# Metabolism of Phthalimidomethyl-O,O-dimethylphosphorodithioate (Imidan) in Cotton Plants

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This investigation was undertaken to determine the metabolic fate of $C^{14}$-Imidan in cotton plants. Imidan was readily absorbed by treated leaves but not translocated in the plant. The major acetone-extractable nonphosphate metabolites of Imidan consisted of phthalic and/or phthalamic acid, benzoic acid, and possibly one or more benzoic acid derivatives. These were determined by radiochromatography and autoradiography. Evolution of $C^{14}O_2$ and evidence for $C^{14}$ incorporation into plant constituents further indicated ready decarboxylation and metabolism of the phthalimide moiety of Imidan in the plant. The thiol analog of Imidan was not discovered in cotton plant tissues; furthermore, the presence of a post-hydrolysis phosphorothionate derivative on paper chromatograms indicated that hydrolysis of Imidan in vivo predominated over oxidation.

IMIDAN [phthalimidomethyl-*O,O*-dimethylphosphorodithioate], trademark of Stauffer Chemical Co. for an insecticide-acaricide, is an experimental insecticide which has undergone extensive laboratory and field trials for the control of insect pests attacking crops and livestock. Excellent control of the boll weevil was reported by Butt and Keller (*3*) in the laboratory and by Davis *et al.* (*4*) in the field. Bottger and Sparks (*2*) showed that Imidan was among the more effective new insecticides controlling *Lygus* species on cotton. Drummond (*5*) reported that Imidan was systemically active in controlling cattle grubs.

This paper reports experiments which describe the fate of $C^{14}$-Imidan in cotton plants following surface application to the leaves. Primary emphasis was placed in tracing the fate of the nonphosphate moiety of Imidan in vivo since the fate of the phosphate moiety has been extensively studied and reviewed by O'Brien (*11*) and others.

## Materials and Methods

**Chemicals.** Purified Imidan (98%) as determined by a column chromatography–ultraviolet spectroscopy method (*13*), its oxygen analog (phthalimidomethyl-*O,O*-dimethylphosphorothiolate), hydroxymethylphthalimide, phthalimide, phthalic acid, and several possible phosphate metabolites were synthesized in the authors' laboratories. The remaining compounds described in this work were obtained from Eastman Organic Chemicals Department, Rochester, N.Y. Radioactive Imidan was prepared by labeling with $C^{14}$ one of the two carbonyl groups of the phthalimide moiety. The material was synthesized and recrystallized by M. A. Leaffer of the Stanford Research Institute, Menlo Park, Calif. The specific activity of the $C^{14}$-Imidan was 1.53 mc. per mmole. An infrared scan of $C^{14}$-Imidan was identical to a pure sample of unlabeled Imidan. Purity of $C^{14}$-Imidan was also determined by radiochromatography using the E/G system described in the section dealing with paper chromatography. By this procedure a purity of 92% was indicated.

**Plant Treatment.** Cotton plants in the 3- to 5-leaf stage were treated topically with 1000 μg. of $C^{14}$-Imidan in 0.2 ml. of acetone per leaf. The plants were kept in an airtight glass container, equipped with air intakes and outlets. Exhausted air was bubbled through NaOH solution to trap any evolved $C^{14}O_2$, which was subsequently precipitated as $BaC^{14}O_3$ for counting purposes. The day temperature in the chamber ranged from 90° to 110° F., and night temperature was approximately 80° F. The atmosphere within the chamber was maintained at 100% relative humidity at all times. At chosen intervals, leaves were sampled for radioassay. In separate experiments, plants were sprayed in the greenhouse with formulated Imidan at a rate of 5 pounds technical per 100 gallons of water. Periodically, leaves were sampled for extraction.

**Extraction and Purification for Paper Chromatography.** Two extracts were prepared from each leaf sample treated with $C^{14}$-Imidan—an external extract obtained by rinsing leaves three times with dried acetone, and an internal acetone extract prepared from homogenized rinsed leaves weighing 1 to 2 grams each. Unrinsed leaf extracts were prepared in the experiments using unlabeled Imidan. Extraction and prechromatographic purification procedures were adapted from Menn *et al.* (*6*). Briefly, crude extracts were spotted on filter paper and eluted with acetonitrile. The solvent selectively extracts Imidan and its metabolites leaving interfering lipids on the filter paper. The purified acetone extracts were concentrated to a final volume of 0.3 ml. of which a 12-μl. aliquot was used for each chromatographic analysis. Recovery of $C^{14}$-Imidan (I) (Table I), its oxygen analog (II), hydroxymethylphthalimide (III), phthalimide (IV), phthalamic acid (V), and phthalic acid (VI) from leaf extracts fortified with these compounds was essentially complete when processed through the extraction and paper chromatographic procedure.

**Paper Chromatography.** A diphase ascending solvent system was employed (*8*). This consisted of 12% v./v. glutaronitrile in acetone as the holder and isopropyl ether saturated with glutaronitrile as the mover (E/G system). One-inch wide Whatman No. 4 paper was used throughout. Following development, the paper chromatograms were sprayed with 0.5% w./v. solution of 2-6-dibromo-*N*-chloro-*p*-quinoneimine (DCQ) in cyclohexane, and developed according to the procedure described by Menn *et al.* (*7*). As little as 1 μg. of Imidan (I) and 5 μg. of its oxygen analog (II) could be detected as red and yellow spots, respectively. Potential hydrolysis products of Imidan containing the nonphosphate moiety were determined by a modification of the Mitchell (*9*) chromogenic agent. Developed chromatograms were sprayed with 3.0% w./v. aqueous solution of $AgNO_3$, followed by exposure to ultraviolet light with greatest emission energy at 2537 A. Phthalic acid and related derivatives appeared as yellow, brown, or gray spots on a light gray background which darkened with time. Approximately 5 to 10 μg. of each derivative could be detected by this method.

An alternative solvent system, ethanol:water:$NH_4OH$ (80:15:5 v./v.) (aqueous system) (*7*), was used to characterize

the more polar potential metabolites of Imidan.

**Radioassay.** Paper chromatograms were scanned with a Geiger tube having a 1.4-mg. per sq. cm. mica window and connected to a Baird Atomic Ratemeter Model 441. Recording of radioactivity was accomplished with a Texas Instruments Servo-riter integrating recorder, Model FWS. Counting scale was set at 300 c.p.m. except for the external extract in Figure 3, where a 1000 c.p.m. scale was used. Time constant setting was 10 seconds and collimation was set at 0.5 inch. $C^{14}O_2$ samples were collected in 5% NaOH solution and precipitated with 10% $BaCl_2$ as $BaC^{14}O_3$. The precipitates were washed with acetone followed with absolute methanol, dried, and counted for radioactivity by using a Baird Atomic shielded dual gas flow counter, Model FC-180A, with an ultra-thin, mylar window less than 100 $\mu$g. per sq. cm. in thickness.

Background corrections were made for samples showing low activity. Self-absorption corrections were made for planchet samples having an infinitely thick layer.

**Autoradiography.** A cotton plant which was treated with $C^{14}$-Imidan in the seedling stage was divided into different plant portions after 54 days holding. The stem and a single boll were sectioned, and all plant parts were dried in a plant press. The dry, pressed plant components were placed in contact with Kodak no-screen x-ray film sheets and exposed in the dark for 14 days. Developed paper chromatograms were also exposed to x-ray film for a period of 7 days. The developed films were then matched with the paper chromatograms for $R_f$ determinations of separated compounds.

## Results

**Chromatography.** Imidan (I), its oxygen analog (II), hydroxymethylphthalimide (III), phthalimide (IV), phthalamic acid (V), and phthalic acid (VI) were characterized with respect to $R_f$ values in the E/G system on paper chromatograms as shown in Table I. Wherever possible, comparable $R_f$ values were shown for the authentic compound and from a purified leaf extract fortified with each compound.

Imidan and compound II were readily separated in the E/G system and detected with DCQ. These compounds did not change their respective $R_f$ values when chromatographed alone or from purified extracts. Compounds III, IV, V, and VI were detected with $AgNO_3$–ultraviolet irradiation and gave similar $R_f$ values when chromatographed alone or from purified leaf extracts. Compounds V and VI did not appear as compact spots on

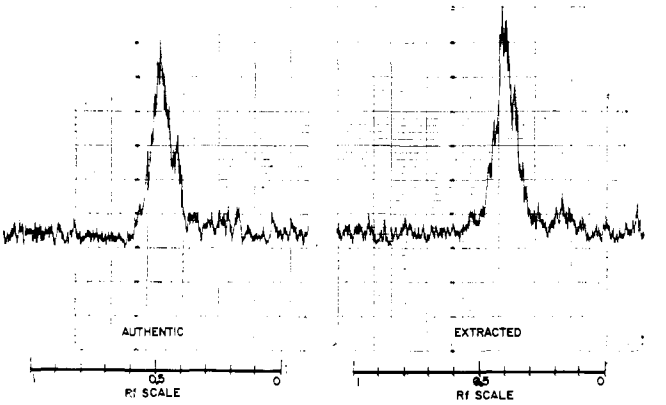



Figure 1. Scans of authentic and extracted $C^{14}$-Imidan chromatographed in the E/G system

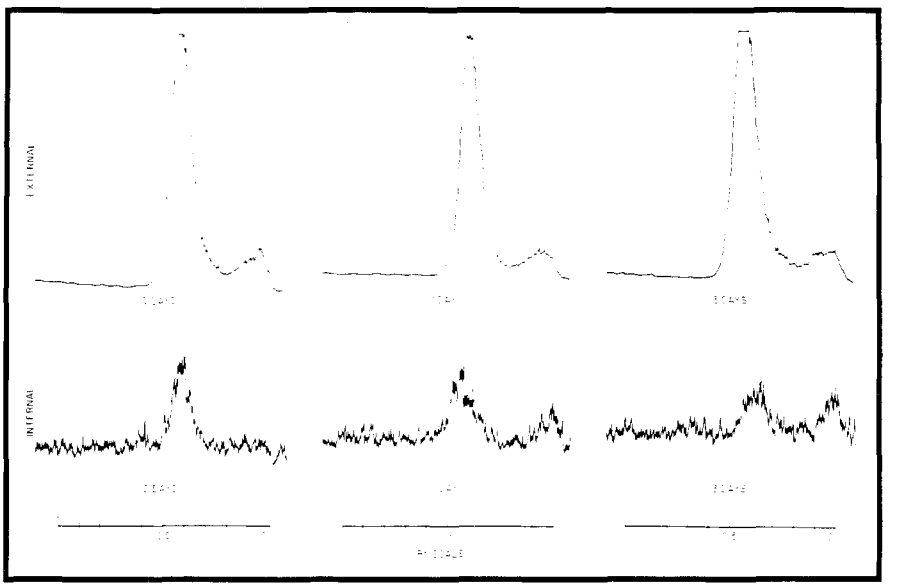


Figure 2. Scans of internal and external extracts of $C^{14}$-Imidan treated cotton leaves chromatographed in the E/G system

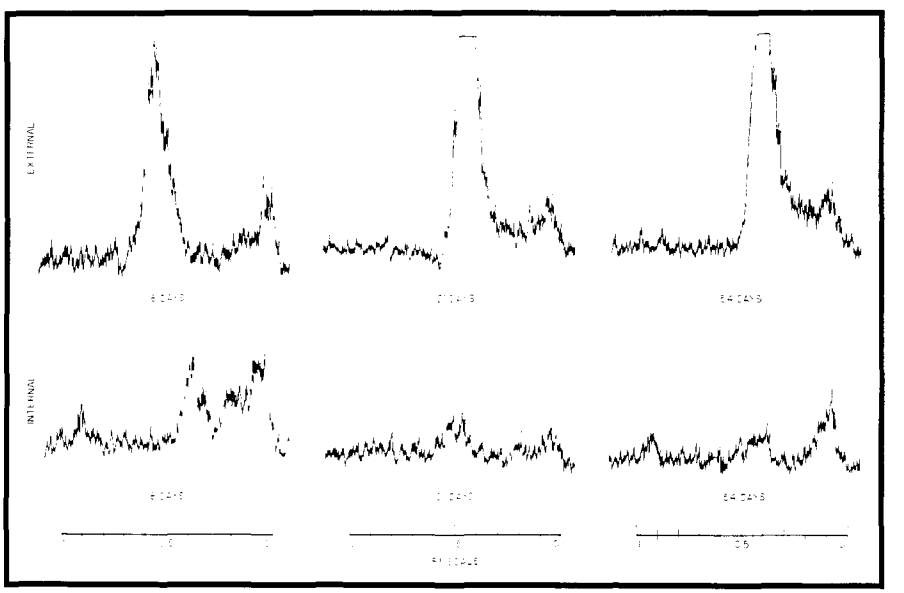


Figure 3. Scans of chromatograms (E/G system) of internal and external extracts of $C^{14}$-Imidan treated cotton leaves

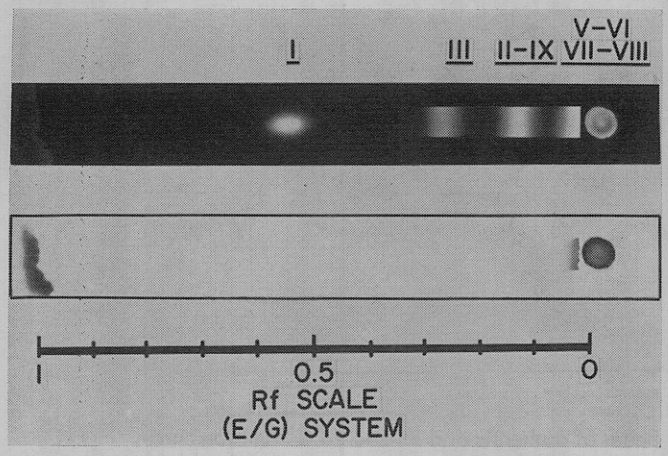



Figure 4. Chromatogram and autoradiogram of an internal extract of a cotton leaf 8 days after treatment with $C^{14}$-Imidan

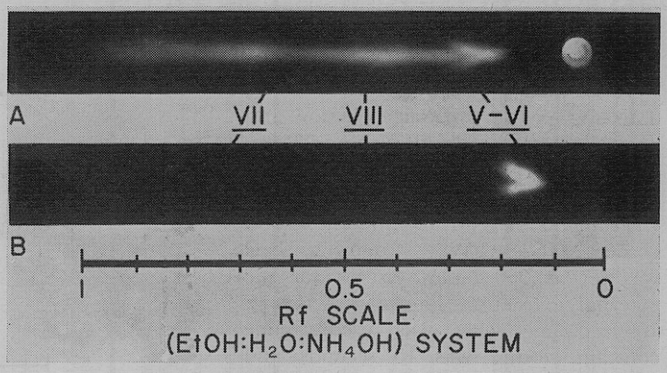



Figure 5. Autoradiograms of extracts of $C^{14}$-Imidan treated and untreated cotton leaves

(A) Polar metabolites from internal extracts of treated leaves; (B) Metabolites from untreated, new leaves

developed chromatograms, but occasionally streaked from the origin to $R_f$ 0.17. Although the latter two compounds overlap the same zone as compound II, they could be separated from compound II using the aqueous solvent system (ethanol:$H_2O$:$NH_4OH$). This system proved to be useful in separating benzoic acid (VII), its hydroxy derivative, *p*-hydroxybenzoic acid (VIII), and benzamide (IX). These three compounds are potential decarboxylation products possibly arising from phthalic (VI) or phthalamic acid (V) (Table I).

**Fate of Imidan in Treated Cotton Leaves.** The radiochromatographic scans of authentic and extracted $C^{14}$-Imidan (E/G system) are presented in Figure 1. Each radiochromatogram represents 2 μg. of $C^{14}$-Imidan. Comparison of both peaks and their respective $R_f$ positions shows that interfering plant tissue extractives were successfully removed by the prechromatographic purification procedure (*6*), and recovery of Imidan was essentially complete.

In Figures 2 and 3 are presented the radiochromatograms of external and internal leaf extracts at 0, 1, 3, 8, 21, and 54 days after treatment.

**External Extracts.** The external radiochromatograms in Figure 2 were scanned at 1000 c.p.m. because of the presence of a large amount of $C^{14}$-Imidan on the leaves. The peak at the origin represents apparent polar impurities in the $C^{14}$-Imidan preparation. The latter did not appear as distinct peaks in Figure 1 since that scan represented only a small fraction of the radioactivity in Figure 2. There was little change throughout the holding period in the profile of the external radiochromatograms with $C^{14}$-Imidan remaining at high levels for the duration of the experiment. Some variation in the peaks apparently was due to variable absorption and dissipation of Imidan by individual leaves at each interval.

**Internal Extracts.** Internally only the $C^{14}$-Imidan peak is evident at 0 days (Figure 2); a gradual radioactive peak buildup is evident at the origin region at 1 and 3 days. Imidan continued to appear at all holding intervals, decreasing gradually after 8 days. The peak at the origin persisted throughout in smaller amounts than the respective $C^{14}$-Imidan peak except at the 54-day interval when the former exceeded the amount of internal $C^{14}$-Imidan. At 8 days, two additional radioactive peaks are evident—one between the peak at the origin and Imidan, conceivably compound III, and another one at the solvent front. The latter peak is also evident at the 54-day interval. Some pigmented plant material always moved with the solvent front in the E/G system.

Figure 4 shows a chromatogram of the 8-day interval and its autoradiogram. The radioactive spot at the solvent front coincided in position and configuration with the greenish, pigmented plant material on the chromatogram. Thus the radioactivity in the solvent front region might be due to $C^{14}$-labeled chlorophyll and acetone-extractable lipoid plant constituents which were not removed by the acetonitrile purification. In addition to the radioactive solvent front, radioactive spots with the following $R_f$ values also appeared: 0.51, 0.24, and 0.12. These three spots corresponded, respectively, based on their $R_f$ values, to Imidan (I), hydroxymethylphthalimide (III), and the oxygen analog (II) and/or benzamide (IX). The material which streaked out at the origin and the spot of application could be phthalic (VI) and/or phthalamic acid (V), and one or more aromatic acid derivatives. There was no evidence in any of the radiochromatograms (Figures 2 and 3) for the formation of phthalimide (IV) which has an $R_f$ of 0.58 (Table I) and would have appeared as a distinct peak or shoulder in front of the $C^{14}$-Imidan peak.

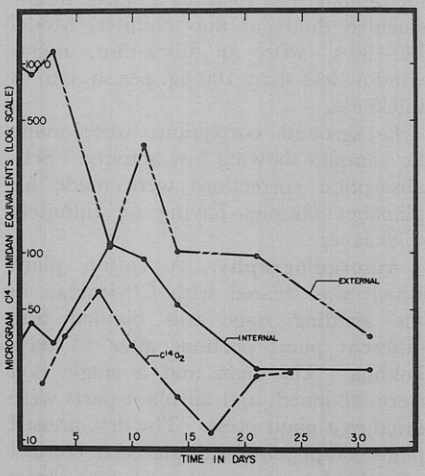
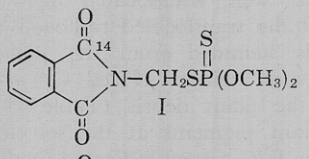
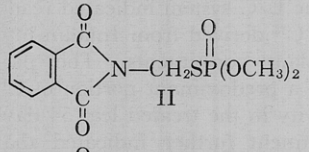
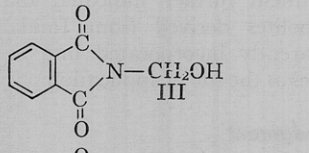
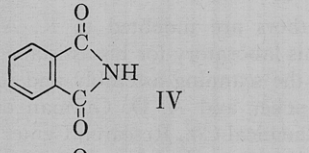
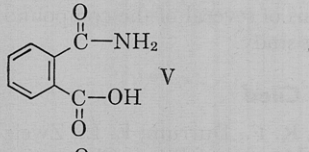
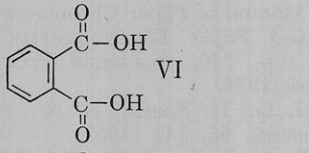
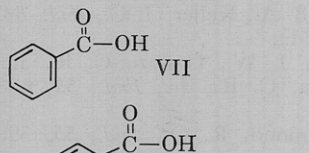
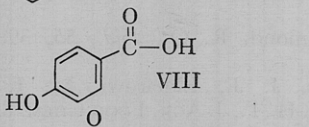
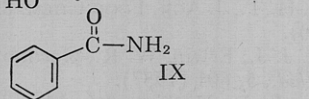



Figure 6. Distribution of radioactivity in leaf extracts and evolved $C^{14}O_2$ at various intervals

The peak areas at the origin of several chromatograms were pooled and extracted with hot methanol and chromatographed using the aqueous system. Autoradiography of the radiochromatograms revealed three spots (Figure 5*A*) with the following $R_f$ values: 0.21, 0.50, and 0.70. These spots correspond in $R_f$ values to the following compounds, respectively (Table I): phthalamic (V) and/or phthalic acid (VI), *p*-hydroxybenzoic acid (VIII), and benzoic acid (VII). Radiochromatograms developed with both solvent systems and sprayed with DCQ failed to reveal the presence of the oxygen analog (II).

A comparison of the hydrolysis rates of Imidan and its oxygen analog (II) showed both compounds undergoing 50% hydrolysis in 12 hours at pH 6.99 (*12*). Essentially complete recovery of compound II from fortified acetone extracts was obtained by paper chromatography with DCQ as the chromogenic agent. Additionally, the dissipation rate of compound II from

**Table I. $R_f$ Values of Imidan and Several Possible Metabolites in Two Solvent Systems**

| Compound | E/G System[a] DCQ Authentic compound | E/G System[a] DCQ Extracted compound | E/G System[a] $AgNO_3$–Ultraviolet Authentic compound | E/G System[a] $AgNO_3$–Ultraviolet Extracted compound | Aqueous System[a] $AgNO_3$–Ultraviolet, Authentic compound |
|---|---|---|---|---|---|
|  I | 0.46 ± 0.05[b] | 0.46 ± 0.05[b] | 0.46 ± 0.05[b] | 0.46 ± 0.05[b] | 0.91 |
|  II | 0.08 | 0.08 | 0.08 | 0.08 | 0.84 |
|  III | None | None | 0.25 | 0.25 | 0.86 |
|  IV | None | None | 0.58 | 0.58 | 0.86 |
|  V | None | None | 0–0.17 | 0–0.17 | 0.24 |
|  VI | None | None | 0–0.17 | 0–0.17 | 0.24 |
|  VII | None | None | 0 | None | 0.71 |
|  VIII | None | None | 0 | None | 0.50 |
|  IX | None | None | 0.12 | None | 0.76 |

[a] $R_f$ values represent average of two or more closely agreeing tests. [b] Std. dev.

treated leaves followed closely that of Imidan, and approximately 15% of the initial dose was recovered after holding 5 weeks in the greenhouse. Based on the foregoing data, the absence of compound II in treated leaf extracts is more likely due to the slow rate of conversion of Imidan to its oxygen analog (II) rather than to rapid breakdown of the latter.

Paper chromatography of cotton plant extracts using nonradioactive Imidan and employing the E/G system, with DCQ as the chromogenic agent, revealed in addition to Imidan the presence of a minor phosphorothionate degradation product with an $R_f$ value of 0.85 ± 0.05 (standard deviation) after holding 3 and 5 weeks in the greenhouse. The presence of the phosphorothionate moiety was indicated by the red spot on the paper. Radiochromatography and autoradiography using $C^{14}$-Imidan failed to show any radioactive material in this $R_f$ region. This suggested that the compound was apparently a post hydrolysis phosphorothionate derivative of Imidan lacking the cyclical moiety.

**Distribution of Radioactivity on and in Treated Cotton Leaves.** Figure 6 shows the changes in total amount of external, and internal radioactivity and evolved $C^{14}O_2$ at various intervals after application of $C^{14}$-Imidan to cotton leaves. Some of the irregularities in external activity levels such as the apparent buildup at 11 days were partially due to sampling of different leaves in the chamber at each time interval. Evidently Imidan penetrated rapidly into the leaves as demonstrated by the rapid buildup of internal activity from 0 days to 8 days.

$C^{14}O_2$ was collected in the exhausted air from the holding chamber at all sampling intervals. Since only one carbonyl group was labeled with $C^{14}$, the actual amount of evolved $CO_2$ should be considered as twice as great since $CO_2$ could arise from either one of the two carbonyl groups of the phthaloyl moiety. The accumulation and decrease of $C^{14}O_2$ closely paralleled the internal activity curve. Thus, evolution of $C^{14}O_2$

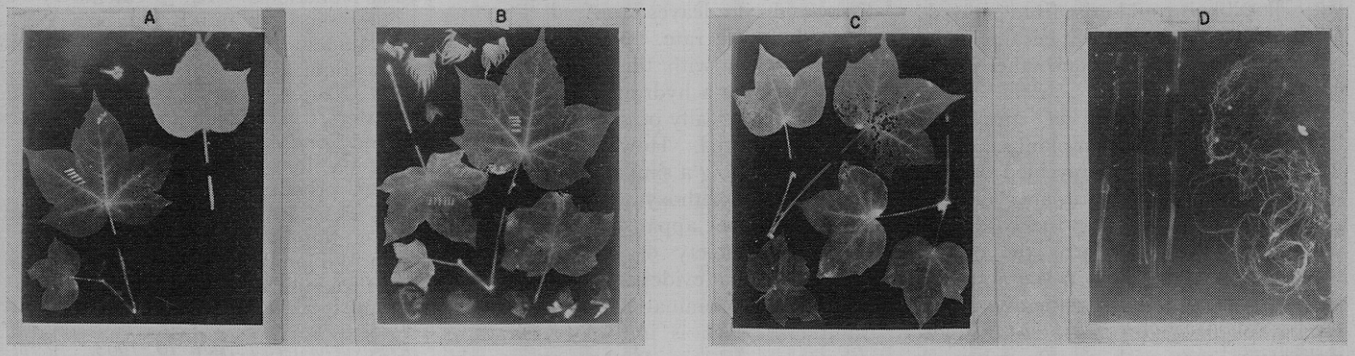



Figure 7. Autoradiograms of a cotton plant 54 days after single leaf treatment with $C^{14}$-Imidan

(A) Upper leaf: treated; lower leaves: untreated, new growth; (B) Untreated new leaves, boll, and bracts; (C) Untreated new leaves; (D) Stem and roots

**Table II. Radioactivity in Plant Debris of Cotton Plant 54 Days after Treatment with $C^{14}$-Imidan**

| Plant Material | Weight, Grams | C.P.M.[a] | Equivalents of $C^{14}$-Imidan, P.P.M. |
|---|---|---|---|
| Leaves[b] | 0.020 | 317 | 7.9 |
| Boll[b] | 0.030 | 147 | 2.5 |
| Bracts[b] | 0.032 | 574 | 8.6 |
| Treated leaf | 0.025 | 53,540 | 1,070 |

[a] Based on 6-minute counting time and corrected for self-absorption and background.
[b] Untreated portions of the cotton plant.

appears to be a function of internal concentration of $C^{14}$-Imidan and its metabolites. However, it is also likely that due to the presence of a diminishing number of leaves in the chamber following each sampling interval and due to plant growth resulting in greater $C^{14}O_2$ fixation, a proportionally lesser amount of evolved $C^{14}O_2$ is to be expected.

**Distribution of $C^{14}$-Imidan Metabolites in Untreated Portions of the Cotton Plant.** Autoradiographs of the $C^{14}$-Imidan treated cotton plant (Figure 7) showed relatively uniform distribution of radioactivity throughout the untreated portions of the plant, including the root system. A high concentration of radioactivity persisted on the treated leaf as indicated by its greater opacity. A greater concentration of radioactivity was also evident in the lateral and terminal growing buds. Radiochromatography of acetone extracts of the untreated leaves, bolls, and bracts of this plant in the E/G system failed to show any radioactive peaks. Planchet radioassay of the acetone unextractable plant debris (Table II) indicated radioactivity in $C^{14}$-Imidan equivalents ranging from 2.5 p.p.m. in the boll to 8.6 p.p.m. in the bracts.

In contrast to the low fixed $C^{14}$ activity in the untreated portions of the cotton plant, a very high level of fixed $C^{14}$ activity was found in the debris of a treated leaf. The nature of the fixed $C^{14}$ activity has not been elucidated yet. Possibly it could arise from evolved $C^{14}O_2$ which probably became incorporated into cellulose and other plant constituents.

Radiochromatography of untreated leaf extracts from a cotton plant sampled 21 days after treatment revealed only a small peak in the origin area of the radiochromatogram using the E/G system. The peak area near the origin was rechromatographed in the aqueous solvent system and subsequently autoradiographed (Figure 5*B*). A distinct radioactive spot with an $R_f$ value of 0.20 was found. This corresponds to phthalic (VI) and/or phthalamic acid (V). Again, no $C^{14}$-Imidan peak was discernible on the radiochromatogram.

## Discussion

Apparently in the case of Imidan, oxidation to the thiol analog (II) was largely bypassed in favor of hydrolysis. This was demonstrated by the apparent absence of compound II in treated cotton leaves when DCQ was used as the chromogenic agent, and in autoradiographs of treated and untreated leaves using the aqueous chromatographic solvent system. In Figure 4, a faint radioactive spot appeared with an $R_f$ value corresponding either to the oxygen analog (II) or to benzamide (IX). However, based on hydrolysis and dissipation rates, compound II would have been readily detected in leaf extracts if it were formed to an appreciable extent. The rapid appearance of the polar metabolites (Figures 2 and 3) suggested that hydrolysis mechanisms predominated over oxidation of Imidan in the plant. Autoradiography (Figure 5*A*) revealed that the nonphosphate hydrolysis products of $C^{14}$-Imidan in the treated leaves corresponded in $R_f$ values to phthalamic (V) and/or phthalic acid (VI) and to the decarboxylation products—*p*-hydroxybenzoic acid (VIII) and benzoic acid (VII). The apparent absence of phthalimide (IV) was demonstrated in Figures 2 and 3. Conceivably in acid media such as the sap of cotton plant (pH 6.0 to 6.5), the *N*-substituted phthalimide could be hydrolyzed directly to phthalic acid (VI) (*10*). The formation of decarboxylated aromatic acids such as benzoic acid (VII) and *p*-hydroxybenzoic acid (VIII) was also suggested by the evolution of $C^{14}O_2$ from the treated plant (Figure 6). The toxicity and elimination of these aromatic acids has been extensively studied in man and animals (*14*). These compounds are relatively nontoxic and readily excreted by mammals, alone or as conjugated metabolites.

Although no attempt was made to characterize the possible phosphate hydrolysis products, a minor thionophosphate degradation product giving an $R_f$ of 0.85 in the E/G system was discovered in leaves sprayed at an elevated dosage rate. No radioactivity was associated with this spot; thus it appeared to be a hydrolyzed derivative. Several theoretically possible compounds were synthesized. However, the only compound giving a similar $R_f$ value was *O,O*-dimethyl methoxymethylphosphorodithioate. The apparent presence of the thiono moiety of this compound served as further evidence that hydrolysis of Imidan predominates over oxidation. Additional work is in progress in an attempt to obtain further structural confirmation of this metabolite.

Entomological investigations conducted in the authors' laboratory and by others have shown that neither Imidan nor its oxygen analog (II) possessed systemic insecticidal or acaricidal activity. Autoradiography (Figure 7) and radiochromatography of untreated plant portions demonstrated that neither Imidan nor its oxygen analog (II) were translocated in the plant, and the translocated radioactive compounds stemmed from the polar metabolites of Imidan. Fixed $C^{14}$ activity in the plant debris (Table II) and in plant pigments at the solvent front in the E/G system indicated ready uptake of $C^{14}$ derived from Imidan into normal plant constituents. The persistence of a predominant portion of $C^{14}$ fixed activity in the treated leaf 54 days after treatment further indicated that $C^{14}$ metabolites derived from Imidan were apparently incorporated into the plant tissues at the site of application.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