Metabolism of carbamate drugs III

Translocation and Degradation of $^{14}$C-Labelled Sevin in Cotton Plant

I. Y. MOSTAFA, A. HASSAN, AND S. M. A. D. ZAYED

Department of Radiobiology, Atomic Energy Establishment, and National Research Centre, U.A.R.

The distribution and metabolic fate of Sevin in cotton plant has been investigated using $^{14}$C-insecticide labelled at two different sites. Sevin is readily absorbed by the root system. Hydrolytic and nonhydrolytic mechanisms contribute almost equally to the metabolism of the insecticide. A specific esterase hydrolyzes the ester bond to produce 1-naphthol, methylamine and CO$_2$ (24% of the absorbed dose). The liberated methylamine undergoes partly a process of oxidative degradation to CO$_2$. A major metabolite possessing the skeleton CO-C(0)-N-C is probably produced by hydroxylation of the naphthalene ring.

Cotton seeds (Gossypium barbadense, v. Ashmouni) were germinated in pots, 15 cm in diameter, and left to grow in the open under field conditions. Treatments were carried out on 2 leaf-stage plants weighing 2.4–3.0 g.

Methods

A. Distribution Studies

Plants were carefully taken out of soil and immersed in a solution of 0.5 mg (≈ 85,000 cpm) Sevin I in 20 ml water. After 1, 3, 5, 24 and 48 hours plants were removed from the solution. The root system was then thoroughly washed in a stream of water, and the different parts of each plant were weighed, dried over P$_2$O$_5$ and the $^{14}$C-activity was determined as described below.

B. Metabolism Studies

For this purpose 5 plants were used; each was left for 72 hours in a solution of 0.5 mg Sevin (I or II) in 20 ml water in a special apparatus designed for the collection of expired $^{14}$CO$_2$. The roots were then thoroughly washed in a stream of water and the whole plant was used, after being weighed, for the characterization and determination of metabolites. The whole plant was extracted 5 times with chloroform (10 ml each) after grinding with fine sand. The combined chloroform extracts were measured for radioactivity and paperchromatographed in systems A (methanol/water; 8:2) and B (methanol/acetic acid/water; 4:1:5). The ascending technique using Schleicher &

Materials

Sevin I (specific activity = $1.7 \times 10^6$ cpm/mg) was prepared by dilution of 0.5 mC 1-naphthyl-N-methyl carbamate-$^{14}$C* with nonradioactive pure Sevin, m.p. 142 °C.

Sevin I was prepared from 1-naphthyl diloroformate (carbamate-$^{14}$C)* with nonradioactive pure Sevin, m.p. 142 °C. Sevin II was prepared from 1-naphthyl diloroformate (carbamate-$^{14}$C)* with nonradioactive pure Sevin, m.p. 142 °C.

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Sevin II was prepared from 1-naphthyl diloroformate (carbamate-$^{14}$C)* with nonradioactive pure Sevin, m.p. 142 °C. It had an activity of $1.0 \times 10^6$ cpm/mg.

* Amersham Buckinghamshire, England.
5 J. A. Lambrecht, 1959, U.S. 2,903,478 (Union Carbide Crop), C. A. 54, 22934c [1960].
Schüll paper 2043 b was adopted. The remaining plant tissues were then extracted 4 times with water (5 ml each) and emulsions were broken by centrifugation. The combined water extracts were measured for radioactivity and chromatographed in the above mentioned systems.

For the determination of free phenolic compounds, a sample of the chloroform extract was evaporated under vacuum till dryness and the phenolic components were then extracted with water. Using 4-aminonaphthalene reagent 6, the amount of phenols present was determined colorimetrically.

**Radiomeasurements**

The expired $^{14}$CO$_2$ was trapped in 1 N NaOH determined as Ba$^{14}$CO$_3$. $^{14}$C-activity in biological samples, aqueous and chloroform extracts was determined as Ba$^{14}$CO$_3$ according to ABONOFF 7 using VAN SLYKE - FOLCH reagent 8. The prepared Ba$^{14}$CO$_3$ plates were analysed for their radioactivity in an endwindow counter. The chromatograms were assayed radiometrically using Frieseke & HÖPNER radioscanner. All radioactivity measurements were carried out under uniform geometrical conditions and corrected for background and selfabsorption.

**Results**

**A. Distribution**

The results obtained show that the insecticide was readily absorbed via the root system of cotton plant. The distribution of Sevin I among the different organs is given in table 1.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Cpm/g dry weight * after [hours]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Leaves</td>
<td>500</td>
</tr>
<tr>
<td>Stem</td>
<td>540</td>
</tr>
<tr>
<td>Root</td>
<td>5350</td>
</tr>
</tbody>
</table>

Table 1. Translocation of Sevin I in cotton plant by absorption through the root system. * Data are mean of 3 replicates.

**B. Metabolism**

1. **Sevin I:** From the initially available dose (500 μg/20 ml water) about 40–47% was taken up in 3 days. 69% of the latter could be recovered in the combined chloroform extracts, while the aqueous fraction contained only 7 per cent. This could be reduced to 3% by exhaustive extraction with chloroform. The rest of $^{14}$C-activity was trapped as $^{14}$CO$_2$. Chromatographic analysis of the chloroform extract revealed the presence of 2 main substances, one of them being Sevin ($R_f$ 0.95 in system A and 0.83 in System B). The second substance showed radioactivity maxima corresponding to $R_f$ 0.81 and 0.53 in systems A and B respectively. From several chromatograms it was estimated that this metabolite accounted for 30 to 45% of the total chloroform $^{14}$C-activity.

- Colorimetric determination of the free α-naphthol (expectedly produced as a metabolite) gave a mean value of 39 μg per plant. This value was corrected for the phenols originally present in the plant tissues.

2. **Sevin II:** The $^{14}$C-activity recovered in the chloroform extracts contributed 73% of the absorbed dose, that in the aqueous extracts 20%, while the expired $^{14}$CO$_2$ accounted for ~4 per cent. The possible elimination of radioactive basic gas(es) was investigated — in a separate experiment — by allowing the expired gases to pass through 6N H$_2$SO$_4$. From each plant 650 cpm could be trapped in sulphuric acid over 3 days.

Paperc chromatographic analysis of the chloroform extract showed the presence of the same metabolite as that obtained from Sevin I ($R_f$ 0.80 in system A and 0.51 in system B). The amount of unchanged insecticide (estimated from several chromatograms) was found to contribute about 60% of the chloroform soluble $^{14}$C-compounds. The radioactivity present in the aqueous extract showed no definite spot (tailing), when chromatographed in the used solvent systems.

**Discussion**

The data presented in this investigation clearly illustrate that Sevin is readily absorbed via the root system of the plant. From 500 μg available insecticide 164 – 180 μg were taken up by the plant in 2 days. Of the total absorbed $^{14}$C-activity, the respiratory $^{14}$CO$_2$ accounted for 18%, whereas the radioactivity retained by the leaves, stem and root contributed 24%, 29% and 29% respectively. The $^{14}$C-activity found in the leaves does not represent the true entering activity, since the evolved $^{14}$CO$_2$ is mainly liberated from the leaves.

The metabolism experiments show that about 50% of the insecticide entering the plant in 3 days, is changed into a variety of metabolites; thus indicating

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6 M. E. MARTIN, Analytic. Chem. 21, 1419 [1949].
a fair rate of degradation. The elimination of $^{14}\text{CO}_2$ from Sevin I indicates that a hydrolytic mechanism is involved in the degradation of the insecticide (scheme). This mechanism represents a major pathway, since it produces 47% of the total metabolites. A specific carbamate esterase catalyzes the hydrolysis of the ester bond; thus liberating $\alpha$-naphthol and $N$-methylcarbamic acid. The latter undergoes spontaneous decarboxylation to give $^{14}\text{CO}_2$. An equivalent amount of free naphthol could be recovered as a metabolite. In this connection it is worthy to mention that, the hydrolytic mechanism plays only a minor rôle in the degradation of Sevin in insects\textsuperscript{8, 9, 10}.

Investigation of the methylamine moiety (from Sevin II) indicates that about 3% of the absorbed insecticide is eliminated as basic volatile $^{14}$C-substance(s), which is probably the unchanged amine. Another part (~ 20%) constitutes a water soluble metabolite(s). Since this substance(s) is absent as a metabolite of Sevin I, it is suggested that methylamine constitutes a precursor for this component.

The elimination of $^{14}\text{CO}_2$ from Sevin II (4%) proves that the methyl group undergoes a process of oxidative degradation. It is unlikely that the intact Sevin molecule suffers such a change, since no $^{14}$C-metabolite lacking the methyl group — other than $\text{CO}_2$ — could be isolated from Sevin I. In other words, it is the methylamine itself rather than Sevin which undergoes the process of oxidative degradation.

Apart from the hydrolytic pathway, a nonhydrolytic mechanism is equally involved in the degradation of Sevin (scheme). Direct evidence for this mechanism has been gained from the fact that, one and the same metabolite has been isolated from Sevin I and Sevin II. This indicates clearly that this major metabolite (53% of the total metabolites) possesses the intact skeleton (C-O-C(0)-N-C). It is probable that this metabolite is produced from Sevin by hydroxylation of the naphthalene ring, and it is rather unlikely that the methyl group has undergone any change. The nonhydrolytic pathway is known to contribute a major mechanism for metabolism of Sevin in mammals\textsuperscript{9, 11} and insects\textsuperscript{9, 10, 12}.

DOROUGH et al.\textsuperscript{9}, working on bean and cotton plants, reported that no large loss of $^{14}\text{CO}_2$ was evolved over a period of 28 days, when the insecticide was injected into the stem. In the present work, however, it could be shown that an appreciable amount of $^{14}\text{CO}_2$ (24% of the absorbed dose) is produced during 3 days.

It is believed, that the carbamate esterase will always catalyze the hydrolysis of both the unchanged insecticide and the main metabolite possessing the skeleton C-O-C(0)-N-C, so that in all probability, the carbamate carbon atom will be destined to leave the plant as $\text{CO}_2$. With this explanation, it may be concluded that the hydrolytic pathway constitutes the major mechanism of degrading Sevin in the cotton plant.

Scheme. Percentages are related to total amount of insecticide entering the plant in 3 days.

\textsuperscript{7} S. ABRONOFF, Techniques of Radiobiocchemistry, the Iowa State College Press, Ames, Iowa 1957.
\textsuperscript{8} D. D. VAN SLYKE and J. FOLCH, J. biol. Chemistry 136, 509 [1940].
\textsuperscript{12} H. W. DOROUGH and J. E. CASIDA, J. agric. Food Chem. 12, 294 [1964].