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HPLC Assay for Avermectin B1a and its Two Photoisomers using a Photo Diode Array Detector

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Avermectins are a new class of biological agents that contain a macrocyclic lactone and are produced by Streptomyces avermitilis (Burg et al.1979). The structures of the avermectins have been elucidated (Albers-Schonberg et al.1981) and some of their biological activities have been reported (Campbell et al.1981). Abamectin is the product that is being developed by Merck and Co., Inc. as an acaricide/insecticide for crop protection (Putter et al.1981). The major component in abamectin is avermectin B1a (Figure 1) which was used in this study and exists in the 8,9-E;10,11-E configuration. The two photoisomers (8,9-Z;10,11-E and 8,9-E;10,11-Z) of avermectin B1a are also shown in Figure 1.

Avermectin B1a has been shown to degrade on citrus fruits to the 8,9-Z isomer of avermectin B1a (8,9-Z-B1a)(Maynard et al.1983). The photolysis of avermectin B1a in organic solution (Mrozik et al.1988), aqueous solution (Ku et al.1983), or as a film (Maynard, unpublished data) also results in the formation of 8,9-Z-B1a. The formation of the 10,11-Z isomer of avermectin B1a (10,11-Z-B1a) was also reported from the photolysis of avermectin B1a in organic solution (Mrozik et al.1988). This report presents an assay which was developed to quantitate the levels of avermectin B1a and these two isomers in an aqueous solution. In addition, the use of the photo diode array detector provided tentative identification of avermectin B1a and its two photoisomers based on the difference in their UV spectra and absorbance maxima.

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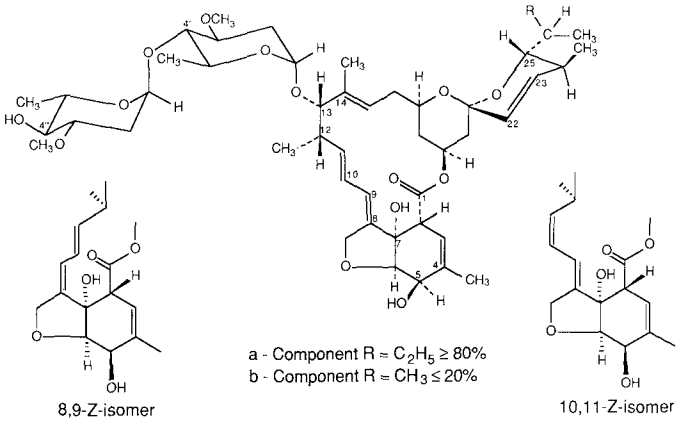

Figure 1. Structures of the Two Major Components of Abamectin and the Two Photoisomers

MATERIAL AND METHODS

Avermectin B1a was purified by HPLC from technical material. 8,9-Z-B1a and 10,11-Z-B1a were supplied by H. Mrozik (Merck & Co., MSDRL, Rahway,NJ). All standards were > 94% pure. Solvents were of high purity and were purchased commercially.

The HPLC system consisted of: Spectra Physics SP8700 pump, Hewlett-Packard (HP) 1040A photo diode array detector, HP85B computer, HP9121 disc drive, HP7475A plotter, HP Thinkjet printer, Rheodyne 7125 injector with a fixed 100-uL injection loop, and a Dupont Zorbax ODS column (4.6 mm x 25 cm). Detector settings were as follows: UV range 200-300 nm, band width + 1 nm, peak width 2, and spectra acquisition 100 msec. The absorbance was monitored at 245 nm and the signal was integrated based on the peak height. The mobile phase was 85/15, methanol/water, and mixed by the pump at a flow rate of 1 mL/min. The system was operated at ambient temperature and was also used for purification of the isomers (Mrozik et al.1988).

A standard solution of avermectin B1a, 10,11-Z-B1a, and 8,9-Z-B1a were prepared in a solution of 50/50, methanol/water. The concentration of the original stock solutions was determined by a UV spectrophotometer using the known extinction coefficient for each compound (Mrozik et al.1988; Albers-Schonberg et al.1981). Aliquots of the original stock solution (4.818 ug/mL, avermectin B1a; 1.156 ug/mL, 10,11-Z-B1a; and 2.500 ug/mL, 8,9-Z-B1a) were diluted with 50/50,

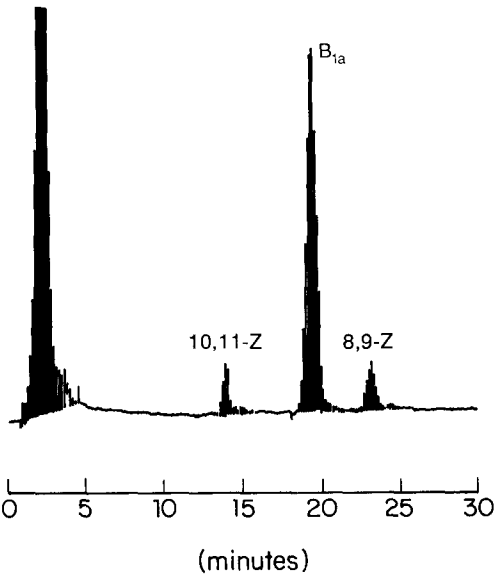

Figure 2. Chromatogram of 10,11-Z-isomer, Avermectin B_{1a}, and 8,9-Z-isomer

methanol/water, to provide standard solutions ranging from 0.02 to 4.8 ug/mL depending upon the compound. Injection volume was 100 uL for all samples and all samples were injected minimally as duplicates.

RESULTS AND DISCUSSION

The RP-HPLC system used for this assay provided excellent separation with all three compounds eluting with baseline separation. A representative chromatogram of a mixture of these three compounds is shown in Figure 2. The retention times are 18.9, 13.8, and 23.0 min for avermectin B1a, 10,11-Z-B1a, and 8,9-Z-B1a, respectively.

The standard curves for these three compounds exhibited excellent linearity over the following concentration ranges: avermectin B1a, 0.05-4.82 ug/mL; 10,11-Z-B1a, 0.02-1.16 ug/mL; and 8,9-Z-B1a, 0.13-1.25 ug/mL. The ranges were selected to approximate the anticipated proportion of parent to photoisomers. The equation and the correlation coefficient for these standard curves were determined by linear regression analysis. The correlation coefficients for avermectin B1a, 10,11-Z-B1a, and 8,9-Z-B1a were greater than 0.999. The limit

of detection, as determined by a signal to noise ratio of two, was 0.005 ug/mL for all three compounds. At selected mid-range concentrations, the intraday and interday reproducibility for the three compounds (CV %) were less than 4.4%. From these results, this assay provided accurate analyses for avermectin B1a and its two photoisomers at concentrations of 0.02 to 4.82 ug/mL in an aqueous solution.

The absorbance spectra generated by the photo diode array detector for avermectin B1a and its two photolytically produced isomers were shown to exhibit absorbance maxima at 244, 248, and 242 nm for avermectin B1a, 10,11-Z-B1a, and 8,9-Z-B1a, respectively (Figure 3). The absorbance maxima for these compounds when measured with a UV spectrophotometer using the same solvent in this study were 245, 247, and 243 nm, respectively. These values have been reported (Mrozik et al.1988). Although the absorbance maxima recorded by the photo diode array detector was slightly different from the spectrophotometer values (+1 nm), they were within the reported accuracy of the detector. The difference in the absorbance maxima among these compounds was differentiated by this detector and therefore can be used for tentative identification purposes. Moreover, the characteristic differences in the shape of the spectra can also be used for tentative identification. In addition, by using the ratio between two selected wavelengths (eg 217, 232, 246, 260 nm) (Figure 3) the three compounds can also be tentatively identified.

The major advantage of this detector is the tentative identification of a compound or its metabolite(s) based on its UV spectra. This identification can be used if the degradation products retain, or change, their UV spectra relative to the parent compound. For example, the metabolism of avermectin B1a (Maynard et al.1985) or 8,9-Z-B1a (Maynard et al.unpublished) by rats resulted in the formation of the 3"-desmethyl and the 24-hydroxymethyl metabolites. These metabolites were both shown to retain their UV spectral properties relative to the corresponding parent compound (eg. UV absorbance maxima, spectra shape, and wavelength ratios). Therefore tentative identification of these metabolites in various tissue and fecal extracts was possible because the UV spectra of the metabolites and parent were unique and different from the natural products found in the extracts. This greatly assisted the subsequent isolation and purification for structure identification.

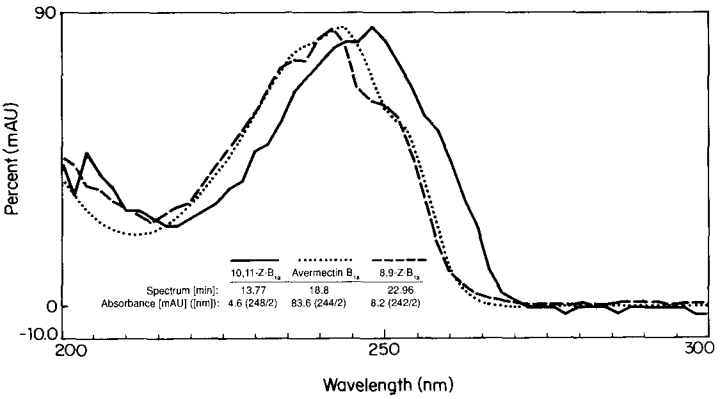

Figure 3. Absorbance of 10,11-Z-B_{1a}, Avermectin B_{1a}, and 8,9-Z-B_{1a} from a Chromatographic Run

This was not the case, however, with avermectin B1a and its two photoisomers. The trans to cis olefin isomerization resulted in not only a change in the elution properties but also a change in the UV spectra properties of the compounds. Because this detector can differentiate these compounds, the characteristic UV changes can be employed when analyzing a sample for either the 8,9-Z or 10,11-Z isomer of avermectin B1a or their metabolites. This would involve determining the absorbance maxima, UV spectra shape, and/or selected wavelenght ratios (260/232 nm for 10,11-Z and 246/217 nm for 8,9-Z-B1a). Therefore, the use of the photo diode array detector provides an opportunity to tentatively identify compounds and their metabolites based on a similar or different UV spectra. This can occur during the HPLC run by monitoring in real time or by data analysis after the run.

In summary, a simple, quick assay was developed for avermectin B1a and its two geometric isomers in an aqueous solution. The use of the photo diode array detector provides an opportunity to tentatively identify avermectin B1a, its two photoisomers, and possibly their degradates in an aqueous solution at the time of HPLC analysis.

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