Photodegradation of Emamectin Benzoate in Aqueous Solutions†

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The half-life of [^{14}C]4″-deoxy-4″-(*epi*-methylamino)avermectin B_{1a} (MAB1a) benzoate (1 ppm) photodegradation in buffer (pH 7), natural pond water, and sensitized buffer (1% acetone in pH 7 buffer) determined at Three Bridges, NJ (latitude ∼40° N) during the fall season under natural sunlight was 22, 7, and 1 days, respectively. The half-life of [^{14}C]MAB1a benzoate (10−12 ppm) photodegradation in buffer (pH 7) containing 1% (v/v) acetonitrile, ethanol, or acetone as cosolvent under continuous exposure with a xenon lamp was 64.5, 8.5, or 0.5 days, respectively. The photoisomer 8,9-Z-MAB1a, 8a-hydroxy-MAB1a, and unknown polar residues were found in light-exposed samples of MAB1a in buffer and natural pond water. In light-exposed sensitized buffer samples, 8a-oxo-MAB1a and MAB1a-10,11-14,15-diepoxide were additional products. Very polar residues found in the organic and aqueous phases after extraction increased with time, and their formation followed the order sensitized buffer ≫ natural pond water > buffer.

Keywords: *MK-0244; emamectin benzoate; photodegradation; residues*

INTRODUCTION

Avermectins are 16-member lactones and are widely used as drugs for the control of parasitic infections in animals and have shown great potential as insecticides for agricultural crops (Mrozik et al., 1989). Avermectin B_1 (B_{1a} and B_{1b}) or abamectin, a crop pesticide and anthelmintic, is a potent miticide (Burg et al., 1979; Campbell et al., 1984) but is less active against other crop pests (Putter et al., 1981). Emamectin, a second-generation product is synthesized from abamectin by substitution of an *epi*-methylamino (−$NHCH_3$) group for a hydroxyl (−OH) group at the 4″-position on the disaccharide and is produced as a benzoate salt (Figure 1). Emamectin benzoate is active against a broad range of lepidopterous larvae and is being developed for use on cole crops and vegetables (Dybas et al., 1989; Lasota and Dybas, 1991). Like abamectin, emamectin benzoate (MK-0244) is composed of a mixture of two homologous compounds, a major (≥90%) constituent 4″-deoxy-4″-(*epi*-methylamino)avermectin B_{1a} (MAB1a) benzoate and a minor (≤10%) constituent 4″-deoxy-4″-(*epi*-methylamino)avermectin B_{1b} (MAB1b) benzoate. MAB1a differs from MAB1b only by the presence of an additional methylene unit on the side chain at C-25 (Figure 1). This is a minor difference in a compound of molecular weight of 886 (MAB1a). Emamectin benzoate photodegraded to many identified products on plants and as thin film on glass, but no photodegradation occurred at the C-25 side chain of emamectin benzoate (Crouch et al., 1991; Crouch and Feely, 1995; Feely et al., 1992). Moreover, it was demonstrated that both homologues were photolyzed in a similar fashion under simulated sunlight (Wrzesinski, unpublished results). It is therefore reasonable to assume that studies of MAB1a benzoate photodegradation will adequately describe the photodegradation of emamectin benzoate.

Emamectin benzoate may be introduced into aquatic environments from agricultural usage where it could hydrolyze and/or undergo photolytic transformation by sunlight. MAB1a benzoate was found to be stable at 25 °C in buffer solutions of pH 5−8 kept in dark for 6 weeks (unpublished results). This study was conducted to investigate photodegradation of MAB1a benzoate under natural sunlight or artificial light in buffer, sensitized buffer, and natural pond water. The half-lives of MAB1a benzoate photodegradation and identification of major photodegradates in buffer, sensitized buffer, and natural pond water are reported.

MATERIALS AND METHODS

Solvents and Reagents. All organic solvents and reagents were of analytical grade purity. Insta-Gel XF and Ultima Gold scintillation cocktail for the determination of radioactivity present in the solutions were obtained from Packard Instrument Company, Downers Grove, IL.

Equipment. The reagent bottles and the quartz tubes (13 mm × 65−67 mm, Ace Glass Inc., Vineland, NJ) were steam sterilized using an autoclave (All American Pressure Steam Sterilizer, model 25X). Spectral irradiance from 280 to 800 nm was measured daily once per hour with a spectroradiometer (Optronics Laboratories, model OL-752). Radioactivity in aqueous samples, extracts, and HPLC eluates was determined by liquid scintillation counting (LSC) on a model 4530, 460, 460M, or 2500TR counter from Packard Instrument Company, Inc. (Downers Grove, IL). A Perkin-Elmer (UV−visible) spectrophotometer (model 320) was used to plot UV−visible absorption spectra of emamectin benzoate solutions and to determine the concentration of test compound in solutions. The pH of the aqueous solutions was measured using a

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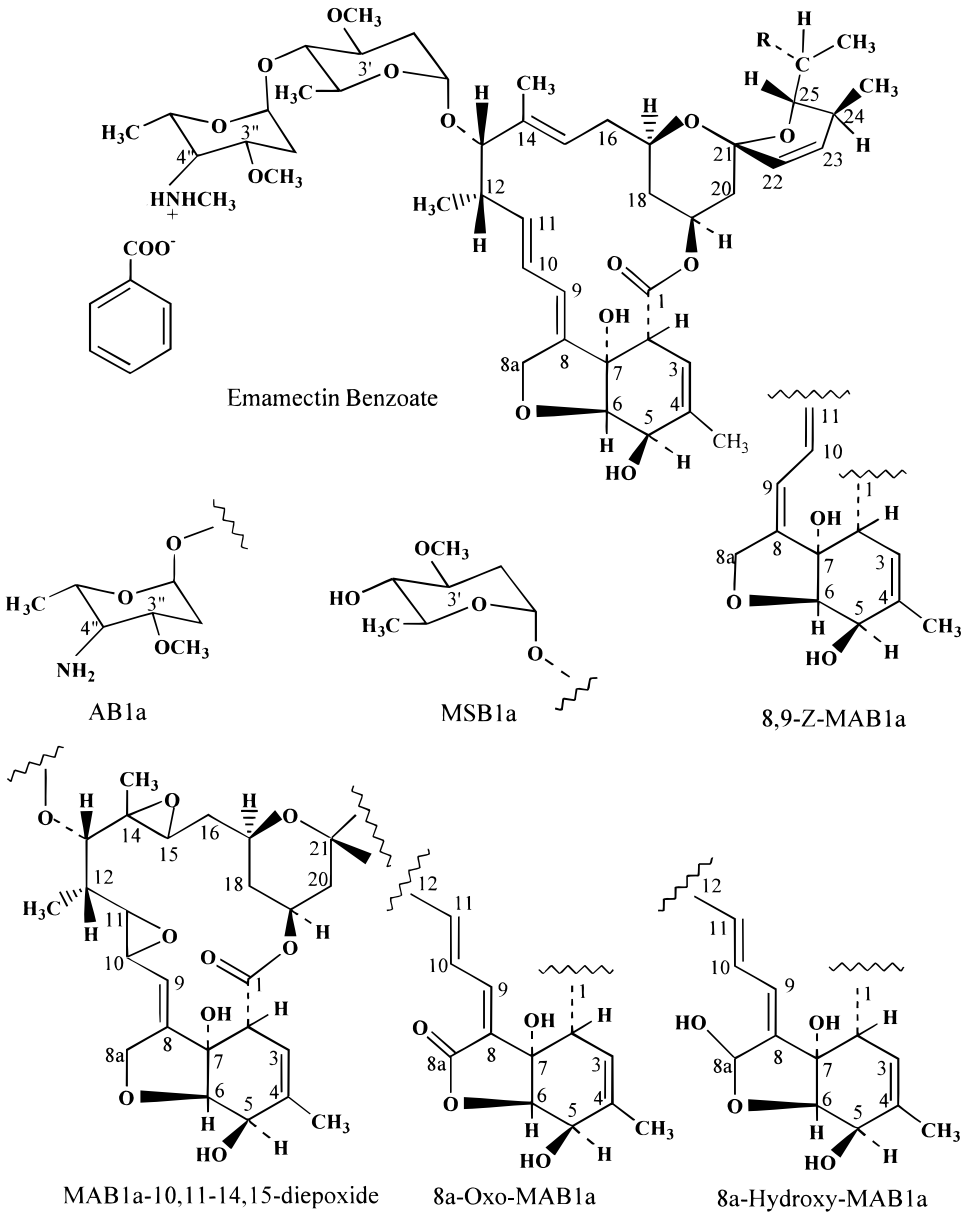

Figure 1. Structure of emamectin benzoate and reference standards. Emamectin benzoate (MK-0244): MAB1a benzoate (≥90%), R = CH_2CH_3; and MAB1b benzoate (≤10%), R = CH_3. [3-, 7-, 11-, 13-, or 23-^{14}C]MAB1a benzoate was added to the aqueous solutions. Emamectin benzoate, 4″-deoxy-4″-(*epi*-amino)avermectin B_{1a} (AB1a), avermectin B_{1a}-monosaccharide (MSB1a), 8,9-Z-MAB1a, 8a-oxo-MAB1a, 8a-hydroxy-MAB1a, and [5-^{3}H]MAB1a-10,11-14,15-diepoxide were used as reference standards.

Beckman (model 43) or Orion (model 420A) pH meter. For HPLC analysis, a Spectra Physics SP-8700 or a SP-4000 solvent delivery system, a Spectra Physics SP-UV2000 detector or a Hewlett-Packard HP-1040A diode array detector, a Rheodyne 7125 injector, and a Pharmacia Frac-100 collector were used. HPLC methods and columns as outlined in Table 1 were used. The eluate was monitored at 215, 245, and/or 280 nm. Appropriate guard columns were used to protect the analytical and preparative columns.

Chemicals. The test chemical [^{14}C]MAB1a benzoate (18−28 μCi/mg) was prepared by the Labeled Compound Synthesis Group, Department of Drug Metabolism, Merck Research Laboratories (Rahway, NJ). The ^{14}C-label was incorporated at the 3-, 7-, 11-, 13-, or 23-position (Figure 1). Unlabeled analytical standards (Figure 1) emamectin benzoate, 4″-deoxy-4″-(*epi*-amino)avermectin B_1 (AB1), avermectin B_{1a}-monosaccharide (MSB1a), 8a-hydroxy-MAB1a, and 8a-oxo-MAB1a were obtained from Chem Data, Merck Research Laboratories (Rahway, NJ). Also, MAB1a and AB1a were isolated by RP−HPLC from emamectin benzoate and AB1 standards, respectively, for use as analytical standards. A [^{3}H]MAB1a-10,11-14,15-diepoxide standard was generated after photodegradation of [5-^{3}H]MAB1a benzoate as described below.

Generation of MAB1a-10,11-14,15-Diepoxide Standard. The UV absorption spectrum of MAB1a (λ_{max} 245 nm) is due to the conjugated 8,9- and 10,11-diene (Figure 1). For MAB1a-10,11-14,15-diepoxide, the diene is absent due to epoxidation at the 10,11-position (Figure 1), and hence the compound has no UV absorption. The compound was therefore generated from [^{3}H]MAB1a benzoate and detected by the presence of tritium. A solution of [^{3}H]MAB1a benzoate (66 ppm), in 0.1 M phosphate buffer (pH 7) containing 1% (v/v) acetone was prepared and divided into 45 quartz tubes (2 mL each). One tube served as the zero time sample, and the remaining 44 quartz tubes were exposed to UV-A340 fluorescent lamps (Q-Panel, Cleveland, OH) for 2 h. All light-exposed samples were pooled and then eluted through a series of water-conditioned Bond-Elut C18 cartridge columns (AnalyticalChem International, Harbor City, CA). Each cartridge column was then eluted twice with 2 mL of water. The aqueous eluant thus collected contained polar photodegradation products that were discarded. The cartridge columns were finally eluted with

Table 1. HPLC Methods[a]

HPLC method	column	eluant A	eluant B	gradient time (min)	% B	flow rate (mL/min)
RP−1	A	H_2O/AA	MeOH/AA	0	82	1
				45	82	
				55	100	
RP−2	A	H_2O/AA	MeOH/AA	0	82	1
				35	82	
				40	100	
RP−3	A	H_2O/AA	MeOH/AA	0	82	1
				35	82	
				45	100	
RP−4	A	H_2O/AA	MeOH/AA	0	0	1
				50	100	
RP−5	B	H_2O/AA	MeOH/AA	0	80	1
				30	80	
				40	100	
RP−6	C	H_2O/AA	MeOH/AA	isocratic	85	3
RP−7	C	H_2O/AA	MeOH/AA	0	80	3
				45	90	
				50	100	
RP−8	A	H_2O/AA	MeOH/AA	0	45	1
				10	45	
				180	100	
RP−9	A	H_2O/AA	MeOH/AA	0	75	1
				35	82	
				45	100	
NP−1	D	Isooct/TEA	EtOH/TEA	isocratic	15	2
NP−2	E	Isooct/TEA	EtOH/TEA	0	10	1
				30	10	
				45	22.5	

[a] 5 mM ammonium acetate (AA) was present in both methanol (MeOH) and water (H_2O) eluants. Similarly 0.4 mM triethylamine (TEA) was present in both ethanol (EtOH) and isooctane (Isooct) eluants. The column was washed for another 10−20 min with the highest concentration of B eluant. Columns A, C, and D were obtained from Analytical Sales and Services, Inc., Mahwah, NJ, and columns B and E were purchased from EM separations, Gibbstown, NJ. A: Axxiom AxxiChrom ODS or Advantage Plus ODS column (4.6 mm × 25 cm, particle size 5 μm). B: LiChrosorb RP-18 column (4.6 mm × 25 cm, particle size 5 μm). C: Semiprep Advantage Plus ODS column (10 mm × 25 cm, particle size 5 μm). D: Axxiom AxxiChrom silica column (4.6 mm × 25 cm, particle size 5 μm). E: LiChrospher Diol column (4 mm × 25 cm, particle size 5 μm).

methanol containing 5 mM ammonium acetate to recover remaining MAB1a with other photodegradation products. The methanol eluates were combined and concentrated by Buchi rotovapor, dried under nitrogen at ~30 °C, and reconstituted in methanol. MAB1a-10,11-14,15-diepoxide was first isolated (~9% of column radioactivity, 38−42 min fractions) from the mixture of residues by repetitive RP-HPLC (method RP-1, Table 1). The isolated product was further purified (~85% of column radioactivity, 43−48 min fractions) using the NP-HPLC (method NP-2, Table 1). The purity of [^{3}H]MAB1a-10, 11-14,15-diepoxide thus generated was determined by the RP-HPLC (method RP-3, Table 1). About 99% of the column radioactivity (17−20 min fractions) was detected under a single radioactive peak. The compound was identified by mass and NMR spectral analyses as described below.

MS and NMR Spectrometric Analyses. Characterization of emamectin benzoate and [^{3}H]MAB1a-10,11-14,15-diepoxide was performed on a Sciex (Thornhill, Ontario, Canada) model API III Plus triple-quadrupole mass spectrometer equipped with a Sciex ionspray source. The nebulizing gas (air) pressure was 40 psi, and the ion spray needle was subjected to a potential of 5 kV. The positive ions generated in the spray were sampled into the mass spectrometer via a 0.0045-in. pinhole aperture. The orifice potential and electron multiplier settings were 65 V and −4.2 kV, respectively. Argon gas was used for collisionally induced dissociation experiments. The NMR spectra of emamectin benzoate and [^{3}H]MAB1a-10, 11-14,15-diepoxide was determined in deuterated chloroform using a Varian Unity 400 MHz spectrometer with the ambient probe temperature regulated at 25 °C. Chemical shift is expressed in ppm scale relative to solvent peak set at 7.26 ppm.

Table 2. Analysis of Natural Water[a] Collected from a Pond

calcium	15.6 mg/L
magnesium	7.8 mg/L
sodium	15.4 mg/L
total organic carbon[b]	45 mg/L
pH	7.53
total hardness[c]	76 mg/L
specific conductance[d]	267 $\mu\Omega$/cm

[a] The natural water was obtained from a pond at the Three Bridges, NJ, facility of Merck Research Laboratories. [b] Total organic carbon (TOC) determined after purging inorganic carbon with nitrogen. [c] Analysis was performed on an unpreserved sample. [d] This sample was field filtered for dissolved metals.

Photolysis under Artificial Light. A phosphate buffer (0.01 M, pH 7) solution was prepared and sterilized by passing through a 0.2 μm Millipore filter. Subsequently, [^{14}C]MAB1a benzoate (10−30 ppm) solutions were prepared in the sterilized buffer containing 1% (v/v) acetonitrile or ethanol as a cosolvent or acetone as a photosensitizer. The solubility of emamectin benzoate in water without any cosolvent is 24 ppm (K. Anderson, unpublished results). Photolysis was conducted in a sterile flow-through 40-mL containers and in sealed glass ampules. For the flow-through system, each reaction vessel was connected to a set of three traps in series (0.1 N sulfuric acid, ethylene glycol, and 2 N sodium hydroxide) for collection of volatile components. A continuous air flow through the system was maintained during the course of the experiment. The reaction vessels were submerged in water in a coolant-jacketed Pyrex vessel connected to a refrigerated circulator. Irradiation was performed with a xenon arc lamp (Hanau Suntest Unit) equipped with filters to remove wavelengths below 290 nm. The reaction vessels were positioned 235 mm from the xenon arc lamp. The samples were irradiated continuously without light/dark cycles. The temperature of the samples was monitored at each sampling interval and periodically between sampling intervals. Dark control reactions were carried out simultaneously in a separate, constant temperature room maintained at 25 ± 1 °C and under flow-through conditions as for the light-exposed samples by wrapping the reaction vessel in aluminum foil. Sealed glass ampules were used later because results of the open system showed that very little (<2%) ^{14}C-labeled volatiles and/or [^{14}C]-carbon dioxide was produced. Each ampule was filled to ~2/3 volume. The sealed samples were submerged and irradiated as described above for the open flow-through system. Dark reactions were carried out in a constant temperature chamber maintained at 25 ± 1 °C.

Photolysis under Natural Sunlight. The natural water was obtained from a pond at the Three Bridges Farm facility of Merck Research Laboratories and was analyzed for pH, hardness, conductivity, calcium, magnesium, sodium, and organic carbon (Table 2) by Lancaster Laboratories, Inc. (Lancaster, PA). The buffer (0.1 M phosphate buffer, pH 7), sensitized buffer [0.1 M phosphate buffer, pH 7 containing 1% (v/v) acetone], and natural pond water were filter sterilized using Nalgene, cellulose nitrate filters (2 μm). [^{14}C]MAB1a benzoate in 0.3 mL of acetonitrile was added to 99.7 mL of each buffer, sensitized buffer, or natural pond water. Thus, three aqueous solutions of [^{14}C]MAB1a benzoate (1 ppm) were prepared, and 0.3% acetonitrile (v/v) was present in each aqueous solution as a cosolvent. It was determined that [^{14}C]-MAB1a benzoate was homogeneously distributed in each aqueous solution by determining the radioactivity in 1-mL aliquots withdrawn at three depths of the solutions. Photodegradation of [^{14}C]MAB1a benzoate in either buffer, natural pond water, or sensitized buffer aqueous solution was conducted with three replicate light-exposed tubes and one dark control (wrapped with aluminum foil) for each time interval. The light and dark quartz tubes containing 2 mL of the test solution were capped, sealed by the parafilm, and placed on the water surface at ~30° angle in a water-filled chamber connected to a temperature-controlled circulator. The temperature of the water-filled chamber was maintained at 25 ±

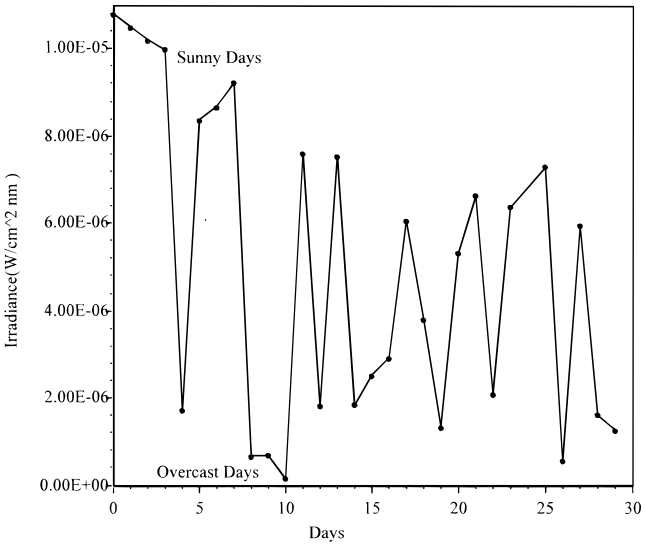

Figure 2. Spectral irradiance of sunlight at 450 nm during exposure period. Spectral irradiance, once per hour during daylight was measured daily at the Three Bridges, NJ, facility of Merck Research Laboratories (latitude ~40 °N). Irradiance values at 450 nm for each day were averaged. As indicated, sunny and overcast days occurred during 30-day exposure period.

1 °C throughout the experiment except for a few occasions when large changes occurred between the day and night outdoor temperatures. All the sample tubes were exposed to natural sunlight (~10 h of light per day) at Three Bridges, NJ (latitude ~40° N) in the fall season. Spectral irradiance from 280 to 800 nm was measured daily once per hour during daylight by a spectroradiometer. The average light intensity varied significantly with weather conditions. The maximum light intensity was observed around 1 p.m. EST on a sunny day, but very little change occurred during the daylight hours of an overcast day. On sunny days there was a 3−5-fold higher light intensity at 450 nm wavelengths in comparison to overcast days (Figure 2). The light-exposed and dark control tubes were removed at selected intervals from day 1 to day 30, and the pH in one of the triplicate light-exposed samples was determined. The pH in buffer and sensitized buffer samples did not change during the experimental period. The pH in natural pond water samples slowly dropped from 8.9 on day 0 to 7.6 on day 30. Triplicate aliquots (0.1 mL) were withdrawn from each dark control or light-exposed specimen for the determination of radioactivity. The remaining aqueous solution was extracted twice with ethyl acetate. The radioactivity in the resulting organic and aqueous phases was determined by LSC, and percent recovery of the sample radioactivity was calculated.

Photodegradation Half-Lives. The organic extracts and organic extracted aqueous-phase samples were spiked with emamectin benzoate and then analyzed by RP-HPLC (method RP-2, 3 or 4, Table 1). Parent MAB1a was only detected in the organic extracts (Figure 3). On the basis of the percent distribution of radioactivity in the organic extracts and percent column radioactivity for MAB1a in the light exposed samples, the total amount of MAB1a remaining in a sample after sunlight exposure was calculated. The samples exposed to artificial light were directly analyzed by RP-HPLC (method RP-7, Table 1) for the quantitation of MAB1a. The amount (μg) of MAB1a (average of three replicates) versus light exposure days was plotted (Figure 4). The half-lives ($t_{1/2}$) via linear regression were calculated assuming first-order kinetics according to the following equations:

$$y = mx + C \quad (1)$$

(where m is the slope from $\log_n$ MAB1a concentration versus time)

$$t_{1/2} = \log_n(2)/\text{slope} \quad (2)$$

Photodegradation Rate of Emamectin Benzoate in Three Seasons. The photodegradation half-life of MAB1a benzoate during fall was extrapolated to summer and winter. Two emamectin benzoate solutions (19.3×10^{-3} and 21.1×10^{-3} M) in methanol were prepared, and the absorbance of each solution at 2.5 nm intervals from 297.5 to 800 nm was determined. The absorbance data were then converted to molar absorbance ($\epsilon\lambda$). The solar irradiance ($L\lambda$) data at the corresponding wavelengths for summer, fall, and winter seasons was from the U.S. EPA (1985). The sum of the products ($\sum\epsilon\lambda \cdot L\lambda$) of the molar absorbance ($\epsilon\lambda$) and the solar irradiance ($L\lambda$) at each wavelength for summer, fall, and winter were calculated for both emamectin benzoate solutions. The average $\sum\epsilon\lambda \cdot L\lambda$ of the two solutions for the summer, fall, and winter were 30.6, 15.8, and 10.0 (1.9:1.0:0.6), respectively. The half-lives of MAB1a benzoate in the summer and winter were then estimated by using the comparative rates of photodegradation in summer, fall, and winter seasons and the half-lives of MAB1a benzoate determined in the fall season.

Identification of Photodegradation Products. The organic extracts from buffer (day 3, 7, and 21), natural pond water (day 1, 3, and 30), or sensitized buffer (day 2, 5, or 7) replicate samples exposed to sunlight were pooled separately. One or more unlabeled reference standards including MAB1a or MK-0244, 8,9-Z-MAB1a, AB1a or AB1, 8a-hydroxy-MAB1a, and/or [^{3}H]MAB1a-10,11-14,15-diepoxide were added to the pooled organic extracts. RP-HPLC analyses (method RP-3, Table 1) were then conducted. The HPLC fractions corresponding to each reference standard were collected and then divided in two for separate RP- and NP-HPLC analyses. The divided fractions were then dried under nitrogen and reconstituted either in methanol for RP-HPLC (method RP-5, Table 1) or in ethanol for NP-HPLC (method NP-1, Table 1). The identity of a photodegradate was thus confirmed by cochromatography of the eluted radioactivity with reference standard by two HPLC methods of different selectivity.

A sterilized solution of [^{14}C]MAB1a benzoate (31 ppm) in 75 mL of sensitized buffer was photolyzed under a xenon arc lamp for 24 h for identification and quantitation of photodegradates by serial RP- and NP-HPLC analyses. The organic extract of the photolyzed sample was first fractionated by a semipreparative RP-HPLC column (method RP-6, Table 1). Based on the retention times of external standards (Figure 8), seven RP-HPLC fractions (P1, P2, P3a, P3b, P3c, MAB1a, and P4 residues) from three separate RP-HPLC runs were isolated, pooled, dried with ethanol under vacuum, and then reconstituted in the starting eluent for HPLC method NP-2 (Table 1). Immediately before the analysis of a residue sample, appropriate reference standards were analyzed. The identification of the photodegradates (except for P1) was made by comparing the retention times of radioactive peaks with those of the external standards and by the diode-array spectra. The very polar P1 fraction (Figure 8) was reconstituted in the starting eluent for RP-8 HPLC method (Table 1) and then analyzed by that method.

RESULTS

Photodegradation under Artificial Light. Since MAB1a was stable in aqueous solutions between pH 5 and pH 8 for at least 6 weeks (unpublished results), the photodegradation of MAB1a benzoate in aqueous solutions was studied at pH 7. Aqueous solutions of [^{14}C]-MAB1a benzoate at 5 and 10 ppm concentrations with ethanol as a cosolvent were exposed to artificial light (simulated sunlight) using a flow-through system. [^{14}C]-MAB1a was essentially stable under dark conditions. MAB1a benzoate at both 5 and 10 ppm concentrations was degraded according to first-order kinetics yielding a half-life of 150 h (6.3 days) and 203 h (8.5 days), respectively (Table 3). The volatile components detected

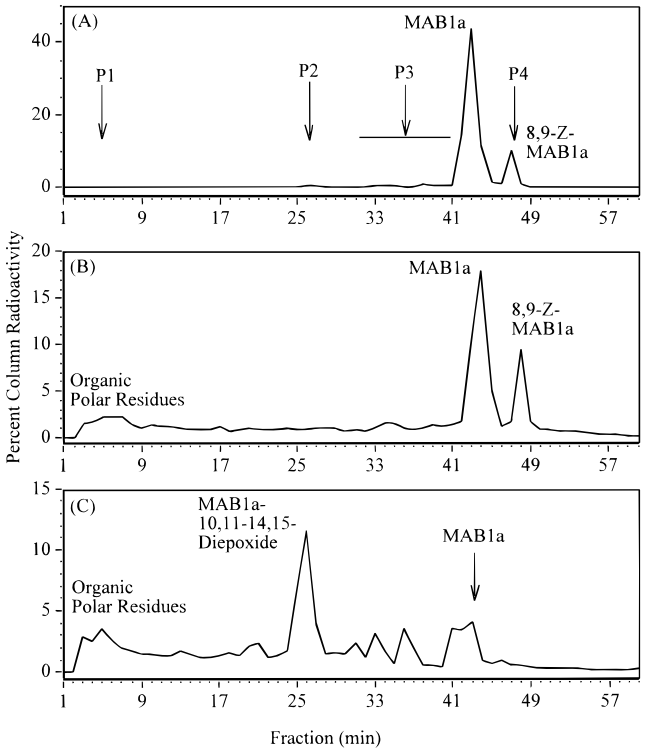

Figure 3. RP-HPLC analyses of organic extracts from day 7 photolyzed [^{14}C]MAB1a benzoate in buffer (A), natural pond water (B), and sensitized buffer (C). The organic extracts of the photolyzed day 7 aqueous samples under natural sunlight were analyzed by RP-HPLC (method RP-1, Table 1). Characterized photodegradates in four HPLC fractions were P1 (organic polar residues), P2 (MAB1a-10,11-14,15-diepoxide), P3 (8a-hydroxy-MAB1a), and P4 (8,9-Z-MAB1a).

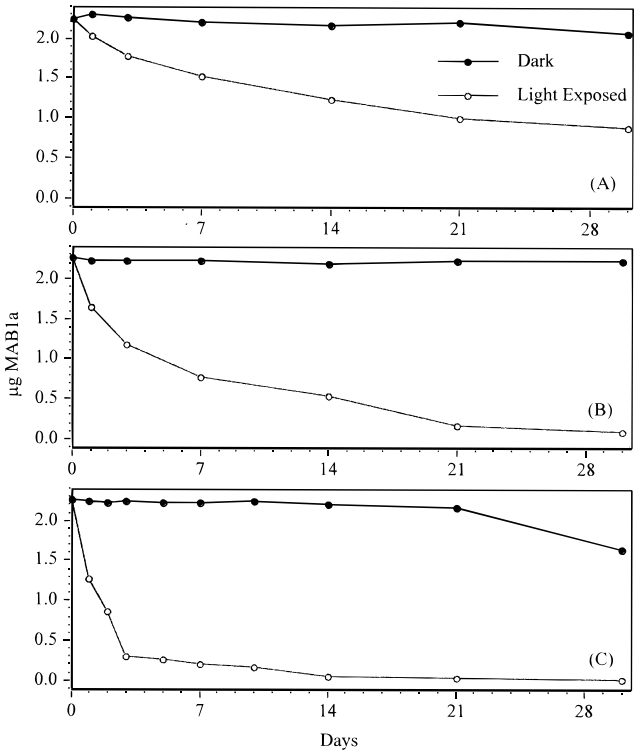

Figure 4. [^{14}C]MAB1a photodegradation with time in buffer (A), natural pond water (B), and sensitized buffer (C). Sterile aqueous solutions (2 mL) of [^{14}C]MAB1a benzoate (1 ppm) in quartz tubes were exposed to natural sunlight at 25 °C. Aluminum foil-wrapped quartz tubes served as dark controls.

were <2% of the applied radioactivity. In the second experiment using sealed glass ampules, aqueous solutions of [^{14}C]MAB1a benzoate at 10 and 30 ppm concentrations and acetonitrile as a cosolvent, the rate of photodegradation was slower with a half-life of 1549 h (64.5 days) and 764 h (31.8 days), respectively. As expected, the photodegradation of MAB1a benzoate at 12 and 32 ppm was rapid in the presence of 1% acetone (v/v) with photodegradation half-lives of 13 h (0.5 day) and 23 h (1.0 day), respectively (Table 3).

Photodegradation under Natural Sunlight. On the basis of the above results with artificial light (Table 3), a photodegradation study was conducted under natural sunlight with [^{14}C]MAB1a benzoate (1 ppm) in buffer, sensitized buffer, or natural pond water. The intensity of the sunlight was monitored throughout the experiment, and as shown in Figure 2, both sunny and overcast days occurred during the 30-day experimental period. Since very little quantities (<2%) of ^{14}C-labeled volatiles and/or [^{14}C]carbon dioxide were detected in the open flow-through system under artificial light, no attempts were made to determine volatiles in this study. The radioactivity in each dark control and light-exposed aqueous sample was determined by directly removing aliquots of the solutions. The recoveries of the applied radioactivity were consistently ~65% by this procedure. However, the recoveries were improved to >90% when the remaining solution in quartz tubes was extracted with ethyl acetate. The above results indicated that some of the parent and/or its photodegradates were adsorbed to the quartz tube surface. The radiocarbon for the parent and photodegradates was therefore quantitated based on the sample volume used for extraction, the percent radioactivity in aqueous and organic phases, and RP-HPLC analyses of the organic extracts. Most (99%) of the applied radioactivity of dark controls was organic extractable. For sunlight-exposed aqueous solutions, the radioactivity in the organic phase (organic extractable residues) decreased with a concurrent increase in the aqueous phase (organic unextractable residues) radioactivity with increasing exposure time. The organic unextractable residues in day 30 sunlight-exposed buffer, natural pond water, and sensitized buffer were 16%, 47% and 57%, respectively. RP-HPLC (method RP-2 or RP-3, Table 1) indicated that MAB1a was not present in these organic unextractable residues. Further RP-HPLC analyses (method RP-9, Table 1) indicated that the organic unextractable residues were a mixture of complex polar residues (chromatograms not shown) and are designated hereafter as aqueous polar residues. RP-HPLC analyses of all dark controls except for day 30 sensitized buffer indicated that MAB1a was essentially stable in the aqueous solutions. However, for day 30 sensitized buffer dark control sample, about 69% of the column radioactivity was associated with the UV peak of MAB1a, and 10% radioactivity was eluted at the retention time of the 8,9-Z-MAB1a. Probably due to an oversight in the later phase of the experiment, this dark sample was exposed to some sunlight and photodegraded. RP-HPLC analysis of the organic extracts from light-exposed specimens indicated that the amount of MAB1a decreased with sunlight exposure time (Figure 4) and that degradation products were formed (Figures 3 and 5). Also, radioactivity in the RP-HPLC polar fractions (hereafter designated as organic polar residues) increased with sunlight exposure time. Aqueous photodegradation of MAB1a was very extensive in sensitized buffer samples

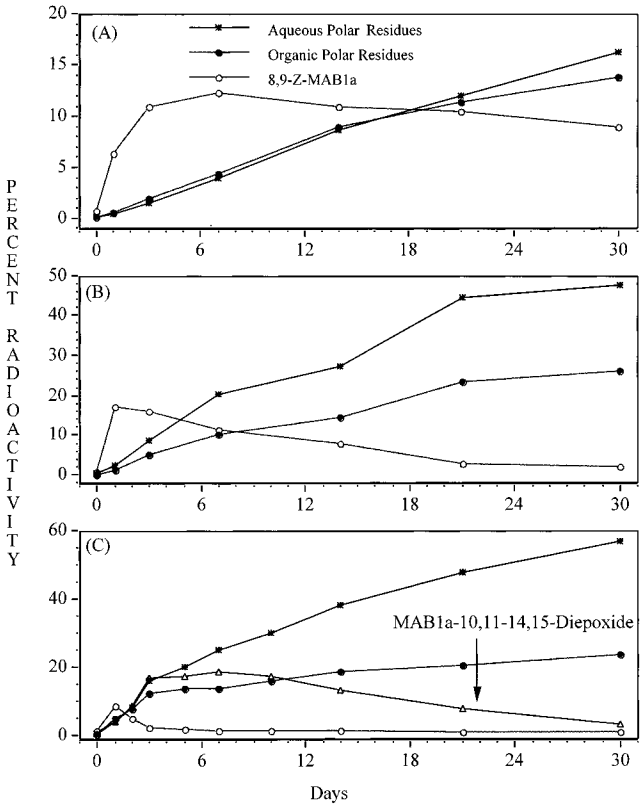

Figure 5. Formation of photodegradates with time under natural sunlight. Major photodegradation products formed with time in buffer (A), natural pond water (B), and sensitized buffer (C) are shown. MAB1a-10,11-14,15-diepoxide was detected only in the sensitized buffer (C).

when compared with the respective natural pond water or buffer samples (Figures 3 and 5). All of the MAB1a and most of its degradation products were further photodegraded to more polar residues (aqueous and organic) by day 30 in light-exposed sensitized buffer samples (Figures 3−5). The amount of MAB1a remaining in light-exposed samples and dark controls was calculated, and then MAB1a quantity (μg) versus light exposure time was plotted (Figure 4). The slopes of the best fit lines of $\log_n$ of MAB1a quantity versus time (0−30 days) for nonsensitized samples were used for the calculations of the half-lives. Since aqueous photodegradation of MAB1a in sensitized buffer was very extensive, $\log_n$ of MAB1a quantity versus time up to 2 days was used. The first-order half-lives of [^{14}C]MAB1a benzoate in buffer, natural pond water, and sensitized buffer under natural sunlight were 22.4, 6.9, and 1.4 days, respectively (Table 3). On the basis of the calculated relative photodegradation rate for summer, winter, and fall (1.9:0.6:1.0), the extrapolated half-lives of aqueous photodegradation of [^{14}C]MAB1a benzoate in buffer, natural pond water, and sensitized buffer were 11.5, 3.6, and 0.7 days in summer and 35.4, 10.9, and 2.2 days in winter, respectively (Table 3).

Photodegradation Products under Natural Sunlight. A primary photodegradate, the 8,9-Z-photoisomer of MAB1a, was accounted for up to 17% of the total radioactivity in the light-exposed buffer and natural pond water samples and was the major degradate at early time points (Figures 3 and 5). In the light-exposed sensitized buffer samples, about 1−8% of 8,9-Z-MAB1a was also present at days 1−3 but reduced to about 1% at later time intervals. Also, a unique photodegradate, MAB1a-10,11-14,15-diepoxide (4−18%), was observed at

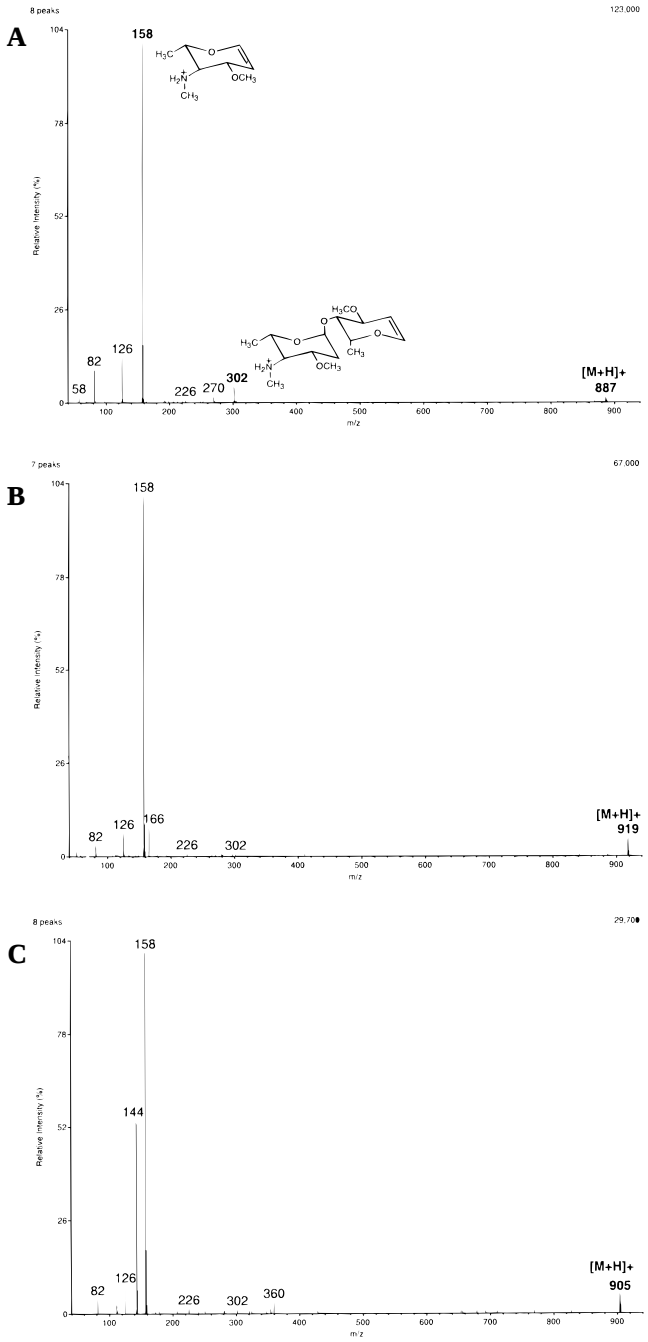

Figure 6. Mass spectra of MAB1a (A), MAB1a-10,11-14,15-diepoxide (B), and MAB1a-8,9-epoxide (C).

day 1 and later time intervals, but its presence in buffer and natural pond water was not confirmed due to low radioactivity in HPLC fractions associated with this photodegradate (Figures 3 and 5). Moreover, 8a-hydroxy-MAB1a and several photodegradates of unknown identity were the minor products in buffer, natural pond water, and sensitized buffer light-exposed samples. The aqueous and organic polar residues steadily increased (sensitized buffer ≫ natural pond water > buffer) with time accounting for up to 37% and 57% of the total radioactivity, respectively. These aqueous and organic polar residues appeared to be the end products in the day 30 light-exposed sensitized buffer samples (Figure 5).

The photodegradates 8,9-Z-MAB1a, 8a-hydroxy-MAB1a, and MAB1a-10,11-14,15-diepoxide were iso-

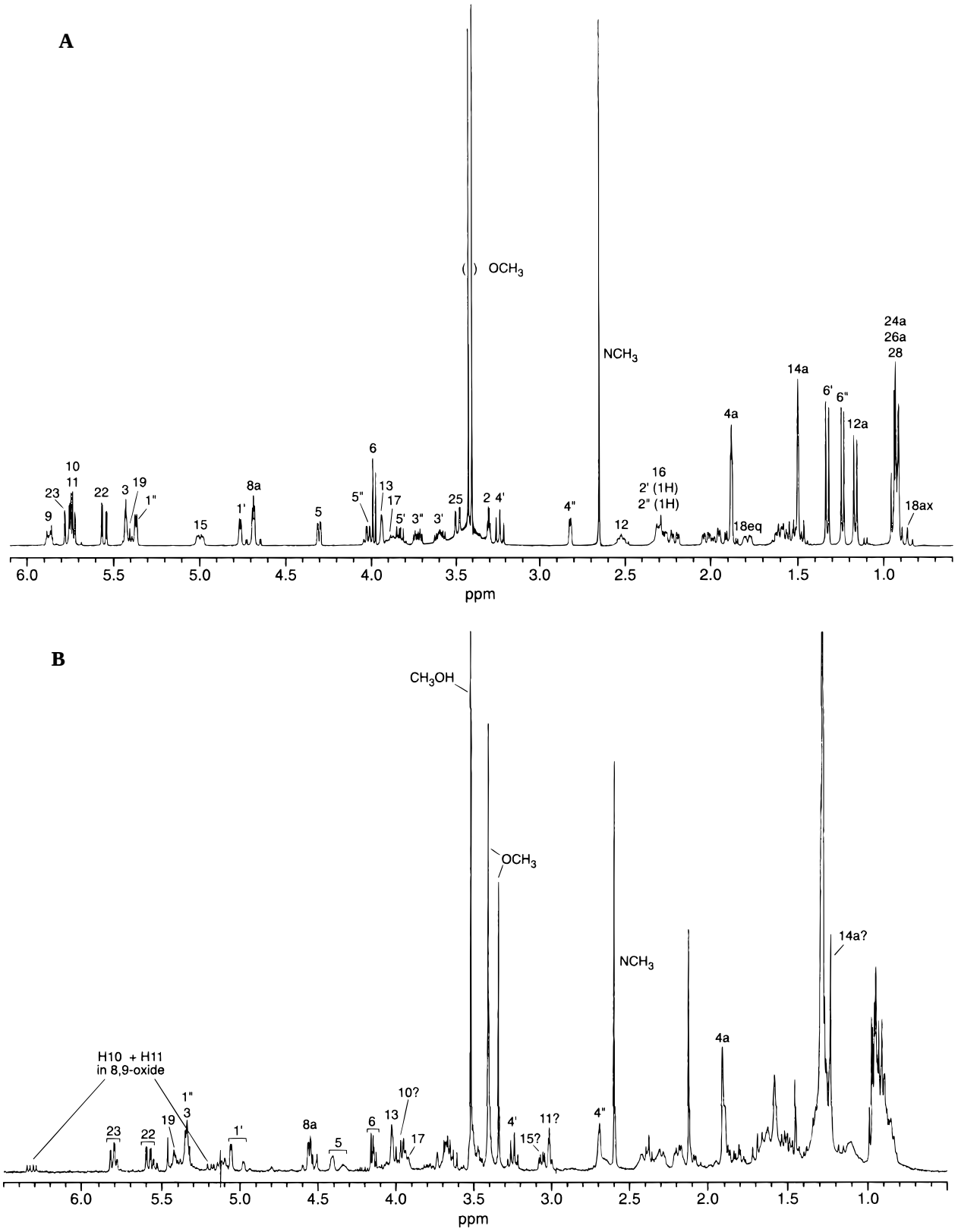

Figure 7. NMR spectra of MAB1a (A) and MAB1a-10,11-14,15-diepoxide/MAB1a-8,9-epoxide (B).

lated by RP-HPLC (method RP-3, Table 1) from the pooled organic extracts, and their identities were confirmed by cochromatography with the reference standard (chromatograms not shown) under two different HPLC systems (methods RP-5 and NP-1, Table 1). The RP-HPLC analyses (method RP-8, Table 1) of the aqueous polar residues and of organic polar residues indicated that these were a mixture of many degradates, each in small quantity (chromatograms not shown).

Photodegradation Products under Artificial Light. Similar results as indicated above for natural sunlight (Figure 3) were obtained with artificial light using MAB1a benzoate aqueous solutions containing 1% (v/v) acetonitrile, ethanol, or acetone. RP-HPLC analy-

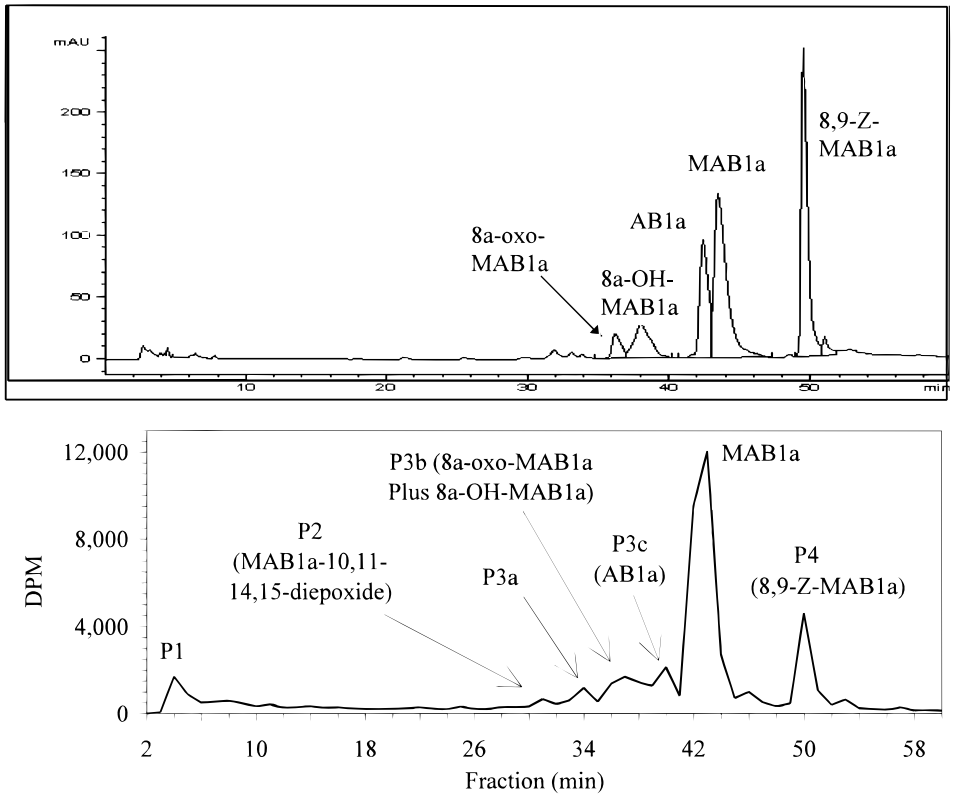

Figure 8. RP-HPLC analyses of the reference standards (top) and of the MAB1a photodegradates isolated from light exposed sensitized buffer (bottom). The reference standards (top) and organic extracts obtained from [^{14}C]MAB1a benzoate irradiated in sensitized buffer (bottom) were analyzed separately by RP-HPLC (method RP-7, Table 1). The diode array UV absorption spectra and retention times of the photodegradates were compared with those of the respective external standards. Based on the comparison, seven apparent residues as shown (bottom) were isolated for rechromatography (see Figure 9).

Table 3. Half-Lives of MAB1A Benzoate Photodegradation in Aqueous Solutions

MAB1a Benzoate concn	aqueous solution	cosolvent	half-life h	half-life days[a]
		Artificial Light[b]		
5 ppm	buffer	1% ethanol	150	6.3
10 ppm	buffer	1% ethanol	203	8.5
10 ppm	buffer	1% acetonitrile	1549	64.5
30 ppm	buffer	1% acetonitrile	764	31.8
12 ppm	sensitized buffer	1% acetone	13	0.5
32 ppm	sensitized buffer	1% acetone	23	1.0

MAB1a benzoate concn	aqueous solution	half-life (days) fall	winter[c]	summer[c]
	Natural Sunlight[b]			
1 ppm	buffer[d]	22.4	25.4	11.5
1 ppm	natural pond water[d]	6.9	10.9	3.6
1 ppm	sensitized buffer[d,e]	1.4	2.2	0.7

[a] The half-life values determined in hours were converted to days (1 day = 24 h). [b] Solutions of [^{14}C]MAB1a benzoate were continuously irradiated under artificial light (top part) or under natural sunlight for 30 calendar days (bottom part). [c] The half-lives for MAB1a benzoate photodegradation were determined in the fall season (~10 h daylight) and were extrapolated for winter and summer from fall half-life (U.S. EPA, 1985). [d] Acetonitrile (0.3%, v/v) as a cosolvent was present in buffer, sensitized buffer and natural pond water samples. [e] Acetone (1%, v/v) was also present in the sensitized buffer samples.

ses (method RP-4, Table 1) of the photolyzed aqueous solutions indicated four crude residues besides parent. Except for organic polar residues (P1 fraction), other residues corresponded to the following reference standards: P2 fraction, MAB1a-10,11-14,15-diepoxide; P3 fraction, 8a-oxo-MAB1a, 8a-hydroxy-MAB1a, and AB1a; P4 fraction, 8,9-Z-MAB1a (similar fractions as described in Figure 3). The organic polar residues (P1 fraction) and 8,9-Z-MAB1a (P4 fraction) were the major photodegradates detected in buffer samples containing acetonitrile or ethanol as a cosolvent. In sensitized buffer samples, all four HPLC fractions (P1−P4) were detected. The rate of MAB1a benzoate photodegradation (Table 3) was dependent on the cosolvent used (acetone ≫ ethanol > acetonitrile).

MS and NMR of [^{3}H]MAB1a-10,11-14,15-Diepoxide Standard. This compound was generated by photolysis for use as a reference standard and was identified by mass and NMR spectral analyses. Figure 6A indicates that the [M + H] ion for MAB1a appeared at *m/z* 887 as expected and a major fragment (*m/z* 158) derived from the terminal oleandrose (Figure 6). The mass spectrum of the [^{3}H]MAB1a-10,11-14,15-diepoxide standard revealed that it had two components, major and minor components with respective molecular weights [M + H] at *m/z* 919 (Figure 6B) and *m/z* 905 (Figure 6C), respectively. The mass increases of 32 (*m/z* 919) for the major component was consistent with the proposed diepoxide of MAB1a. The [M + H] of the minor component (Figure 6C) suggested hydration (+18 Da) or oxidation with a double bond reduction (+16 +2 Da). The mass spectra of two degradation products (Figure 6B,C) revealed the fragment at *m/z* = 158 as for MAB1a (Figure 6A), suggesting that the transformations had occurred on the aglycone portion in both cases. Further structural characterization of the [^{3}H]MAB1a-10,11-14,15-diepoxide standard was conducted by NMR. The NMR spectroscopic analysis also indicated that it was a mixture of two components (2:1), the major

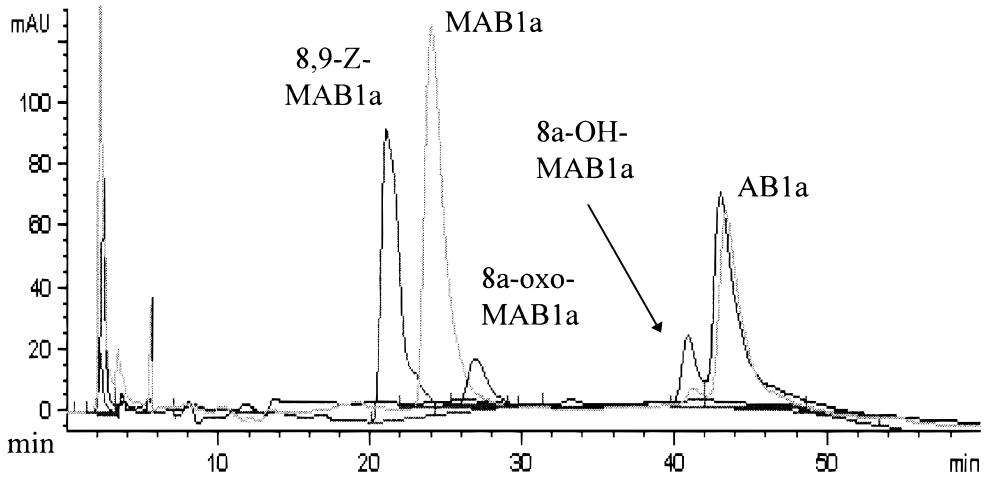

Figure 9. NP-HPLC separation of reference standards. Reference standards were separately analyzed by NP-HPLC (method NP-2, Table 1), and repeat analyses of some standards are shown. Each residue was isolated by RP-HPLC (Figure 8) and was then analyzed by method NP-2 (Table 1). The identity of each photodegradate was confirmed by its UV−visible absorption spectrum and retention time as compared to its external standard. The UV−visible absorption spectra of the standards were reported earlier (Wrzesinski et al., 1996).

component being the 10,11-14,15-diepoxide derivative of MAB1a, and the minor component being the 8,9-epoxide of MAB1a. Key NMR features of the diepoxide were (Figure 7B) as follows: (1) absence of characteristic signals for H10, H11, H15, and the 14a allylic methyl, (2) appearance of a novel, unsplit methyl peak at 1.23 ppm consistent with CH_3CO, and (3) appearance of new methines at 3.97 ppm (d, 6.5), 3.07 ppm (dd, 10.0, 4.2), and 3.02 ppm (t, ca. 1.8) reasonably assigned to H10, H15, and H11, respectively, each consistent with an attached oxygen. These findings suggested incorporation of at least two oxygens, one at C14/C15 and one at C10/C11. Therefore, the mass and NMR results were consistent with the proposed diepoxide derivative of MAB1a. The molecular weight of the minor component suggested that it may have been epoxidated and reduced (+16 +2 Da). Due to limited quantity, the minor component was only partially characterized by NMR as an 8,9-epoxide derivative of MAB1a. The major and minor components were not resolved either by the RP- or NP-HPLC conditions used.

Identification of Photodegradates. As described before, MAB1a benzoate in sensitized buffer solution was extensively photodegraded under artificial light and contained many residues including P1−P4 residues (Figure 3). To produce photodegradates for identification, [^{14}C]MAB1a benzoate in sensitized buffer solution was photolyzed under a xenon arc lamp for 24 h and seven apparent residues were isolated (Figure 8). Out of seven, three apparent residues (Figure 8) corresponded with P1 (organic polar residues), P2 (MAB1a-10,11-14,15-diepoxide), and P4 (8,9-Z-MAB1a) similarly as observed previously (Figure 3). The parent MAB1a and P4 residues (Figure 8) had the same retention times and UV absorption spectra (not shown) as the reference standards, MAB1a, and 8,9-Z-MAB1a, respectively. The P2 residues had the same retention time as [^{3}H]MAB1a-10,11-14,15-diepoxide standard (detected by ^{3}H-radioactivity) and had no UV absorbance consistent with the absence of the conjugated diene. The P3 residues were subdivided into three different residues: P3a, P3b (8a-hydroxy-MAB1a/8a-oxo-MAB1a), and P3c (AB1a, minor component). The P3a residues did not correspond to any available reference standards, but it had characteristic avermectin UV spectra (not shown). AB1a and the

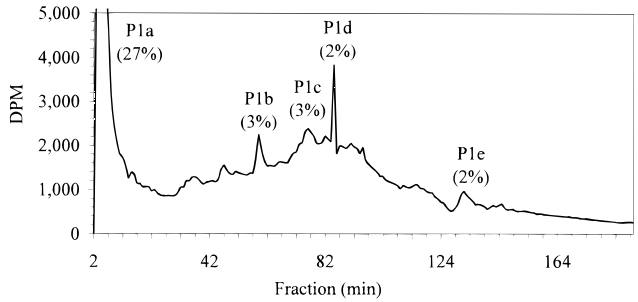

Figure 10. Rechromatography of the P1 residues. The organic polar residues (P1) isolated from semi-prep HPLC (Figure 8) was rechromatographed by RP-HPLC (method RP-8, Table 1). Among the five residues (P1a−P1e), the major P1a residue was eluted in the column void volume. Similar results were obtained when aqueous polar residues (organic-unextractable) were analyzed under similar conditions (chromatogram not shown).

closely eluting 8a-hydroxy-MAB1a and 8a-oxo-MAB1a residues had retention times and UV spectra of the respective reference standards (Figure 8). The identity of each residue indicated in Figure 8 was confirmed by NP-HPLC (method NP-2, Table 1 and Figure 9). The NP-HPLC analyses indicated that each isolated residue was further fractionated into one or more residues (chromatograms not shown). Thus, at least 16 residues were characterized after RP- and NP-HPLC and out of which six (8,9-Z-MAB1a, MAB1a, AB1a, 8a-hydroxy-MAB1a, 8a-oxo-MAB1a, MAB1a-10,11-14,15-diepoxide) were identified by UV spectra (not shown) and retention times corresponding to external standards (Figures 8 and 9). The aqueous polar residues were analyzed directly, and organic polar residues (P1) were analyzed after RP-HPLC isolation (see Figure 8) by RP-HPLC (methods RP-8 and RP-9, Table 1). Both types of polar residues were extremely complex with numerous minor peaks. RP-HPLC analysis of P1 residues indicated a high background with a major residue (P1a, 27%) which eluted in the void volume (Figure 10). Therefore, P1a is likely to be comprised of many components. The other four residues (P1b, P1c, P1d, and P1e) ranged between 2 and 3% of eluted radioactivity, but because of the high background these percentages are undoubtedly overstated.

Table 4. Quantitation of [^{14}C]MAB1A Benzoate Residues in Sensitized Buffer after Irradiation under Artificial Light for 24 h

residue	% amount[a]
MAB1a	36.2
AB1a	0.5
8,9-Z-MAB1a	6.6
8a-oxo-MAB1a	2.4
8a-OH-MAB1a	1.0
MAB1a−10,11-14,15-diepoxide	1.1
characterized residues[b]	15.5
undefined residues[c]	36.7
total	100

[a] The residues identified by RP-HPLC (Figure 8) were confirmed by NP-HPLC (Figure 9). The quantitation of each residue as identified in this table was done from the combined results of RP- and NP-HPLC analyses (data not shown). [b] Additionally 10 minor unidentified residues (each <1%) eluting between MAB1a-10,11-14,15-diepoxide and 8,9-Z-MAB1a (Figure 8) were also resolved by NP-HPLC (method NP-2). [c] Most of the undefined residues were a mixture of unknown organic polar residues that were eluted before MAB1a-10,11-14,15-diepoxide (Figure 8). These polar residues were a complex mixture of many unknown residues (Figure 10).

The percent composition of the identified residues (Table 4) present in sensitized buffer after 24-h artificial light exposure was calculated from the combined results of the RP- and NP-HPLC analyses (Figures 8 and 9). MAB1a was the major residue (36.2%), followed by 8,9-Z-MAB1a (6.6%), 8a-oxo-MAB1a (2.4%), MAB1a-10,11-14,15-diepoxide (1.1%), 8a-hydroxy-MAB1a (1.0%), and AB1a (0.5%). Approximately 10 minor unidentified residues (15.5%) eluted between MAB1a-10,11-14,15-diepoxide and 8,9-Z-MAB1a (Figure 8). The remaining undefined residues (36.7%) were very polar organic residues (Table 4) that were eluted before MAB1a-10, 11-14,15-diepoxide (Figure 8). Due to the low quantity and complex nature of these polar residues, no attempts were made for further identification.

DISCUSSION

There was no degradation or hydrolysis of MAB1a benzoate in aqueous solutions as indicated by the RP-HPLC analyses of dark controls. The photodegradation half-lives of [^{14}C]MAB1a benzoate at 10 and 30 ppm concentration in aqueous buffer containing 1% acetonitrile as a cosolvent under artificial light with continuous irradiation were 64.5 and 31.8 days, respectively (Table 3). The photodegradation of [^{14}C]MAB1a benzoate at 5 and 10 ppm with 1% ethanol as a cosolvent under the same experimental conditions was substantially increased with half-lives of 6.3 and 8.5 days, respectively. The rate of photodegradation of [^{14}C]-MAB1a benzoate at 12 and 32 ppm with 1% acetone as a cosolvent was very rapid with half-lives of 0.5 and 1.0 day, respectively. Apparently, acetonitrile had no effect on the photodegradation of MAB1a benzoate. Acetonitrile (0.3%, v/v) was present as a cosolvent in buffer, natural pond water, and sensitized buffer aqueous solutions that were exposed to natural sunlight up to 30 calendar days; the photodegradation half-lives at a 1 ppm concentration of [^{14}C]MAB1a were 22.4, 6.9, and 1.4 days, respectively. The results suggest that the photodegradation of [^{14}C]MAB1a benzoate under artificial light with continuous irradiation or under natural sunlight was dependent upon the concentration of the parent and the cosolvent used. A compound undergoes direct photolysis if it absorbs light at wavelengths

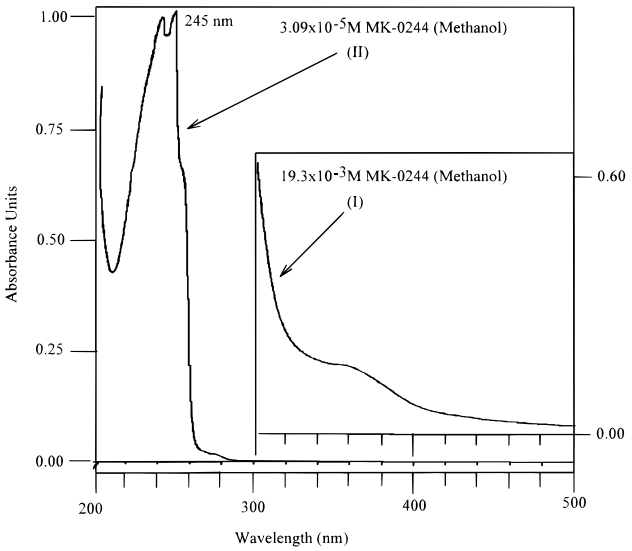

Figure 11. UV−visible absorption spectra of emamectin benzoate in methanol. UV−visible absorption spectra of emamectin benzoate (MK-0244) at high concentration indicate absorption above 290 nm. [^{14}C]MAB1a benzoate at very low concentration (1−32 ppm) will have very low absorption above 290 nm and hence lower photodegradation rate of MAB1a benzoate in buffer solution.

greater than 290 nm (Miller and Zepp, 1983). Figure 11 shows the UV−visible absorption spectra of two emamectin benzoate solutions (19.3×10^{-3} and 3.09×10^{-5} M) in methanol. Apparently, no absorbance could be observed at wavelengths greater than 290 nm in the UV−visible absorption spectrum of 3.09×10^{-5} M emamectin benzoate solution in methanol. But, at higher concentration (19.3×10^{-3} M emamectin benzoate solution in methanol), absorbance bands were visible at wavelengths greater than 290 nm (Figure 11). The MAB1a benzoate was used at low concentration (1−30 ppm) and therefore had very weak absorption above 290 nm, which resulted in lower photodegradation of MAB1a in buffer solutions. Miller and Zepp (1983) also reported that indirect photolysis by the action of a photosensitizer such as acetone or humic acids in natural water could be much more rapid depending on the compound. The results of this study indicated that the rate of MAB1a benzoate photodegradation in the presence of acetone (a photosensitizer) or ethanol (a radical hydrogen donor) in buffer was substantially increased (Table 3). Moreover, the rate of MAB1a benzoate photodegradation in natural pond water was faster in comparison to buffer solution with no photosensitizer. Probably, humic acids present in the organic matter (Table 2) of the pond water acted as photosensitizers. Moreover, photodegradation of [^{14}C]MAB1a benzoate under natural sunlight was proportional to sunlight intensities (summer > fall > winter). In comparison to fall, MAB1a benzoate is expected to photodegrade under natural sunlight about 2 times more in summer and about 0.5 times less in winter (Table 3).

The photodegradation of MAB1a benzoate aqueous solutions under natural sunlight or artificial light followed at least two pathways. The first pathway involves isomerization of MAB1a to its 8,9-Z isomer (Figures 3 and 5). Feely et al. (1992) also reported 8,9-Z-MAB1a as a photodegradate of MAB1a exposed to light as a thin film on glass. The 8,9-Z- and the 10,11-Z-photoisomers of the structurally related compound

avermectin B1a were reported by Mrozik et al. (1988). Crouch et al. (1991) identified both the 8,9-Z- and 10, 11-Z-photoisomer as well as seven monooxygenated derivatives and one dealkylated derivative in the photodegradation of avermectin B1a thin film on glass. Similarly, 8,9-Z- and 10,11-Z-photoisomers were also reported in the photodegradation of avermectin B1a and 22,23-dihydroavermectin B1a (ivermectin B1a) in aqueous solutions under natural sunlight (Halley, unpublished results). Although, the 10,11-Z-isomer of avermectin B1a was detected in all the photodegradation studies (Mrozik et al., 1988; Crouch et al., 1991; Maynard and Maynard, 1989), the 10,11-Z-isomer of MAB1a was not detected as a photodegradate in this study and in other published reports. The second photodegradation pathway probably involved highly reactive free radical intermediates and/or singlet-oxygen ($^1O_2$) with the formation of more polar residues such as epoxides and hydroxy derivatives of MAB1a. Feely et al. (1992) reported AB1a, 4″-*N*-methyl-*N*-formylavermectin B1a (MFB1a), 4″-*N*-formylavermectin B1a (FAB1a), their 8,9-Z-isomers, and MSB1a as the additional photodegradation products of MAB1a thin film on glass. Crouch and Feely (1995) and Wrzesinski et al. (1996) identified MSB1a, FAB1a, MFB1a, 8a-oxo-MAB1a, 8a-hydroxy-MAB1a, AB1a, MAB1a, and 8,9-Z-MAB1a as the extractable residues from lettuce and cabbage. In the present study, 8a-oxo-MAB1a, 8a-hydroxy-MAB1a, AB1a, and very polar residues were identified while MSB1a, FAB1a and MFB1a, were not detected. A unique photodegradation product, MAB1a-10,11-14,15-diepoxide, was identified only in the present study. Moreover, the polar residues increased steadily with the light exposure time and were the end products in the photodegradation of MAB1a benzoate (Figures 3, 5, and 8; Table 4).

Since emamectin benzoate is immobile in soils (Mushtaq et al., 1995), contamination of water through leaching and runoff is expected to be minimal. However, contamination of water through air drift is possible. Nonetheless in the natural waters, emamectin benzoate would be expected to be well below 1 ppb as a result of agricultural use. The natural organic matters such as humic acids present in natural water will serve as photosensitizers and will increase the photodegradation rate of emamectin benzoate. Thus, emamectin benzoate in natural water will be rapidly eliminated as a mixture of very polar residues.

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