

Fraction D was investigated only to the extent of using paper chromatography in benzene-water and paper electrophoresis with 5*N* acetic acid as electrolyte. In the

former case, the radioactivity moved with the solvent front; in the latter, the peak activity stayed at the origin. This fraction contained lipophilic material.

The radioactivity in fractions E and F was submitted to paper electrophoresis using 5*N* acetic acid as electrolyte and operating parameters identical to those described for this system (Table V). A good material balance was maintained throughout the isolation of fractions E and F and in the electrophoresis work. This suggests that no radioactivity was lost through volatilization. The compound in fraction E, having an apparent mobility of 7.5 cm. (−), and hydrogen phenyl *N*-methylphosphoramidate were submitted to paper chromatography using solvents 2 and 3 (Table IV). The coincidence of the radioactive material was exact with that of the known compound.

Fraction G contained a relatively small amount of radioactivity and was shown to be Nellite nematocide.

Paper electrophoresis and chromatography strongly suggested that fraction H, lipophilic material, was similar to that found in fraction D. This material was not investigated further.

Fraction J was not investigated.

The extractive-free residue, fraction K, was delignified by the process of Wise *et al.* (1946) to give holocellulose and a chlorite extract. The extract was dialyzed exhaustively against running water, and this yielded a small amount of water-soluble polysaccharide. The chlorite holocellulose was fractionated by successive extractions with 5 and 24% potassium hydroxide solutions (Wise *et al.*, 1946). This fractionation yielded three materials, referred to as hemicellulose A (soluble in 5% potassium hydroxide), hemicellulose B, and α-cellulose (insoluble in 24% potassium hydroxide). The carbohydrate material which passed into the chlorite liquors and was isolated by dialysis might also be regarded as a fraction of the holocellulose.

The water-soluble carbohydrate material from the chlorite liquors and hemicellulose A was hydrolyzed using the procedure described by Monier-Williams (1921). As a result of these hydrolyses, 39 and 77% of the radioactivity, respectively, were lost from these two samples.

Isopropylidine derivatives were prepared from the water-soluble polysaccharide hydrolyzate using the procedure of Bell (1947), but none of these derivatives proved to be radioactive. This hydrolyzate was submitted to paper chromatography and paper electrophoresis (Table VI). The hemicellulose A hydrolyzate was run through a column of Dowex 50 resin (H^+ form) and the eluate, along with water washings, was then run through a column of Dowex 3 resin (OH^- form). The eluate from the latter resin plus washings contained neutral sugars. This column was then eluted with 4*N* ammonium hydroxide; the eluate contained acidic materials, probably uronic acids.

The specific activities of the various fractions derived from crude cellulose, fraction K, were determined; these are recorded in Table VII on a relative scale, using the specific activity of the holocellulose as 1.

Another experiment was carried out, designed to find how much Nellite nematocide was in the plant, relative to the total activity, at a very early stage of growth. Cucumber plants grown in soil treated with dimethyl-^{14}C were sampled at 8 days. The leaves were cut up and continuously extracted in a Soxhlet extractor with 80% ethanol for 4 hours. The extract was concentrated in vacuo at 40° C. and was then submitted to paper electrophoresis and paper chromatography (Table VIII). The compound in the benzene-water solvent system, R_f 0.25, was Nellite nematocide, shown by procedures described above. Eighty-two per cent of the radioactivity was extractable by the ethanol solvent. Since 84% of this value is repre-

Table V. Apparent Electrophoretic Mobility of Radioactive Material in Fractions E and F

Dimethyl-^{14}C Experiment

Fraction	Peak Mobility, Cm.[a]	% of Total Radioactivity
E	0.5 (−)	90
	7.5 (−)	10
F	0.5 (+)	85
	6.5 (−)	15

[a] Electrolyte, 5*N* acetic acid; ambient temperature, 0.16 ma./cm of paper width (Whatman No. 1); time, 5 2/3 hours.

Table VI. R_f and Apparent Electrophoretic Mobility for Water-Soluble Polysaccharide Hydrolyzate

Dimethyl-^{14}C Experiment

Solvent Systems[a] and Electrolyte	Peak R_f and Apparent Mobility	% of Total Activity
2	0.08	55
	0.44	20
	0.78	25
3	0.10	7
	0.48	27
	0.77	66
5	0.10	15
	0.80	85
5*N* acetic acid	0.5 cm. (−)	100

[a] Numbers representing solvent systems for paper chromatography keyed to Table IV. Operating parameters for paper electrophoresis, using 5*N* acetic acid, same as in Table V.

Table VII. Relative Specific Activities of Fractions from Degradation of Crude Cellulose (Fraction K)

Dimethyl-^{14}C Experiment

Fraction	Relative Specific Activity
Crude cellulose	2.0 ± 0.3[a]
Holocellulose	1.0 ± 0.2[a]
Water-soluble polysaccharide	1.3 ± 0.3[a]
Lignin	3.8 ± 0.2[a]
Hemicellulose A	0.8
Hemicellulose B	0.5
α-Cellulose	0.5
Uronic acids (?)	0.6
Neutral sugars	0.2

[a] ± standard error, three replicates.

sented by nematocide, 69% of the total radioactivity associated with this 8-day plant is due to the nematocide.

RESULTS AND DISCUSSION

These experiments demonstrate that radioactivity is taken up rapidly from solution by cucumber plants; that radioactive carbon dioxide may be one of the products of degradation of the nematocide in the cucumber plant; that Nellite nematocide and/or its radioactive metabolites accumulate around the margin, and especially at the tips, of the leaf; that no detectable quantity of the nematocide or its metabolites carrying at least one intact *N*-methyl group accumulates in the fruit; and that the nematocide is metabolized by the cucumber plant.

The rate at which the cucumber plant takes up radioactivity from solution is shown in Table I. While the total radioactivity in the plant increases with time, the specific activity reaches a maximum at 384 hours. This behavior is commonly observed in experiments of this type. The plant growth rate between 384 and 720 hours is greater than the rate of uptake of radioactivity during this same time period, and this is reflected in the decrease in specific activity.

Radioactive carbon dioxide seems to be evolved from cucumber plants treated with both methyl-labeled and ring-labeled Nellite nematocide to the extent shown in Table II. A comparison of the relative rates of radioactive carbon dioxide evolution shows that the methyl-labeled compound contributes radioactive carbon dioxide at more than four times the rate of the other compound. It has not been proved that an aromatic ring can be opened by plants. Therefore, the present phenomenon, evolution of radioactive carbon dioxide from the plant treated with the phenyl-labeled compound, implying catabolism of the benzene ring, needs to be more vigorously investigated before such a claim can be made.

The radioautograph shown in Figure 1 demonstrates that radioactivity taken up through the roots accumulates in the meristematic tissue of leaves, areas of high metabolic activity where growth is taking place; there is a high concentration of radioactivity in the leaf margins and tips. It is in these areas of high metabolic activity where the greatest rates of decomposition would be expected. The picture of radioactivity distribution when phenyl-1-^{14}C was used was exactly the same.

There was no detectable radioactivity found in the mature cucumber fruit. The limit of detection was 0.03 p.p.m. as the equivalent of Nellite nematocide. Since this experiment involved the *N*-methyl–labeled compound, only the nematocide and those metabolites carrying at least one intact *N*-methyl group would be represented.

The extent to which Nellite nematocide is metabolized by the cucumber plant is seen in Table III. An apparent discrepancy in the data, which is immediately noticeable, is the difference in the total radioactive content of the dried plant material for the two different treatments with the compound. That for the dimethyl-^{14}C experiment is substantially lower than that for the phenyl-1-^{14}C treatment. The lower content of discrete radioactive compounds in the former case may be due, in part, to its higher rate of radioactive carbon dioxide evolution. While the amounts of Nellite nematocide present in the two experiments are roughly the same, the proportion in the dimethyl-^{14}C treatment is, accordingly, a much larger part of the total—i.e., 79%. This fact is offered as evidence that the *N,N′*-dimethyl portion of the Nellite molecule becomes assimilated in the metabolic pool of the plant faster than does the phenyl ring, a phenomenon already suggested by the difference in radioactive carbon dioxide evolution rates.

Nellite nematocide appears to undergo hydrolysis to yield all possible simple breakdown products expected from this reaction. For identification purposes, attempts were made to synthesize the two *N*-methylamidophosphoric acids through the interaction of methylamine and phosphoryl chloride, but the products were mixtures. Attempts to isolate pure compounds from these mixtures were unsuccessful. However, paper chromatography resolved the crude product sufficiently to permit a tentative identification of these two compounds. The R_f data are shown in Table IV. Even so, with the exception of hydrogen phenyl *N*-methylphosphordiamidate, the phosphoramidates listed in Table III (dimethyl-^{14}C experiment) are not well characterized, indicated by question marks after the compounds.

The presence of a relatively large amount of free phenol (Table III, phenyl-1-^{14}C experiment) indicates that phenol is more slowly disposed of than the *N*-methyl groups of Nellite nematocide. Under the conditions used to isolate phenol and demonstrate its presence, Nellite nematocide, the parent compound, does not decompose. Therefore, phenol is a true metabolite and not an artifact.

In both labeled experiments, a small amount of lipophilic material was isolated (Table III). The material detected in the phenyl-labeled experiment appeared to contain at least three radioactive entities as deduced from paper chromatographic evidence. Alkaline hydrolysis of this extract resulted in the recovery of phenol and dihydrogen phenyl phosphate. Thus, these two compounds apparently were in some way tied up with lipide-like material.

Of what might be called the minor metabolites in Table

Table VIII. R_f and Apparent Electrophoretic Mobility for Ethanol Extract of 8-Day Cucumber Plants

Dimethyl-^{14}C Experiment

Solvent Systems[a] and Electrolyte	Peak R_f and Apparent Mobility	% of Total Activity
2	Origin	10
	0.92	90
3	0.86	100
6	Origin	16
	0.25	84
5*N* acetic acid	Origin	85
	14.5 cm. +	15

[a] Numbers representing solvent systems for paper chromatography keyed to Table IV. Operating parameters for paper electrophoresis, using 5*N* acetic acid, same as in Table V.

III (phenyl-1-^{14}C experiment), the water-soluble neutral material (Unknown 1) is present in the largest quantity. Paper chromatographic evidence suggests that this is a single compound. Acid hydrolysis yielded phenol. However, paper chromatography clearly shows that the compound is not phenyl-β-D-glucopyranocide. This radioactive compound was not present in the experiments conducted with dimethyl-^{14}C and remains unidentified. The water-soluble, neutral material in this latter experiment contained at least two radioactive entities (Table V, fraction F), neither one of which has been identified.

A very small amount of radioactive acidic material is present in the methyl-labeled experiment and, in part, this also remains unidentified. Under fraction E of Table V, the unidentified acidic material appears to have no apparent electrophoretic mobility. In Table V, the acidic compound with an apparent mobility of 7.5 cm. (−) appeared to be hydrogen phenyl *N*-methylphosphordiamidate.

The plant material was further extracted with 10% acetic acid in a serial manner to give fraction J (Figure 2). Fraction J, water-soluble polysaccharide and protein in Table III, contained a small amount of radioactivity in both the labeled experiments. The material was not investigated further except to show by dialysis experiments that it was polymeric. Thus, the remaining residue (fraction K of Figure 2) was considered to be extractive-free. This residue, designated as crude cellulose (Table III), contained some radioactive material.

Crude cellulose is defined as a fusion of α-cellulose, hemicellulose, and lignin by Wise *et al.* (1946) and this definition is rather widely accepted for its convenience. The crude cellulose was broken down by well defined procedures, and the distribution of radioactivity was determined.

In the case of phenyl-labeled Nellite nematocide, the lignin had a specific activity 7.7 times that of the holocellulose; holocellulose consists of α-cellulose and a mixture of other polysaccharides called hemicellulose. Hydrolysis of the holocellulose resulted in a release of radioactive fragments, so that about half were lost by volatilization. Nothing is known about how the radioactivity is bound to the holocellulose.

The high specific activity of the lignin from the phenyl-labeled experiment, relative to that of the holocellulose (7.1 to 1), strongly suggests that phenol has in some way been directly involved in the lignification process in the plant. It has been well demonstrated, for instance, that phenolic cinnamic acids are important intermediates in the formation of lignin (Brown, 1961). In addition, Brown and Neish (1955) have shown that a large variety of aromatic compounds can be transformed into lignin residues, albeit rather inefficiently. Siegel (1955) has suggested that phenolic compounds are potential lignin precursors, but their success in this role depends greatly upon their behavior as a substrate for peroxidase. This enzyme may operate as a degrading, dehydrogenating, hydroxylating, or polymerizing catalyst; and the particular reaction rates probably determine to a large extent the efficiency with which the substrate is transformed into part of the lignin structure. In addition, blockage of the hydroxyl group by methylation or glycoside formation prevents incorporation into lignin. At any rate, about 3% of the radioactivity was associated with the lignin fraction in this experiment.

The crude cellulose from the dimethyl-^{14}C experiment (fraction K, Table III) was also broken down to holocellulose and lignin. Holocellulose was fractionated by successive extractions with 5 and 24% potassium hydroxide solutions to give hemicellulose A and B and α-cellulose. Insolubility in this strength of alkali has, ever since the work of Cross and Bevan (1903), served as the definition of α-cellulose. It is, in fact, a measure of the amount of cell wall material resistant to the types of chemical attack normally encountered in commercial operations. Additionally, a small amount of carbohydrate material passed into the chlorite delignification liquors and was isolated by dialysis; this may be regarded as a fraction of the holocellulose.

When the water-soluble polysaccharide from the chlorite liquors was hydrolyzed, 39% of its radioactivity was lost. In addition, the diisopropylidine derivatives prepared from the hydrolyzate were not radioactive. These facts suggest that the radioactivity was not associated with the skeletal carbon of the sugar molecules but, more likely, was present as *O*-methyl derivatives. Diisopropylidine derivatives form only with sugar molecules having free pairs of *cis,cis*-hydroxyl groups. *O*-Methyl groups would either prevent the formation of these derivatives or the radioactive methyl group might be displaced in the derivative formation. Further, the loss of radioactivity on hydrolysis seems to offer additional evidence that radioactive *O*-methyl groups are present in this polysaccharide.

When this same polysaccharide hydrolyzate was submitted to paper electrophoresis and chromatography (Table VI), there were at least three radioactive entities present in the mixture, and none has any apparent electrophoretic mobility. With the electrolyte used, neither glucuronic acid nor a variety of neutral sugars has any mobility.

Hemicellulose A was also hydrolyzed with an attendant 77% loss of radioactivity. The products were separated into neutral and acidic materials by ion exchange. The latter are probably uronic acids, since these compounds are known to constitute a sizable portion of the hemicellulose molecule.

Comparison of the relative specific activities of all the fractions derived from crude cellulose (Table VII) shows that lignin has a relative specific activity significantly higher than any other fraction. This suggests that there is a substantially higher concentration of what are probably radioactive *O*-methyl groups in the lignin than in other fractions associated with crude cellulose. The holocellulose and water-soluble polysaccharide are not significantly different with respect to their specific activities, and hemicellulose A is probably not different from these. The feature which allows hemicelluloses A and B to be separated from each other—i.e., their differential solubility in potassium hydroxide solutions—is generally attributable to their uronic acid content. Hemicellulose B, presumably the least acidic material, would have the lower uronic acid content. If the relative specific activities of these two materials are significantly different, these data reflect this difference because the uronic acids would tend to be richer

in *O*-methyl groups than the sugar portion of the hemicellulose.

As a study of the rapidity with which Nellite nematocide was degraded by the cucumber plant, it was found that only 8 days after treatment with the nematocide the concentration of the compound represented 69% of the total radioactive burden; this is a very rapid rate and is similar to the values found for the 49-day plant. R_f values for paper chromatography and apparent mobility for paper electrophoresis of the extracts of this early sampling are shown in Table VIII.

Although plants do not possess well defined organs to remove potentially injurious compounds, foreign substances are rendered innocuous or translocated within the plant where they can do no harm. These materials are transported, usually in an inactivated state, to regions of active secondary growth where they are stored either temporarily or permanently as polymeric substances of the cell wall or as materials extraneous to the cell wall. Lignin, as a part of the cell wall structure, is a complex polymer of condensed, substituted phenols. The lignification process, in so far as it proceeds by the utilization of phenolic substances from the sap stream, may be essentially a process of waste disposal.

The results of this work show that many other reactions can occur which remove foreign materials. In addition to the radioactivity appearing in the lignin fraction, radioactive carbon dioxide was detected in the experiment with the phenyl-labeled compound, at least suggesting that the benzene ring is being degraded. A small portion seems to be in the form of a water-soluble conjugate, some appears as lipide-like material, and a small amount is associated with holocellulose. Finally, there remains a rather large amount of free phenol. Apparently, at this stage of growth, the plant has not yet been able to cope with the large amount of phenol present as a result of hydrolysis of the Nellite nematocide.

These same remarks apply to the amide portion of the nematocide molecule. In this case, the distribution of radioactivity varies somewhat from the phenyl-labeled material, but this is to be expected, since it is an entirely different carbon fragment.

CONCLUSIONS

All manner of reactions inherent to a biological system can operate on a foreign substance. The distribution of radioactivity that results because of these reactions simply reflects the rates at which these reactions take place. Of course, the rates, in turn, are a function of many things such as enzymatic activity, concentration of substrate, and concentration of products. These principles almost certainly apply to degradation efforts made by plants, as well as by animals and bacteria.

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