Relative Stability, Toxicity, and Penetrability of Abamectin and Its 8,9-Oxide

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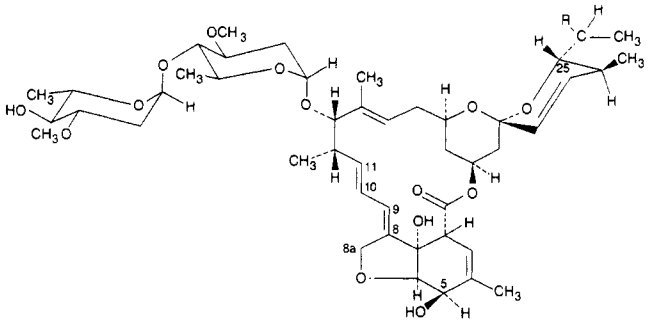
Experiments have been conducted to compare abamectin and its 8,9-oxide derivative in terms of ingestion toxicity to two-spotted spider mites, stability on Petri dishes, and penetration into bean leaves. The observed higher miticidal potency of the 8,9-oxide is explained by greater penetrability and stability. For both compounds, the lethal dose by ingestion is about 0.8 pg/mite.

Avermectin B1 (abamectin, I) has been shown to have excellent activity against phytophagous mites and selected insect species of economic importance in agriculture (Putter et al., 1981; Dybas, 1983). It is currently under development worldwide for control of arthropod pests on several horticultural and agronomic crops (Dybas and Green, 1984); however, its utility on certain crops is limited by poor stability to light and oxygen (Bull et al., 1984; Iwata et al., 1985).



a-Component R = C_2H_5 ≥ 80%
b-Component R = CH_3 ≤ 20%

I

In both laboratory and field studies, abamectin is rapidly degraded by exposure to light and air. Bull and coworkers (1984) found rapid dissipation on cotton leaves and in soils; Iwata et al. (1985) reported very rapid initial dissipation from orange and lemon leaves and fruit; MacConnell (1985, unpublished observations) measured a half-life for abamectin in a fire ant bait formulation of about 12 h when exposed to summer sun. Laboratory studies in solution have shown that light causes isomerization about the 8,9 and 10,11 double bonds (Mrozik et al., 1988) and the 8,9-isomer has been detected in extracts of abamectin-treated produce exposed to natural sunlight (Maynard et al., 1983). Other laboratory investigations of the dark degradation of abamectin indicate that the 8a-position is particularly susceptible to oxidative attack; several 8a-oxidized degradants have been identified [for instance, Bull et al. (1984) identified the 8a-hydroxy compound and the 8a-keto compound has been identified in numerous unpublished studies]. This is not unexpected since the 8a-carbon is allylic to the 1,3-diene system and adjacent to an ethereal oxygen atom, both of which activate the position for oxidative attack.

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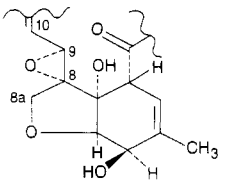
In an attempt to stabilize the avermectin molecule by modifying the conjugated diene chromophore, abamectin 8,9-oxide (II) was prepared (Mrozik, 1985; Blizzard et al., 1988). In laboratory foliar residual bioassays against



II

the two-spotted spider mite (*Tetranychus urticae* Koch), which measures the persistence of the test compound, the 8,9-oxide derivative was 2–4 times as potent as abamectin (Preiser, F. A., unpublished results, 1985; Dybas et al., 1989). Our interest in increased potency against arthropods and stability on plant foliage prompted us to seek the cause for this difference in residual biological activity between the two compounds.

We have performed experiments to separate several key factors affecting activity including inherent ingestion toxicity to mites, chemical stability, and foliar penetration and to provide discrete comparisons for these factors.

EXPERIMENTAL SECTION

Formulations. Separate 18 g/L emulsifiable concentrate (EC) formulations of abamectin and its 8,9-oxide were prepared by removing the solvent from 1 μCi of either radiolabeled avermectin B_{1a} or its 8,9-oxide (both labeled with ^3H at the 5-position) and redissolving the residue in 100 μL of "cold" 18 g/L EC. Nominal 18 mg/L aqueous dilutions of the abamectin EC (emulsion 1) and its 8,9-oxide (emulsion 2) were used in the plant experiments (toxicity and penetration).

A separate formulation was made for the Petri dish stability study. A mixed abamectin/8,9-oxide emulsion was prepared as follows. A portion of the radiolabeled 8,9-oxide EC prepared above was diluted with cold 8,9-oxide EC (10 volumes). An aliquot (20 μL) was mixed with water (20 mL); to this 8,9-oxide emulsion was added cold abamectin EC (20 μL). The resulting mixed system (emulsion 3) is thus nominally 18 μg/mL in both cold abamectin and radiolabeled abamectin 8,9-oxide.

Stability Study. Eight glass Petri dishes (Kimax, 150-mm diameter × 15-mm depth) were cleaned with chromic acid, rinsed with distilled water, and air-dried. To each dish was added a 2.0-mL aliquot of emulsion 3, and the dishes were dried for 1 h in a darkened hood. Two zero-time dishes were then processed as follows. The residue was washed from the dishes with meth-

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anol (3 × 5.0 mL) and concentrated to dryness at 40 °C. The residue was redissolved in methanol (2.0 mL), and the resulting solution was quantitated by high-performance liquid chromatographic (HPLC) analysis.

The remaining six dishes were treated as follows. Three were placed in a dark drawer at room temperature; one was processed 24 h, one 48 h, and one 72 h after zero-time. The remaining three dishes were placed about 125 cm below the output of a Kratos Model LH 153 solar simulator (beam diameter at dish level ~25 cm). One dish was processed as above after 6-, 24-, and 48-h exposure to the simulated sunlight.

HPLC–radiomonitor analysis was necessary only for the stability study. The column was a 5-μm Zorbax (Du Pont) C_{18}, 4.6 mm (i.d.) × 25 cm, maintained at 40 °C. The mobile phase was acetonitrile–water (85:15, v/v), flowing at 0.5 mL/min. The ultraviolet detector (LDC Spectromonitor III, Model 1204A) was set at 245 nm, 0.02 absorption unit full scale. A nominal 10-μL loop was used for all samples. The effluent from the UV detector was directed to a Berthold LB504 radiomonitor, equipped with a 800-μL flow cell, where it was mixed with 1.50 mL/min of Aquasol 2 (New England Nuclear) liquid scintillator, provided by a Kratos Spectroflow 400 pump. The ultraviolet and radiomonitor signals were analyzed on separate channels by Nelson Analytical software and a Hewlett-Packard 9816 computer.

Ingestion Toxicity Study. Nonresistant mites, primarily adult females, were taken from a laboratory colony maintained for several years on bush bean plants. Two-week-old bush bean seedlings (*Phaseolus vulgarus* L. var. Tendercrop) (24) were prepared by cutting the two leaves of each plant down to 2 × 2 cm squares centered around the midrib. Tanglefoot was applied around the edges to confine applied mites to the ventral surface. The 24 plants were divided into groups representing six replicates of four treatments.

Miticide application involved 64 1-μL drops of emulsions 1 and 2 applied in a 8 × 8 pattern (8 × 4 on each side of the midrib) to the dorsal surface of each leaf. As a background correction for the three other treatments, an 18 μg/mL emulsion of cold abamectin was pipetted onto each of the 12 leaves of treatment group 1. Emulsion 1 was applied to leaves of treatment groups 2 and 3; emulsion 2 was applied to the 12 leaves of treatment group 4. When the leaves were dry (~1 h after application), they were infested on the ventral surfaces with mites (approximately 150–200/leaf) to recover a total of at least 2 × 500 pooled dead mites (two replicates) from the 12 leaves of each treatment group. The 18 plants of groups 1, 3, and 4 were put in a dark growth chamber overnight. The six plants of group 2 were placed in an illuminated chamber. Both chambers are maintained at 22.5 °C. Group 2 was included to estimate the effects of photodegradation on toxicity.

The following morning, the dead mites were brushed from the ventral leaf surfaces and individually counted into sample oxidizer cones. The cones were combusted using a Packard Tri-Carb B-306 sample oxidizer. All samples were counted in a Packard Tri-Carb 460 liquid scintillation counter.

Penetration Study. Eight similar bush bean seedlings were used in the penetration study, but here each of the 16 leaves was cut down to a 1 × 1 cm square. Sixteen 1-μL drops of emulsion 1 were applied to the dorsal surface of each of eight leaves on four plants, and emulsion 2 was similarly applied to the eight leaves of the other four plants.

Leaves were processed at dry-down (1 h after application) and 24 h after application. The 24-h plants were kept in a dark growth chamber. For processing, each leaf was dipped into methanol (10.0 mL) and gently agitated for 5 s. An aliquot (1.0 mL) of the methanol rinse was drawn from each of the 16 vials and added to 10.0 mL of Insta-gel liquid scintillation cocktail. Recoveries of 100% were determined from aliquots of the applied labeled emulsions. All samples were counted in a Packard Tri-Carb 460 liquid scintillation counter.

RESULTS

Stability Study. Abamectin is composed of two components, avermectin B_{1a} and B_{1b}; however, the results given are for avermectin B_{1a} only. This is further addressed in the Discussion.

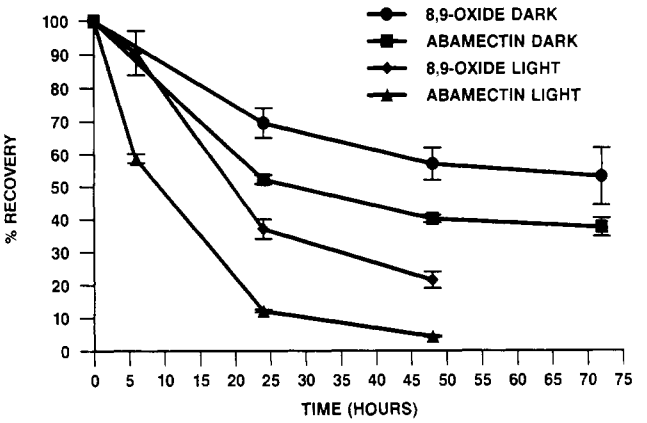

Figure 1. Stability of abamectin and its 8,9-oxide in Petri dishes. The data for the top pair of curves (dark) were generated from one set of three dishes, each containing mixed 18 ppm emulsions of abamectin and its 8,9-oxide. The three light-exposed dishes, containing the same mixed emulsion, are represented by the bottom pair of curves. In all cases, abamectin was quantitated by UV detection and abamectin 8,9-oxide by flow scintillation counting in the radiomonitor, using peak heights.

The results of the stability study are shown graphically in Figure 1. Percent recovery was determined by taking radiomonitor or UV detector peak heights for avermectin B_{1a} as a percent of the B_{1a} peak height for the corresponding zero-time sample. The error bars represent standard deviations for measurements on the same dish. The initial half-lives vary from less than 10 h to more than 72; of the four treatments, the order of stability is (most stable first) 8,9-oxide, dark > abamectin, dark > 8,9-oxide, light > abamectin, light.

Ingestion Toxicity Study. The data for the ingestion toxicity study of abamectin and its 8,9-oxide are presented in Table I. For mites exposed to plants held in the dark, there was no significant difference between the amount of radiolabeled abamectin and its 8,9-oxide ingested. However, mites ingested a significantly greater amount of radioactivity from abamectin-treated plants held in the light. The radioactivity counted in the control group mites was not significantly above the background count. These results are based on 100-min counts of each of the sample oxidizer vials. From the accumulated counts, uncertainties of ~2% are due to the random nature of radioactive decay. Another ~5% represents possible sampling and pipetting errors. The sample oxidizer may contribute another 5–8%, reflecting precision (repeatability) and accuracy (recovery) deficiencies. Overall, we believe our values are accurate to ±15%.

Penetration Study. The results of the bean leaf penetration study are presented in Table II. The percent recoveries given are the total radioactivity counted in the methanol rinse as a fraction of that originally applied to the leaf. At both time points, significantly greater abamectin was recovered in the methanol than 8,9-oxide, at the 95% confidence level. This means that significantly more 8,9-oxide was associated with the leaf.

DISCUSSION

General Discussion. Excluding parent compound volatility and contact activity [which are regarded as equivalent for both compounds (Dybas et al., 1989)], there are only three mutually independent factors responsible for a difference in miticidal activity between two compounds. The first is the compound's inherent miticidal potency, that is the amount actually needed to kill a mite.

Table I. Accumulation of Tritium-Labeled Abamectin and Its 8,9-Oxide in Two-Spotted Spider Mites

treatment group	wt ingested/mite, pg
2 (abamectin, light)	1.13, 0.96 (2 replicates)
3 (abamectin, dark)	0.83, contaminated
4 (8,9-oxide, dark)	0.85, 0.80

The second factor is the chemical stability of the miticide on and in the plant leaf tissues. Stability is especially important for materials like abamectin, whose toxicity is expressed slowly at field concentrations (Putter et al., 1981). The third factor is penetrability; this is the toxicant's movement from the leaf surface through the cuticular waxes into the lower layers where the mites feed (Jeppson et al., 1975; Brito et al., 1986).

In the interpretation of these results, one concern relevant in all three studies is how well the behavior of avermectin B_{1a} predicts the behavior of abamectin. Our conclusions are based primarily on the behavior of radiolabeled avermectin B_{1a}, so the question is appropriate. Abamectin consists of a mixture of at least 80% avermectin B_{1a} and no more than 20% avermectin B_{1b}. Avermectin B_{1a} differs from the B_{1b} component only in having as the C-25 substituent *sec*-butyl (R = C_2H_5) as opposed to isopropyl (R = CH_3). Numerous studies of abamectin's in vivo and in vitro metabolism and/or degradation have failed to detect a statistically significant change in the B_{1a} to B_{1b} ratio as a function of abamectin loss (Bull et al., 1984; many unpublished Merck studies). A ratio change would occur if the two components were degrading at different rates. On this basis, we believe the behavior of avermectin B_{1a} is an excellent predictor for that of abamectin.

A second concern is how well stabilities on Petri dishes predict stabilities on bean leaves. Third, in the penetration experiment, only the nonbound levels of abamectin and its 8,9-oxide were measured. We did not distinguish between miticide adhering to the superficial wax layer from that that may have migrated into deeper strata of the leaf where the mites feed. Fourth, the value measured (~0.8 pg/mite) for both compounds in the toxicity experiment must represent an upper limit for two possible reasons. First, degradation to nontoxic but still radioactive products would necessarily increase the uptake of radioactivity by a mite before a lethal dose were obtained. Second, a mite could conceivably ingest a supratoxic amount before dying, especially if the toxicant (as abamectin) is relatively slow-acting. These factors would not greatly obscure or over-emphasize the potency difference between these compounds but would have an effect on the magnitude of the potencies measured.

Stability Study. There are two sources of possible error in using stabilities on Petri dishes to predict stabilities on leaf surfaces. First, one could envision some degradative process catalyzed by glass that cannot occur on the surface of a bean leaf. On the other hand, the opposite is also true: An enzyme-catalyzed reaction could occur on a leaf that would not occur on a Petri dish surface. However, our goal in this study was to establish not absolute degradation rates but only relative rates for abamectin and its 8,9-oxide. It seems reasonable to assume that the compound that degrades more slowly on Petri dishes will decompose more slowly on plant leaves as well. We have data to support this assumption (Demchak and MacConnell, 1985, unpublished observations). Abamectin 8,9-oxide is more stable on both Petri dishes and the leaves of rough lemon seedlings than is abamectin. With use of techniques not importantly different from those described here, the 8,9-oxide analogue was found to have a half-life of about 50 h on Petri dishes (compared to 12 h for abamectin) and of 22 h on lemon leaves (compared to 10 h for abamectin). These experiments were also conducted under the solar simulator. We thus believe Petri dishes are satisfactory predictors of relative rates of degradation on leaves. Therefore, considering stability alone, we should expect greater (and/or longer lasting) miticidal activity from abamectin 8,9-oxide than from abamectin.

Table II. Percent Recovery of Tritium-Labeled Abamectin and Its 8,9-Oxide in Methanol Rinses of Treated Bean Leaves

1-h data	24-h data
Abamectin	
100	96.4
96.8	100
100	85.7
92.8	85.9
97.4 ± 1.7[a]	92.0 ± 3.7[a]
Abamectin 8,9-Oxide	
72.1	87.8
87.0	78.3
92.3	73.0
92.5	79.7
86.0 ± 4.8[a]	79.7 ± 3.1[a]

[a] Mean ± SEM.

Penetration Study. In the penetration experiment, we measured the amount of radiolabeled miticide associated with plant foliage, either bound to epicuticular waxes or associated with the lower layers. We chose not to pursue methods for stripping successive layers of leaf cuticle to obtain a more complete profile of toxicant penetration [see, for instance, Silcox and Holloway (1986)]. We elected instead to take a very simple approach and generate data indicative of penetration. Although the data from nominally identical leaves are rather imprecise, there is little doubt that under similar formulation, concentration, and contact conditions more 8,9-oxide is unextracted by methanol. Thus, to the extent that better penetration yields a better miticide, the penetration study results favor the 8,9-oxide.

Ingestion Toxicity Study. We conducted the toxicity study at 18 ppm to achieve a quick kill and thus reduce the influence of degradation. Nevertheless, we included two abamectin replicates held overnight in light to help gauge the magnitude of the effect. The intake per mite went from about 0.83 pg/mite in the dark to 1.05 pg in the light. If degradation is the only difference, this means that 21% of the material ingested had decomposed to inactive substances. To the extent that degradation did occur in the dark, and thus diluted the compound with radioactive but nontoxic degradants, the estimate of ~0.8 pg/mite is an upper limit.

The toxicity results allow further calculations and access to information that would otherwise remain unavailable. McEnroe (1961) found that active two-spotted spider mites weigh 17–22 μg each and imbibe about 3 nL/h under simulated feeding conditions. The absolute toxicity is thus about 40 ppb (nanograms of abamectin or 8,9-oxide per gram of mites). Now, abamectin has a water solubility of about 8 ng/mL (Maynard, 1982, unpublished observations). From this value and the intake value provided by McEnroe, an individual mite would have to feed continuously for about 33 h to acquire a toxic dose of ~800 fg. Since the mites in this experiment had no source of toxicant except through feeding and fed no longer than about 22 h, either they took in the plant fluids more rapidly or the abamectin concentration in the imbibed fluid

exceeded the 8 ng/mL solubility in pure water. We strongly suspect the latter.

In conclusion, the results of these studies show that (1) abamectin 8,9-oxide is more stable than abamectin in Petri dishes and on plant leaves, (2) abamectin 8,9-oxide is associated with plant foliage to a greater extent than is abamectin, and (3) abamectin and its 8,9-oxide have equivalent ingestion toxicity for the two-spotted spider mite. Both penetration and stability are probably the determinants of the improved residual activity of the 8,9-oxide over abamectin. At this time, however, it is not possible to determine the relative contributions of these factors to the increased activity of the 8,9-oxide in laboratory bioassays.

ACKNOWLEDGMENT

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Registry No. I, 71751-41-2; avermectin B_{1a}, 65195-55-3; avermectin B_{1a} 8,9-epoxide, 98734-60-2.

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Synthesis of a 3-Acyl-4-hydroxycyclohex-3-en-1-one

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A synthesis of the title compounds from the isomeric 2-acyl-3-hydroxycyclohex-2-en-1-ones 1 has been developed. The 1,3-diketone system of 1 was preserved as its isoxazole derivative **2**, facilitating manipulation of the remaining carbonyl, which in five steps was removed and reintroduced at the adjacent position. The carbonyl of the resulting 5-ketotetrahydrobenzisoxazole **11a** was sensitive to reduction and was protected as a ketal during reductive disassembly of the isoxazole ring.

A number of 2-acyl-3-hydroxycyclohex-2-en-1-ones 1 and 2-acyl-3,6-dihydroxycyclohex-2-en-1-ones **6** in which R is a saturated or unsaturated long-chain hydrocarbon radical are natural products isolated from insects (Lusby et al., 1987; Mudd, 1978, 1981) and plants (Nemoto et al., 1987). In a recent publication we described the synthesis of **6** starting with the 4-ketotetrahydrobenzisox-

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