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Note

Normal-phase chromatography and post-column colorimetric detection of abamectin-8,9-oxide

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The avermectins[1] are a class of macrocyclic lactone, natural products. They have generated much interest as a broad-spectrum anthelmintic (ivermectin) and as an acaricide/insecticide (abamectin or avermectin B_1). The 8,9,10,11-diene chromophore of avermectin B_1, which is the basis for its UV detection at 245 nm in reversed-phase high-performance liquid chromatography (HPLC), contributes[2] to the rapid degradation of the avermectins in the environment[3]. Thus, attempts to prolong their activity have involved the synthetic modification of the diene[4].

Recent work has shown that the 8,9-oxide of avermectin B_1 (structure 1, Fig. 1) can be easily synthesized[5], is biologically active[6] and is chemically more stable[7] than the parent compound, avermectin B_1. However, the lack of the diene chromophore produces an analytical problem. Abamectin-8,9-oxide can be detected at 210 nm, but in the presence of formulation excipients this wavelength has a low specificity for the analyte. One course of action is to separate the analyte from the excipients and utilize reversed-phase HPLC with UV detection at 210 nm. We present an alternative for detecting abamectin-8,9-oxide and similar analytes, namely, normal-phase HPLC with no separation of analyte and excipients and with post-column detection at 570 nm. Post-column reactions have been used to detect several pesticides, including carbamates[8,9] and glyphosate[10]. Additionally, they have been useful for the analysis of drugs in animal feeds[11,12].

We recently discovered a novel color reaction between trichloroacetic acid (TCA) and several avermectins[13]. The reaction conditions for abamectin-8,9-oxide are similar to those in the colorimetric determination of vitamin A[14,15], despite that the former contains no conjugated double bonds whereas the latter contains five conjugated double bonds. The reaction between TCA and abamectin-8,9-oxide forms the most intense colors in chlorinated solvents and requires a few minutes of mild heating, which is not necessary with vitamin A. The reaction is inhibited, to varying extents, by typical solvents used in reversed-phase HPLC, *i.e.*, water, acetonitrile, tetrahydrofuran and alcohols.

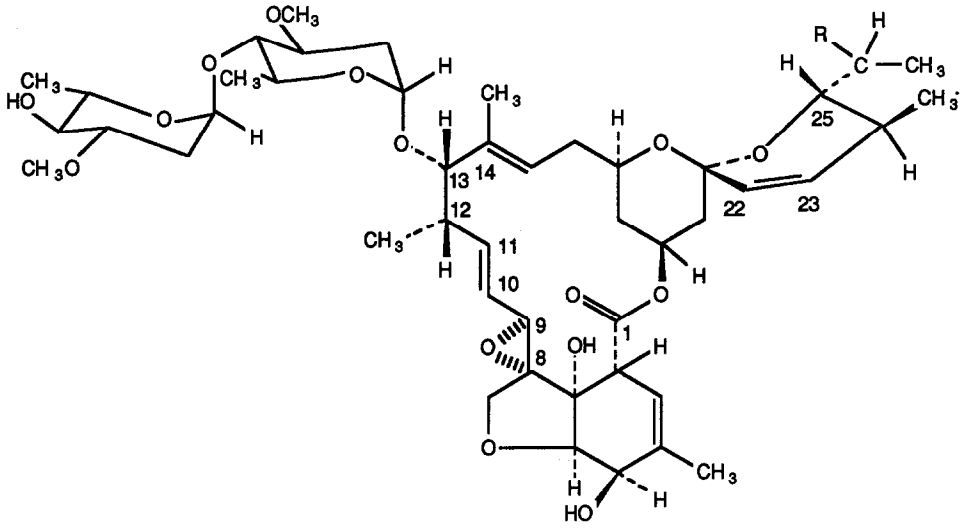

Fig. 1. Structure 1 (abamectin-8,9-oxide). B_{1a} component: R = C_2H_5 ($\geqslant$ 80%); B_{1b} component: R = CH_3 ($\leqslant$ 20%).

EXPERIMENTAL

The HPLC apparatus consisted of an LDC Constametric III pump, a Kratos Spectroflow 757 dual-lamp (deuterium and tungsten) detector, a Micromeritics 725 autoinjector connected to a Valco EC6W valve and a Nelson Analytical data system with XTRA-CHROM software. The silica column was 25 cm × 4.6 mm, packed with 3-μm Hypersil (Keystone Scientific). The post-column reaction system (PCRS) consisted of a Kratos Spectroflow 400 pump and a Kratos Model 525 dual-oven heating unit. The reaction coils were constructed from PTFE PFA tubing (0.020 in. × 1/16 in.) that had been made with a melt process (Zeus Industries). Two knitted reaction coils, each 2.5 ml in volume and the maximum allowed by the apparatus, were fabricated and connected in series. All solvents were HPLC grade and the TCA (Fisher), 2-naphthalenesulfonic acid 1-hydrate (NSA) (Kodak) and 3-hydroxypropionitrile (Aldrich, Gold Label) were used without further purification.

The mobile phase consisted of 1.1% (v/v) 3-hydroxypropionitrile in a 5:2 (v/v) mixture of 1-chlorobutane–1,2-dichloroethane. The 3-hydroxypropionitrile was more soluble in 1,2-dichloroethane than in 1-chlorobutane and this blend yielded a modifier concentration that was close to saturation. The PCRS reactant solution consisted of 5% TCA (w/v) in 1,2-dichloroethane, which was then saturated with NSA ($\sim$500 ppm). Both the mobile phase and PCRS reactant solution were filtered through a 0.2-μm PTFE filter (Millipore) prior to use. No attempts were made to eliminate water from these solutions, other than to minimize its introduction by using a previously unopened bottle of TCA.

The abamectin-8,9-oxide was dissolved in the mobile phases at a nominal concentration of 100 ppm. The solutions used to demonstrate linearity were made by diluting the 100 ppm solution with mobile phase.

RESULTS

Fig. 2 depicts the visible absorption spectra for one concentration of abamectin-8,9-oxide under different reaction conditions, in 1-cm cuvettes. The amount of color formation is directly proportional to the concentration of TCA, and the maximum absorption decreases (curves 2 and 3) as one reduces the level of TCA from 10 to 5%. However, 5% TCA solutions that are saturated with NSA or 4-biphenylsulfonic acid yield essentially the same absorption maximum as that with 10% TCA. The higher absorbance at 400 nm in curve 1 is not due to the absorption from the NSA itself; it is due to a change in the nature of the chromophore(s).

The HPLC conditions were chosen so as to achieve a sensitivity comparable to that in UV detection and to minimize wear-and-tear on the instrumentation. For example, the Tefzel coils originally supplied with the Kratos PCRS were subject to leakage with the halogenated solvents, and TCA is a very corrosive reagent. The custom-fabricated PTFE PFA coils were more robust, and use of NSA lowered the TCA concentration. The coil volume of 5 ml was the maximum allowed by the apparatus, so adequate sensitivity was achieved by raising the temperature.

Fig. 3 depicts the normal-phase chromatography of an abamectin-8,9-oxide solution (100 ppm in mobile phase) with detection at 210 nm using a typical mobile phase. There was no separation between the B_{1a} and B_{1b} components at a retention time of 28–29 min. Fig. 4 displays the chromatography obtained for a similar abamectin-8,9-oxide solution (100 ppm in mobile phase) with post-column reaction detection at 570 nm using the tungsten lamp. Based upon the flow-rates and coil volumes, the reaction time was 3–4 min. At retention times of 34–37 min there was near baseline separation of the B_{1a} and B_{1b} components. The B_{1a} component eluted before the B_{1b} component, which is opposite to the elution order in reversed-phase HPLC. The response shown in Fig. 4 is linear (correlation coefficient = 0.9996) over the range 20–100 ppm, and one can easily detect 1.0 μg (20 ppm × 50 μl) of analyte.

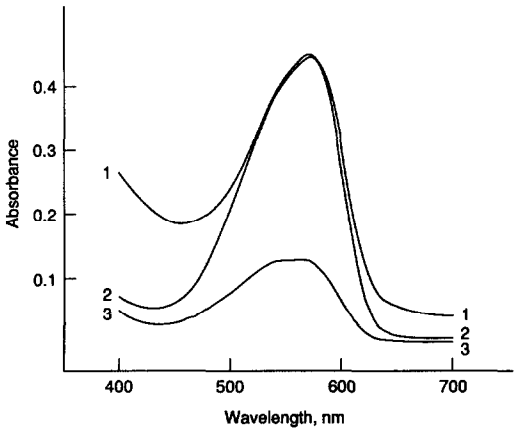

Fig. 2. Color formation with abamectin-8,9-oxide after 5 min at 50°C. Curve 1: 1 ml of abamectin-8,9-oxide (75 ppm in mobile phase) + 0.5 ml of 5% TCA, saturated with NSA. Curve 2: 1 ml of abamectin-8,9-oxide (75 ppm in mobile phase) + 0.5 ml of 10% TCA. Curve 3: 1 ml of abamectin-8,9-oxide (75 ppm in mobile phase) + 0.5 ml of 5% TCA.

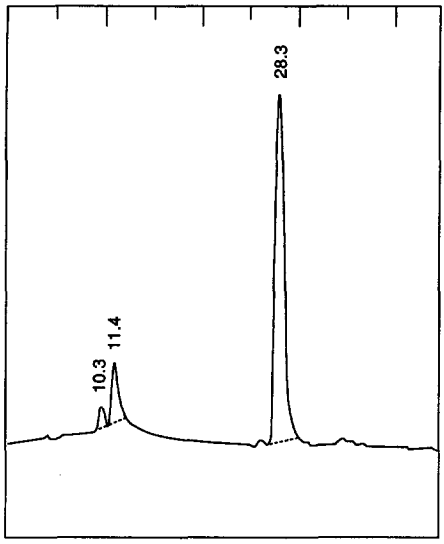

Fig. 3. Abamectin-8,9-oxide with UV detection at 210 nm. HPLC conditions: mobile phase, cyclohexane–isopropyl alcohol (90:10); flow-rate, 0.5 ml/min; column temperature, 30°C; PCRS solution, mobile phase flowing at 0.5 ml/min; PCRS temperature, ambient; loop size, 50 μl; detector sensitivity, 0.02 a.u.f.s. Numbers at peaks indicate retention times in min.

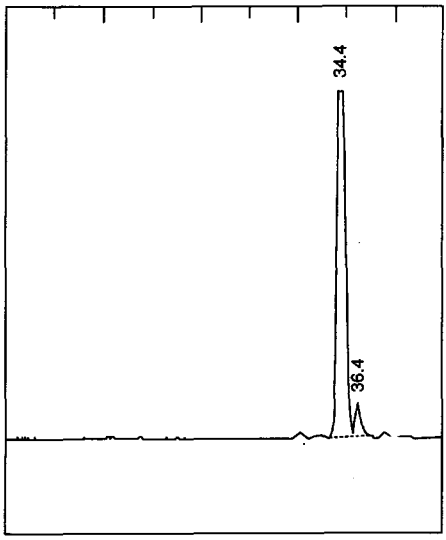

Fig. 4. Abamectin-8,9-oxide with detection at 570 nm, after post-column reaction. HPLC conditions: mobile phase, 1.1% 3-hydroxypropionitrile in 1-chlorobutane-1,2-dichloroethane (5:2); flow-rate, 1.0 ml/min; column temperature, 30°C; PCRS solution, 5% TCA in 1,2-dichloroethane saturated with NSA flowing at 0.5 ml/min; PCRS temperature, 45°C; loop size, 50 μl; detector sensitivity, 0.02 a.u.f.s.

DISCUSSION

The nature of the chromophore that is formed in these reactions merits some discussion. Although abamectin-8,9-oxide is a much larger molecule than vitamin A, not all of the avermectin structure is necessary for color formation. In fact, structure 2 (Fig. 5) shows an avermectin fragment, kindly supplied by Dr. S. Hanessian[16], that also gives a color reaction. Color formation with fragments frequently occurs at ambient temperatures. In contrast, 22,23-dihydroavermectin B_1 (ivermectin), lacking the double bond at C-22–C-23, does not undergo this color reaction. Now, it has been shown[17] that under strongly acidic conditions, the C-22–C-23 double bond participates in the opening of the neighboring ketal functionality. Also, the silyl-protecting groups would be removed from structure 2 under these conditions. Since small amounts of sulfonic acids catalyze the reaction, thus implying some elimination step(s), one can at least speculate on the formation of some chromophore similar to that which occurs in the reaction of TCA with vitamin A. No reaction products of this color formation have been isolated; however, this is also true of the long-studied vitamin A color reaction. The end result of the work is that one has a post-column procedure that is highly specific for avermectin-like structures.

The development of the mobile phase used in the colorimetric detection of abamectin-8,9-oxide deserves some explanation. It has been stated in the literature[18,19] that a silica surface is modified by adsorption of a polar solvent from a typical hydrocarbon mobile phase. One view is that the modifier is adsorbed much more strongly than the analyte, and does not compete with it for adsorption sites[20]. However, Scott[21] has shown that there are conditions under which an analyte does displace a polar solvent used as a modifier. The advantage of this latter concept is that it is easier to understand how modifier composition can affect chromatographic selectivity. Our experience is that a mobile phase that contains a nitrile modifier such as 3-hydroxypropionitrile provides better resolution for the avermectin components than does a typical alcohol–hydrocarbon mobile phase. The major advantage of 3-hydroxypropionitrile over other nitrile modifiers, such as 3,3′-oxydipropionitrile or glutaronitrile, is that it does not have to be purified for most chromatography with UV detection. We suspect that a nitrile-deactivated silica will be a useful alternative to an alcohol-deativated silica in many instances.

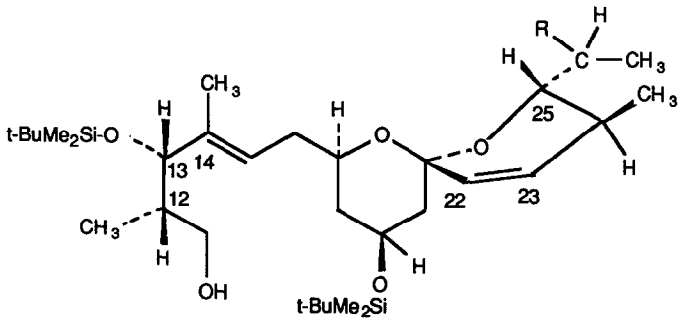

Fig. 5. Structure 2. B_{1a} component: R = C_2H_5; t-Bu = *tert.*-Butyl; Me = methyl.

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