Incorporation of Emamectin Benzoate (MK-0244) Residues into Soluble Sugars of Plants

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The semisynthetic avermectin, emamectin benzoate (MK-0244), as well as other avermectins, is known to degrade rapidly and photolytically on plants, mostly to complex polar extractable residues. After [^{14}C]emamectin benzoate treatment, extractable residues from foliage of lettuce and cabbage and from fodder, kernels, and cobs of sweet corn were studied to determine the nature of a specific subfraction of these polar residues. These subfractions of polar extractable residues were water soluble and retained by aminopropyl solid phase extraction columns. HPLC analysis showed peaks of radioactivity corresponding to those of xylose, fructose, glucose, galactose, and/or sucrose standards. Several other discrete but unidentified radioactive components were also seen. This incorporation of radioactivity into simple sugars indicated that extensive fragmentation of emamectin had occurred. The formation of radioactive sugars in plants after [^{14}C]emamectin treatment may indicate the source of radioactivity for other classes of natural products in these treated plants for which bioincorporation has also been reported.

Keywords: *MK-0244; emamectin; avermectin; residue incorporation; sugars*

INTRODUCTION

The avermectins are a class of natural products consisting of a disaccharide linked to a pentacyclic 16-membered lactone ring and are produced by the soil microorganism *Streptomyces avermitilis*. Abamectin, consisting of a mixture of the natural avermectins B_{1a} and B_{1b}, is a commercial miticide and insecticide. Emamectin benzoate (MK-0244) is a broad-spectrum and potent insecticide active against lepidopterous larvae on crops (Lasota and Dybas, 1991) and is currently under development at Novartis Crop Protection. Emamectin benzoate is synthesized from abamectin by modification of the terminal disaccharide; an *epi*-aminomethyl (−$NHCH_3$) group is substituted for a hydroxyl (−OH) group at the 4″-position. Emamectin benzoate is composed of two homologous compounds; a major constituent (≥90%), 4″-deoxy-4″-*epi*-(methylamino)avermectin B_{1a} (MAB1a) benzoate, and a minor constituent (≤10%), 4″-deoxy-4″-*epi*-(methylamino)avermectin B_{1b} (MAB1b) benzoate (Figure 1). Since the homologs differ by only by a single methylene unit on the C-25 side chain, the degradation or metabolism would be expected to be the same or similar. Emamectin benzoate degrades rapidly and photolytically in plants and on plant surfaces, mostly to an extremely complex group of polar extractable residues (Crouch and Feely, 1995; Crouch et al., 1995, 1997; Feely et al., 1995; Wrzesinski et al., 1996). A similarly rapid and predominant degradation to polar extractable residues was also observed in abamectin-treated crops (Bull et al., 1984; Moye et al., 1990). In the present study a subfraction of polar radioactive residues extracted from the foliage of [^{14}C]-emamectin benzoate-treated lettuce, cabbage, and sweet corn and from sweet corn cobs and kernels was identified as soluble sugars. Incorporation of radioactivity into other natural products present in unextractable residue fractions was previously shown in lettuce (Crouch and Feely, 1995), cabbage (Feely and Crouch, 1997), and sweet corn (Feely et al., 1995) treated with [^{14}C]-emamectin benzoate.

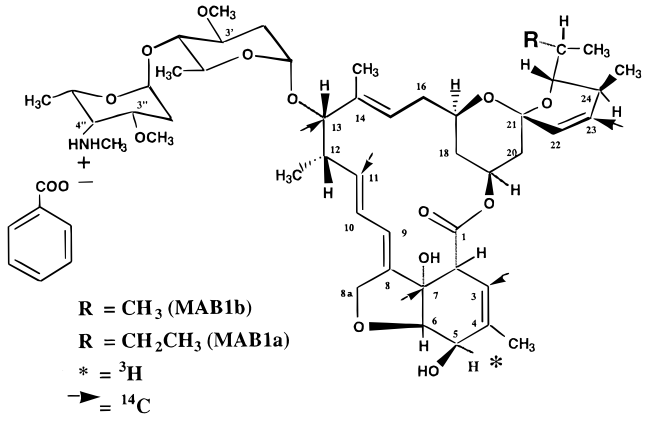

Figure 1. Structure of emamectin benzoate. Emamectin benzoate consists of ≥90% of the MAB1a homolog and ≤10% of the MAB1b homologue. All plants were treated only with the MAB1a homolog. MAB1a benzoate was labeled with either [3,7,11,13,23-^{14}C] or [5-^{3}H] as indicated above. For sweet corn, all applications (six) were of [^{14}C]MAB1a benzoate. For lettuce and cabbage, [^{14}C/^{3}H]MAB1a benzoate was used at the last application and [^{14}C]MAB1a benzoate for all previous (seven) applications.

MATERIALS AND METHODS

Test Compounds and Reference Standards. The test compounds, [*C3, C7, C11, C13, and C23*-^{14}C]MAB1a benzoate

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(approximately 30 μCi/mg) and [5-^{3}H]MAB1a benzoate (approximately 10 mCi/mg), were supplied by the Labeled Compound Synthesis Group, Merck Research Laboratories, Rahway, NJ. The sugars used as external standards were D-(+)-glucose, D-(−)-fructose, D-(+)-galactose, D-(+)-xylose, and sucrose (Sigma Chemical Co., St. Louis, MO).

Chemicals and Solvents. All chemicals used were of reagent grade unless otherwise specified. All solvents used were of HPLC grade. Water was purified to 18 MΩ resistance using a Waters Milli-Q system.

Treatment and Harvest of Crops and Specimen Processing. Multiple applications of MAB1a benzoate were made at 0.075 lb of active ingredient (ai)/acre (85 g/ha) to sweet corn (Silver Queen variety, *Zea mays*), cabbage (Copenhagen market variety, *Brassica oleracea* L.), and lettuce (Great Lakes variety, *Latuca sativa*) growing outdoors in direct sunlight during the normal growing season. This application was 5-fold the maximal proposed for a single application to each of these crops. Cabbage and lettuce were treated as previously described for lettuce (Crouch and Feely, 1995) with seven weekly applications of [^{14}C]MAB1a benzoate followed by a final application of [^{14}C/^{3}H]MAB1a benzoate (1:1 ratio of specific activity) one week later. Sweet corn received six applications of [^{14}C]MAB1a benzoate spaced at approximately 3 day intervals. All applications were made with the radiolabeled MAB1a benzoate being first dissolved in an agricultural formulation and then diluted with water for spraying as previously described in detail for cabbage (Crouch et al., 1997).

Harvesting was performed at 3 days after the last application for each crop. Three (cabbage, lettuce) or five (sweet corn) plants were harvested. For cabbage and lettuce the heads and wrapper leaves were combined as a single foliage specimen for each plant. For sweet corn, four individual specimens of tissues, leaf plus stalk (combined), husk plus silk (combined), kernels, and cobs were obtained from each plant.

All plant specimens were homogenized and extracted similarly using a method described previously (Crouch and Feely, 1995). Briefly, the individual plant specimens were homogenized separately with methanol, and the homogenates were stored in a freezer when not in use. Residues were extracted from all plant tissues at room temperature using various solutions of methanol/water containing ammonium acetate. For extraction, portions of the individual tissue homogenates from all lettuce and cabbage plants harvested were composited. For extraction of sweet corn tissues, portions of the individual homogenates of the leaf plus stalk and husk plus silk specimens were composited from all plants as a combined leaf/stalk/husk/silk (fodder) sample. The homogenates of kernels and cobs from all sweet corn plants were also separately composited prior to extraction.

Quantitation of Radioactivity. The total radioactivity of the various homogenized tissue specimens from lettuce, cabbage, and sweet corn were determined by radiocombustion analysis (RCA) of the methanol homogenates as described previously (Crouch and Feely, 1995). RCA was performed by oxidation using a Packard Model 306 or 307 oxidizer followed by liquid scintillation counting (LSC) in a Packard Model 4530, 460, or TR-2500 instrument. The total radioactivity of extractable residues and subfractions thereof as well as of HPLC fractions (see below) was determined by direct LSC.

Fractionation of Extractable Residues by Solid Phase Extraction. C_{18} solid phase extraction (SPE) was used to fractionate the extractable residues from lettuce, cabbage, and sweet corn fodder into four residue fractions. They were, in order of decreasing polarity, sugar polar, extreme polar, retained polar, and avermectin-like residue fractions. The polar extractable residues were thus subdivided into three fractions (sugar polar, extreme polar, and retained polar) by this method. The SPE columns (Varian, 0.5 g, 6 cm^3) were conditioned by washing with methanol followed by water. Aliquots of the extractable residues were applied to the conditioned columns in water, and the eluate was collected. The columns were then eluted with water, and this eluate was combined with the previous eluate and designated the sugar polar residues fraction. The column was then eluted with 25:75 methanol/water containing 5 mM ammonium acetate, and this eluate was the extreme polar residues fraction. Next, the retained polar residues fraction was eluted using a solution 70:30 methanol/water containing 5 mM ammonium acetate. Finally, the avermectin-like residues fraction was eluted with methanol containing 5 mM ammonium acetate.

Aminopropyl SPE was used to fractionate the total extractable residues from sweet corn kernel and cobs and the sugar polar residues fractions from lettuce, cabbage, and sweet corn fodder. The total extractable residues from sweet corn kernel and cob had chromatographic properties similar to those of the sugar polar residues fractions from the other plant tissues (data not shown). The aminopropyl SPE columns (Bakerbond, J. T. Baker, 500 mg) were conditioned by washing successively with acetonitrile, water, and 80:20 (v:v) acetonitrile/water. Aliquots of the total extractable residues, or their sugar polar residues fractions from C_{18} SPE, were applied to the conditioned columns in 80:20 acetonitrile/water. The resulting breakthrough eluates were termed the nonretained amino SPE residues. Each column was then eluted with water and the resultant eluates were referred to as the retained amino SPE residue or plant sugar fraction. Amino SPE of sugar standards demonstrated that up to 40 mg of fructose, glucose, galactose, and xylose could be applied with 75−90% being retained. The plant sugar fractions were analyzed by high-pressure liquid chromatography (HPLC) as described below.

HPLC Analyses of Plant Sugar Fractions. The plant sugar fractions were mixed with ethanol and/or acetonitrile and taken to dryness under nitrogen or reduced pressure. The dried plant sugar fractions were then reconstituted in an acetonitrile/water solution of the same composition as the starting eluent for the HPLC analysis. HPLC was performed using a system comprised of a Rheodyne 7125 injector, a Perkin-Elmer (PE) Nelson Model 1020 Integrator or a Hewlett-Packard (HP) ChemStation data station, a Spectra Physics (SP) P4000 gradient controller or a SP 8700 solvent delivery system, a SP UV2000 dual wavelength UV detector or a HP 1040A diode array detector with optical upgrades, Supelco amino semipreparative column (240 × 10 mm), and a Brownlee amino guard column. The column was eluted at a flow rate of 3 mL/min with various mixtures of water (solvent A) and acetonitrile (solvent B) as described below. The eluate absorbance was monitored at 192 nm with the SP UV2000 detector and at 192, 200, and 210 nm with the HP 1040A detector. A Pharmacia Frac-100 fraction collector was used to collect 0.5 min fractions in 6 mL plastic minivials (Packard). Radioactivity in eluate fractions or aliquots thereof was determined by LSC. Two HPLC methods (1 and 2) were used for analysis, which varied only in the gradient program used. The gradient for method 1 was as follows: 0−15 min, isocratic at 80% B; 15−20 min, decrease linearly to 70% B; 20−30 min, isocratic at 70% B; 30−35 min, increase linearly to 100% B; 35−45 min, wash the column at 100% B. The gradient used for method 2 was as follows: 0−30 min, isocratic at 80% B; 30−40 min, increase linearly to 100% A; 40−45 min, wash the column at 100% A. The HPLC system operation was verified daily prior to sample analysis using a standard mixture containing xylose, fructose, glucose, and galactose. In addition, a sucrose standard was chromatographed on the day of analysis for certain samples.

Sugar Assay and Invertase Incubation. A Trinder reagent kit (Sigma) was used to quantify glucose (Trinder, 1969) in the various SPE and HPLC fractions of extractable residues. Absorbance at 505 nm (A_{505}) was measured on a Perkin-Elmer spectrophotometer, Model 320. With each set of samples analyzed for glucose, a standard curve using glucose was also generated. Invertase (baker's yeast, Sigma) was used to indicate the presence of sucrose in aliquots of various SPE and HPLC fractions of extractable residues. All samples were incubated with invertase in 50 mM sodium acetate buffer (pH 4.5) with approximately 800 units/mL of invertase at 55 °C for 3 h with gentle shaking. A sucrose standard, with and without invertase, was also incubated at the time of each sucrose assay to verify enzyme activity. After incubation, samples were stored frozen until analyzed, either by the Trinder assay for glucose or by HPLC.

Table 1. SPE Fractionation of Extractable Residues[a]

extractable residue fraction	lettuce	cabbage	sweet corn fodder	sweet corn kernels	sweet corn cobs
C_{18} SPE					
sugar polar[b]	11.9	3.9	5.2	NA[j]	NA
extreme polar[c]	2.7	2.5	1.9		
retained polar[d]	31.1	39.1	37.2		
avermectin-like[e]	54.3	54.4	55.6		
amino SPE					
nonretained[f]	11.2	10.7[h]	24.2[i]	38.7	40.9
retained[g] (plant sugar)	88.8	89.3[h]	75.8[i]	61.3	59.2

[a] The total extractable residues from lettuce and cabbage foliage and sweet corn fodder were fractionated by C_{18} SPE, and the resultant sugar polar residue fractions were further fractionated by amino SPE. All values are expressed as percent of recovered ^{14}C radioactivity. The four C_{18} SPE fractions obtained were defined as all radioactivity eluted sequentially with [b]water, [c]25:75 methanol/water containing 5 mM ammonium acetate (AA), [d]70:30 methanol/water containing 5 mM AA, and [e]methanol containing 5 mM AA. The two amino SPE fractions obtained were defined as all radioactivity eluting sequentially with [f]application solution (80:20 acetonitrile/water) and [g]water. All values are for single fractionation procedures except as noted for [h]average of 4 and [i]average of 2. Recoveries ranged from 93 to 108% for C_{18} SPE and from 49 to 84% for amino SPE. [j] NA, not applicable as extractable residues were not subjected to C_{18} SPE.

NMR Analysis. The sugars xylose, fructose, glucose, and galactose were purified from corn fodder extractable residues by a combination of SPE and HPLC method 2. Proton nuclear magnetic resonance (NMR) analysis was conducted at a field strength of 400 MHz using a Varian Unity-400 spectrometer. The purified sugars were dissolved in D_2O, and the NMR analysis was performed at 25 °C. NMR spectra of the xylose, fructose, glucose, and galactose standards were obtained at the same time for comparison with the corresponding sugars isolated from corn fodder.

RESULTS

Extractable Residues and SPE Fractionations. The total extractable residues were approximately 88, 77, 82, 42, and 53% for lettuce, cabbage, sweet corn fodder, sweet corn kernels, and sweet corn cobs, respectively. Recoveries from these extractions averaged 115% and ranged from 105 to 127%. After C_{18} SPE of the total extractable residues when appropriate, the resultant fractions ranged from approximately 54 to 56% for the avermectin-like residues, from 31 to 39% for the retained polar residues, at values <3% for the extreme polar residues, and from 4 to 12% for the sugar polar residues (Table 1). The avermectin-like residue fraction contained the parent MAB1a and a number of minor residues of closely related structure (not shown), which were the same as those previously detailed for emamectin-treated lettuce (Crouch and Feely, 1995) and cabbage (Wrzesinski et al., 1996; Crouch and Feely, 1997). The total extractable residues from sweet corn cobs and kernels corresponded to the C_{18} SPE sugar polar residues fraction from the other specimens (Table 1) and no MAB1a or avermectin-like residues were present (not shown).

The plant sugar fractions from amino SPE of the total extractable residues from sweet corn kernels and cob contained 61.3 and 59.2% of the recovered radioactivity, respectively. The plant sugar fractions from amino SPE of the C_{18} SPE sugar polar fractions were 88.8, 89.3, and 75.8%, respectively, for lettuce, cabbage, and sweet corn fodder (Table 1).

Analyses of Plant Sugars. Essentially, quantitative recovery of the glucose present in the extractable residues from lettuce, cabbage, and sweet corn fodder was observed for the C_{18} SPE sugar polar residues fraction. Values of 5, 11, 27, 25, and 44 mg of glucose/g of wet weight were obtained, respectively, for lettuce, cabbage, and sweet corn fodder, kernels, and cobs (Table 2). The values obtained for the glucose content of lettuce and cabbage were similar to those previously reported (Table 2).

Table 2. Glucose Content of Extractable Residues[a]

analysis	lettuce	cabbage	sweet corn fodder	sweet corn kernels	sweet corn cobs
sugar polar residue fraction (% total glucose)[b]	99	97	99	NA[c]	NA
tissue glucose content (mg/g of wet weight)[d]	5 (2.5)[e]	11 (15.8)	27	25	44

[a] The glucose content of the C_{18} SPE sugar polar residue fractions and/or the total extractable residues from each plant was determined according to the Trinder method. [b] Percent of total glucose in extractable residues present in C_{18} SPE sugar polar residues fraction. [c] NA, not applicable as extractable residues were not subjected to C_{18} SPE. [d] Assayed glucose content of extractable residues expressed as unit of tissue wet weight. [e] Values in parentheses are reported glucose content (Ockerman, 1978).

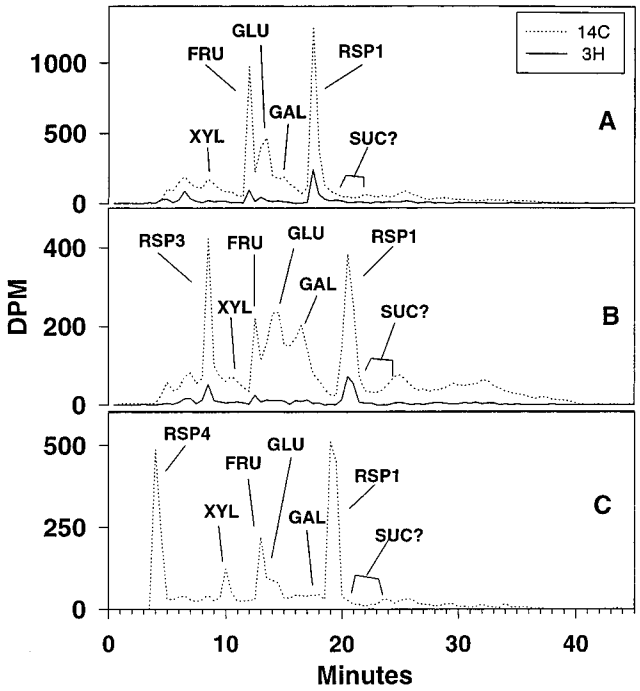

Figure 2. HPLC analysis of plant sugar fractions from foliage and fodder. The amino SPE plant sugar fractions from (A) lettuce, (B) cabbage, and (C) corn fodder were assayed by HPLC method 1. Sugars indicated were identified by comparison with external standards assayed on the same day. Standard retention times varied slightly between days but were approximately as follows: xylose (XYL), 9.8 min; fructose (FRU), 12.2 min; glucose (GLU), 14.3 min; galactose (GAL), 15.2 min; and sucrose (SUC), 20.8 min. RSP1 and RSP4 are unidentified residues.

Analysis of the plant sugar fraction from lettuce by HPLC method 1 indicated the presence of radioactive glucose, fructose, and possibly xylose and galactose. The major component, however, was an unknown residue designated RSP1 (Figure 2A). Analysis of the plant sugar fraction from cabbage by HPLC method 1 revealed the presence of radioactive fructose, glucose, galactose, and possibly xylose, RSP1 as for lettuce, and an additional unknown residue designated RSP3 (Figure 2B). Analysis of the plant sugar fraction from sweet corn fodder by HPLC method 1 showed radioactive glucose, fructose, xylose, and possibly galactose, RSP1, and an

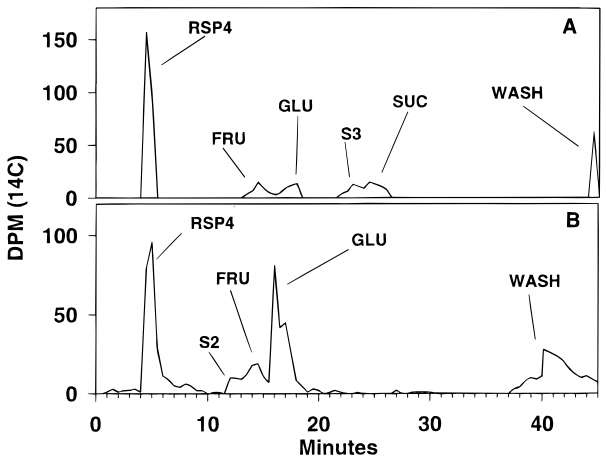

Figure 3. HPLC analysis of plant sugar fraction from kernels. Amino SPE fractions from sweet corn kernels (A) before and (B) after invertase treatment were assayed by HPLC method 2. Sugars were identified by comparison to external standards assayed on the same day. Standard retention times varied slightly between days but were approximately as follows: xylose (XYL), 9.5 min; fructose (FRU), 11.9 min; glucose (GLU), 13.5 min; galactose (GAL), 16.0 min; and sucrose (SUC), 24.0 min. S2, S3, and RSP4 are unidentified residues.

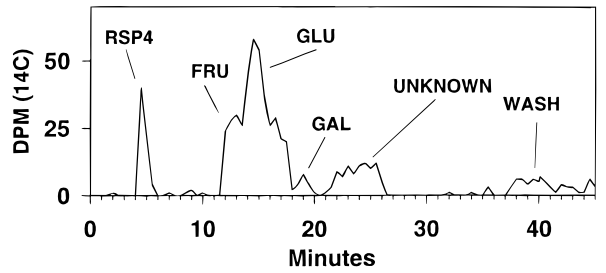

Figure 4. HPLC analysis of plant sugar fraction from cobs. The amino SPE plant fraction from corn cobs was assayed by HPLC method 2. Sugars were identified by comparison to external standards assayed on the same day. Standard retention times were as for Figure 3. RSP4 is an unknown residue.

additional unknown residue designated RSP4 (Figure 2C). Analysis of the plant sugar fraction from sweet corn kernels by HPLC method 2 indicated the presence of radioactive sucrose, fructose, and glucose, RSP4, and an additional unknown residue designated S3 (Figure 3A). After incubation with invertase, the sucrose and S3 peaks were no longer present and the fructose and glucose peaks were enhanced. A third unknown residue designated S2 appeared, and RSP4 was unchanged (Figure 3B). Analysis of the plant sugar fraction from sweet corn cobs by HPLC method 2 was consistent with the presence of radioactive fructose, glucose, and possibly galactose, along with RSP4 as observed for sweet corn fodder and kernels (Figure 4).

Glucose ranged from 2.5 to 44.9% of the plant sugar fraction's radioactivity, with sweet corn fodder the lowest and sweet corn cobs the highest (Table 3). Fructose ranged from 6.6 to 16.8% of the plant sugar fraction's radioactivity, with cabbage the lowest and sweet corn cobs the highest as for glucose. Xylose was a prominent sugar in the plant sugar fraction from sweet corn fodder at 4.3% but was minor or absent in sweet corn kernels, cobs, lettuce, and cabbage. Galactose was a relatively prominent sugar in the plant sugar fraction from cabbage but was less so in lettuce and sweet corn cobs and fodder and was low or absent in sweet corn kernels. With the exception of sweet corn

Table 3. HPLC Analysis of Plant Sugar Fractions[a]

sugar or residue component	lettuce[b]	cabbage[b]	sweet corn fodder[c]	sweet corn kernels[b]	sweet corn cobs[b]
xylose, %[d]	2.3	2.4	4.3	NP[f]	NP
ratio[e]	ND[f]	ND			
fructose, %	13.0	6.6	8.2	8.2	16.8
ratio	10.5	11.5			
glucose, %	13.6	14.0	2.5	9.0	44.9
ratio	13.5	18.5			
galactose, %	4.6	5.8	2.9	NP	3.0
ratio	12.9	29.1			
sucrose, %	trace?	trace?	trace?	9.6	NP
ratio	ND	ND			
RSP1,[g] %	20.9	14.0	30.2	NP	NP
ratio	5.4	5.6			
RSP3,[g] %	NP	10.3	NP	NP	NP
ratio		8.4			
RSP4,[g] %	NP	NP	14.4	51.8	10.3
ratio					
S3,[g] %	NP	NP	NP	8.8	NP
ratio					

[a] The plant sugar fractions from amino SPE were analyzed by HPLC method 1 or 2. Lettuce and cabbage were treated at the last application only with [^{14}C/^{3}H]emamectin B1a benzoate, while sweet corn was treated with [^{14}C]emamectin B1a benzoate at all applications. All radioactivity not accounted for was present as background with no significant peaks. [b] Value from single analysis. [c] Value average of two analyses. [d] Percent of eluted ^{14}C radioactivity. [e] [^{14}C]:[^{3}H] ratio in peak. [f] NP, apparently not present; ND, not determined because of low radioactivity. [g] Unidentified residues. Recoveries from the HPLC analyses ranged from 55 to 92%.

kernels, radioactive sucrose was present at only trace levels or was absent (Table 3).

The plant sugar fraction from corn fodder was also fractionated by HPLC method 2 to obtain purified sugars for NMR analysis. The NMR analysis confirmed the identity of fructose and glucose, but the xylose analyzed was of insufficient purity for identification. The NMR analysis of the isolated galactose indicated that it was apparently glucose. However, when the HPLC fractions corresponding to the peaks of radioactivity attributed to glucose and galactose were analyzed for glucose by the Trinder method, although the great majority of the glucose was indeed associated with the radioactive glucose peak, there was significant tailing of the glucose peak into the galactose fractions (data not shown). Therefore, it was not clear whether the peak of radioactivity from corn fodder corresponding to the galactose standard was actually galactose.

DISCUSSION

The photodegradation of avermectins such as emamectin (Feely et al., 1992) and abamectin (Crouch et al., 1991) as thin films on glass surfaces is rapid and characterized by the initial formation of a number of minor identified products which are of similar structure to the parent avermectin. As avermectin photodegradation on glass proceeds, polar residues of greater complexity and apparently of much less structural similarity to the parent avermectin accumulate (Crouch et al., 1991, 1992; Feely et al., 1992). The fate of avermectins applied to foliar surfaces of plants largely parallels that observed for photolysis on glass inasmuch as the degradation is rapid, the same minor products are formed, and polar residues accumulate as a function of time (Bull et al., 1984; Moye at al., 1990; Crouch et al., 1995; Wrzesinski et al., 1996). However, in [^{14}C]-avermectin-treated plants significant unextractable residues also occur (Bull et al., 1984; Maynard et al., 1989; Moye et al., 1990; Crouch and Feely, 1995), which

indicates that photodegradation is not the only degradative route for avermectins in plants. Characterization of unextractable residues from [^{14}C]avermectin-treated plants has indicated that these may largely be the result of incorporation of radioactivity into natural products such as cellulose (Feely and Wislocki, 1991; Crouch et al., 1995; Crouch and Feely, 1995; Feely and Crouch, 1997), lignin (Feely and Wislocki, 1991; Feely and Crouch, 1997), proteins (Crouch et al., 1995; Feely and Crouch, 1997), starch (Feely and Crouch, 1997), and phytoglycogen (Wrzesinski and Crouch, unpublished results). In addition, incorporation of radioactivity into the fatty acids of cottonseed oils after foliar treatment of cotton with [^{14}C]abamectin has been demonstrated (Alvaro, Merck Research Laboratories, personal communication). The present results establishing the presence of soluble sugars with incorporated radioactivity after [^{14}C]emamectin treatment of lettuce, cabbage, and sweet corn indicate the likely source for formation of the radioactive polysaccharide natural products, which are present in the unextractable residues. The same mechanism is likely operative for formation of radioactive polysaccharide natural products observed in abamectin-treated plants since radiolabeled glucose was found after acid hydrolysis of unextractable residues from celery (Feely and Wislocki, 1991). Since in plants the aromatic amino acids and *p*-coumaric acid are derived biosynthetically from glucose via the shikamate pathway (Goodwin and Mercer, 1983), it is possible that proteins and lignin present in [^{14}C]avermectin-treated plants may have incorporated radioactivity from the observed radioactive sugars by this mechanism.

The percent radioactive fructose in the plant sugar fraction from lettuce was approximately equal to that of glucose (Table 3), although the fructose content of lettuce has been reported as approximately 1.8-fold that of glucose (Ockerman, 1978). For cabbage, the percent radioactive glucose in the plant sugar fraction was approximately twice that of fructose (Table 3), whereas previous results indicate that these sugars are approximately equal (Ockerman, 1978). The low levels or absence of radioactive sucrose relative to fructose and glucose in cabbage and lettuce (Figure 2; Table 3) is consistent with reported values for cabbage (Ockerman, 1978), but the sucrose content of lettuce has been reported as approximately one-third that of glucose (Ockerman, 1978), which is not readily apparent in the present study (Figure 2A). However, the results for the relative amounts of radioactive fructose, glucose, and sucrose in these species as well as the glucose content determined (Table 2) in the present study agree reasonably well with the reported values for these sugars (Ockerman, 1978). Furthermore, the differences observed with the previously reported values could be simply a result of natural variation or the inclusion of the wrapper leaf with the cabbage and lettuce heads in the present study. In contrast to lettuce and cabbage, in corn fodder the amount of radioactive fructose was much higher relative to radioactive glucose (Table 3) but reported values for corn fodder were not found. The sucrose content of sweet corn kernels has been reported as approximately 10-fold that of fructose or glucose (Ockerman, 1978), whereas in the present study the percentages of radioactivity for these three sugars in the plant sugar fraction were approximately equal (Table 3). However, others have reported lower ratios of sucrose to fructose and glucose as a result of strain differences or postharvest storage conditions (Garwood et al., 1976).

The mechanism of incorporation of radioactivity from avermectins applied to plants into soluble sugars is unknown. Since the [^{14}C]MAB1a benzoate used in the present study (Figure 1) is labeled at carbons 3, 7, 11, 13, and 23, as was the abamectin used in previous studies (Feely and Wislocki, 1991), it is apparent that any of a number of possible macrocycle fragments could be the source for the observed radioincorporation. The observation that some tritium incorporation may have occurred in fructose, glucose, and galactose from lettuce and cabbage (Table 3; Figure 2) suggests that fragments from the hexohydrofuran portion of the macrocycle (carbons 2−8, Figure 1) were utilized. The carbon-14 to tritium ratio for these sugars was about 10−30 (Table 3) versus approximately 1:1 in the MAB1a benzoate applied once in the last application prior to harvest. Therefore, fragments from other portions of the macrocycle may be predominantly incorporated or the tritium label could be mostly lost prior to incorporation. Interestingly, the unknown RSP1 residue from lettuce and cabbage and the RSP3 residue from cabbage have a carbon-14 to tritium ratio of about 5−8 (Table 3). As these isotope ratios are significantly different from those of all identified sugars, it is possible that RSP1 and RSP3 are natural products other than sugars or are residues more closely related to MAB1a which have not been utilized in plant biosynthetic pathways. The unknown RSP4 was unique to sweet corn and was a major component of the plant sugar fraction of all tissues (Figures 2−4; Table 3). The presence of RSP4 in kernels and cobs, which were not directly exposed to the MAB1a benzoate application, as well as in fodder that was directly exposed suggests that RSP4 may be a natural product synthesized in foliage and subsequently transported to other parts of the plants. This is in keeping with corn foliage as the site of synthesis for sugars which are subsequently transported to sink tissues such as kernels and cobs. The observation that MAB1a and degradates of related structure are absent from sweet corn kernels and cobs further suggests that RSP4 is a natural product and not a residue resulting from MAB1a photolysis since transport of avermectin-like residues from their site of application and formation to kernels and cobs apparently did not occur. However, as RSP1 was observed in foliage from all plants (Figure 3) but not in kernels or cobs (Figures 3 and 4), it is possible that this residue is derived from MAB1a photodegradation without subsequent bioincorporation and thus remains at its site of formation.

The avermectin macrocyclic fragments that are incorporated into plant sugars may well result entirely or in large part from photolysis. Studies in progress have shown that polar residues which are apparent end products from extended photolysis of [^{14}C]MAB1a benzoate on glass, when applied to immature lettuce plants grown under fluorescent light, result in the formation of radioactive fructose and glucose (Wrzesinski and Crouch, unpublished results), as was observed after MAB1a treatment of lettuce in the present study. However, since structural determinations of residues in this subfraction of polar avermectin residues which apparently serve as precursors for sugar formation in lettuce have not been made, it is not known whether the required macrocycle fragmentation results only from photolysis or also from metabolism. It is hoped that future studies of the bioincorporation of subfractions of

polar emamectin photodegradates into plant sugars may lead to a general understanding of the mechanisms involved in the observed bioincorporation of avermectin residues into a wide range of natural products in crops treated with avermectins.

ABBREVIATIONS USED

FRU, fructose; GAL, galactose; GLU, glucose; HPLC, high-pressure liquid chromatography; LSC, liquid scintillation counting; MAB1a, 4″-deoxy-4″-*epi*-(methylamino)avermectin B_{1a}; MAB1b, 4″-deoxy-4″-*epi*-(methylamino)avermectin B_{1b}; RCA, radiocombustion analysis; SPE, solid phase extraction; SUC, sucrose; XYL, xylose.

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