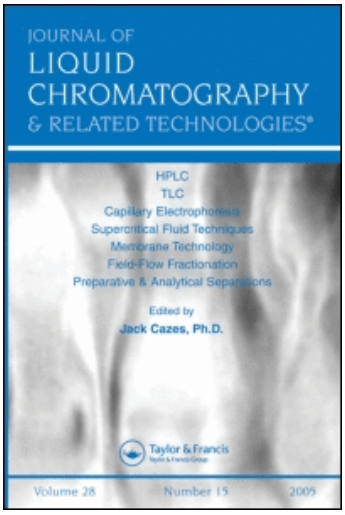

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HPLC SEPARATION OF THE ZZ, ZE, EZ, AND EE GEOMETRIC ISOMERS AND EE ISOMER ENANTIOMERS OF A SUBSTITUTED PENTADIENYL CARBOXAMIDE USING ACHIRAL/CHIRAL COLUMN SWITCHING

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ABSTRACT

The four geometric isomers of a substituted pentadienyl carboxamide were separated on an achiral aminopropyl column coupled with a silica precolumn. The R and S enantiomers of the biologically active EE isomer (RO 24-0238 and RO 24-2099) were resolved (R_s 1.65) on a cellulose-based chiral stationary phase (Chiracel OF). The coupled silica and aminopropyl columns were connected to the Chiralcel OF column through a six port switching valve that enabled transfer to the EE isomer to the chiral phase for enantiomer resolution. By examining the selectivity for separation of the geometric isomers of various achiral stationary phases using hexane-isopropanol mobile phases, a method was developed which linked the achiral separation of the geometric isomers with the chiral separation of the EE enantiomers.

INTRODUCTION

Platelet activating factor (1-O-alkyl-sn-glycero-3-phosphocholine, PAF) is a phospholipid mediator produced in inflammatory and allergic reactions. The biological activity consists of hypotension and increased vascular permeability, bronchoconstriction, coronary vasoconstriction and platelet aggregation. As a result of these actions, PAF is a mediator in septic shock, asthma, coronary artery disease, and stroke (1-4). RO 24-0238, 5-(4-methoxyphenyl)-N-[1-methyl-4-(3-pyridinyl)-butyl]-2,4-decadienamide, is a pentadienyl carboxamide PAF antagonist with a stereoselective bioactivity profile (see Fig. 1). The R enantiomer (RO 24-0238) not only causes a greater inhibition of PAF induced bronchoconstriction, but also has a substantially longer duration of action than the S enantiomer (RO 24-2099) (5). Both enantiomers have the EE configuration. The remaining geometric isomers shown in Fig. 1 are biologically inactive.

Column switching in HPLC has been reported in the literature for such applications as sample preparation, direct injection of serum samples and for the separation of drugs and metabolites. Oda et al separated verapamil from its metabolites on a reversed phase octadecylsilane column and then transferred the drug via column switching to an ovomucoid chiral stationary phase (CSP) for separation of the enantiomers (6). Stalcup et al used column switching to determine enantiomeric purity of scopolamine on a beta cyclodextrin

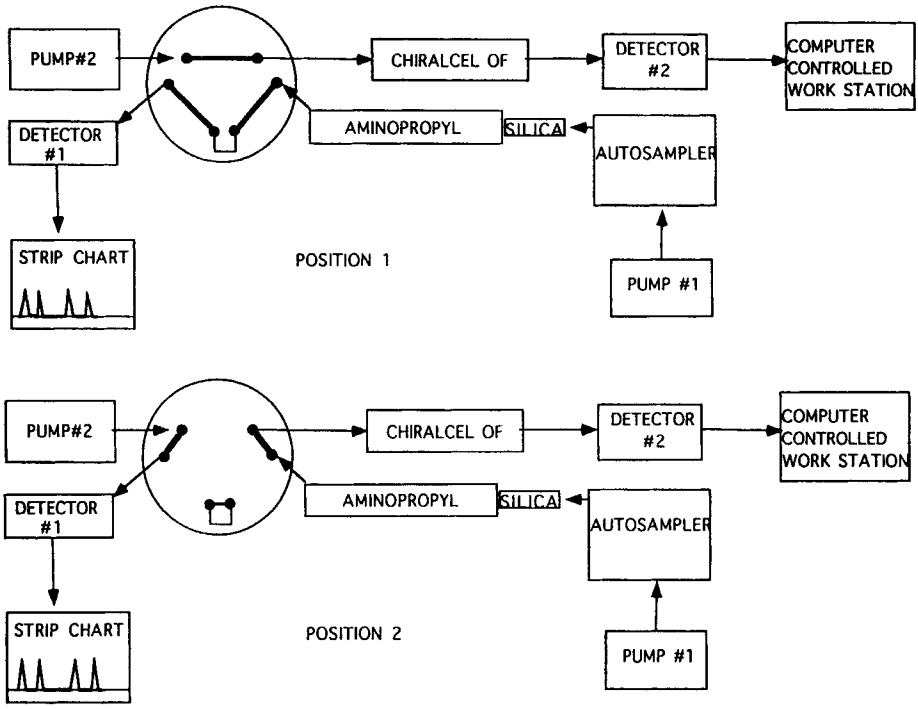


Figure 1 - The chemical structure of a substituted Pentadienyl Carboxamide and its ZZ, ZE, EZ and EE Geometric Isomers and the R and S enantiomers of the EE isomer.

column after separation of the drug from other alkaloids and biological material in a vegetal extract (7).

Individual methods for the separation of the four geometric isomers (ZZ, ZE, EZ and EE) and chiral resolution of the R and S enantiomers of the EE isomer have previously been performed by Aggarwal et al (8). The geometric isomers were separated on a cyanopropyl column using a mobile phase of 60:35:5:0.2 v/v/v/v

hexane-methylene chloride-ethyl acetate-triethylamine. The enantiomers of the EE isomer (RO 24-0238 and RO 24-2099) were separated on a Chiralcel OF column using a mobile phase of 70:30 v/v hexane-isopropanol. Since the mobile phase used in the normal phase separation of the geometric isomers was not compatible with the Chiralcel OF column, other stationary phases were investigated that might be useful in the separation of the geometric isomers and yet be compatible with the solvent restrictions of the Chiralcel OF column. In this paper, a column switching method is reported for the on-line analysis of the four geometric isomers of a substituted pentadienyl carboxamide including a chiral separation of the R and S enantiomers of the EE isomer.

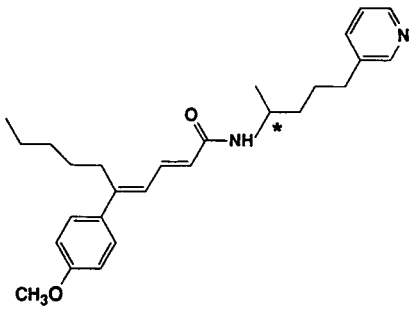
EXPERIMENTAL

Reagents and Chemicals

The R and S enantiomers of the EE isomer were provided as RO 24-0238 and RO 24-2099 respectively, by Hoffmann-LaRoche (Nutley, NJ). The geometric isomers RO 24-6106 (ZZ), RO 24-6105 (ZE) and RO 24-1553 (EZ) as racemates were also supplied by Hoffmann-LaRoche. HPLC grade hexane and isopropanol were obtained from J.T. Baker Inc. (Phillipsburg, NJ). Diethylamine was obtained from Eastman Kodak Co., (Rochester, NY).

Instrumentation

Figure 2 shows schematics of the two HPLC systems used. HPLC system #1 for the achiral separation consisted of a Spectra-Physics



RO 24-0238, EE (R)
RO 24-2099, EE (S)
RO 24-6106, ZZ (R,S)
RO 24-6105, ZE (R,S)
RO 24-1553, EZ (R,S)

Figure 2 - Schematics of the achiral HPLC system #1 and the chiral HPLC system #2.

Model SP 8800 Ternary pump (San Jose, CA), a Lambda-Max Model 400 UV Spectrophotometer (Waters Associates, Milford, MA) set at 322 nm, a Kipp and Zonen Model BD41 chart recorder, and a Perkin-Elmer Model 1SS 100 autosampler set for a 10 μL injection. HPLC System #2 consisted of a Beckman Model-110B pump (Fullterton, CA), a Waters Model 440 UV detector set at 322 nm, and a Nelson Model 2600 Chromatography Data System to accumulate data from the chiral separation and relay a signal to activate the Rheodyne Model 7010 six port pneumatic actuated column switching valve (Cotati, CA).

The chiral stationary phase was cellulose tris(4-chlorophenyl carbamate) bonded to silica gel commercially available as Chiralcel OF

(Daicel Inc., Fort Lee, NJ, USA). The achiral stationary phase was a Zorbax 4 cm x 60 mm i.d., 3 μm particle size silica column coupled to a μ-Bondapak aminopropyl 30 cm x 3.9 mm i.d., 10 μm particle size column (Waters Inc., Milford, MA). The HPLC pump in system #1 delivered a mobile phase of hexane-isopropanol (92:8) containing 1 mM diethylamine to the achiral coupled columns and the HPLC pump in system #2 delivered a mobile phase of hexane-isopropanol (70:30) containing 1 mM diethylamine to the chiral column. A sample containing the four geometric isomers was initially injected onto the coupled silica-aminopropyl stationary phases. Retention times for each geometric isomer were monitored by the detector in system #1 and the chromatograms recorded on the chart recorder with the switching valve in position #1(see Fig. 2). The valve was switched to position #2 just before the EE geometric isomer peak eluted from the column (approx. 40 min). This allowed for transfer of the EE isomer to the chiral column for separation of the R and S enantiomers. The valve was then switched back to position #1 for the remaining detection of the EZ peak (approx. 44 min).

Preparation of Standard Solutions

Approximately 5 mg of each geometric isomer (ZZ, ZE and EZ) and the R and S enantiomers of the EE isomer were dissolved in 70:30 hexane-isopropanol in a 10 mL volumetric flask and the solvent was added to volume. The samples were sonicated for 10 min and then

filtered through a 0.5 μm Millex SR syringe filter (Millipore Co., Rutherford, NJ) attached to a 20 mL glass syringe (Becton Dickinson Co., Rutherford, NJ).

RESULTS AND DISCUSSION

An achiral/chiral column switching HPLC method was developed to separate the biologically active EE isomer from its three other geometric isomers including a transfer of the EE isomer to a CSP for resolution of the R and S enantiomers. It had been previously shown that a chiral separation of the R and S enantiomers of the EE isomer was achieved on a cellulose tris(4-chlorophenyl carbamate) CSP using hexane-isopropanol (70:30) containing 1 mM diethylamine. Since the choices of mobile phase solvents with the Chiracel OF CSP are essentially limited to hexane, isopropanol and small amounts of diethylamine, various achiral stationary phases applicable to normal phase chromatography were initially examined for geometric isomer separation prior to transfer and separation of the EE enantiomers on the Chiracel OF column. It was found that a silica column used in the normal phase mode provided a partial separation of the geometric isomers. A beta cyclodextrin stationary phase, which had been reported to separate geometric isomers in the normal phase mode (9), was also investigated. However, when this stationary phase was used in our laboratories, coelution of the EE and EZ isomers occurred. An aminopropyl stationary phase with various combinations of hexane-

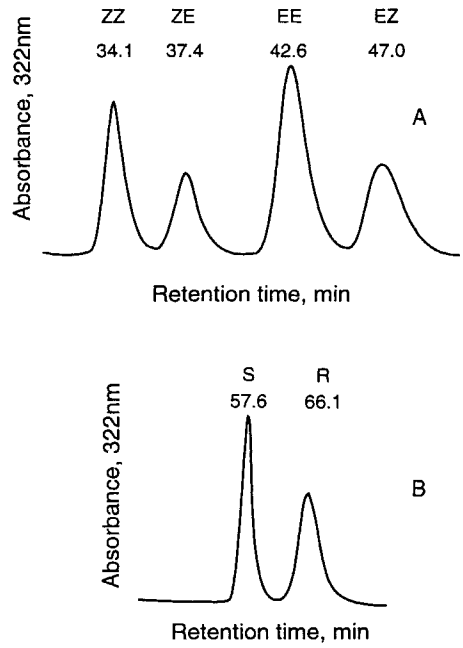


Figure 3 - (A) Chromatogram of the HPLC separation of the ZZ, ZE, EE and EZ isomers of a substituted pentadienyl carboxamide on tandem silica-aminopropyl columns using a mobile phase of 92:8 hexane-isopropanol.

(B) Chromatogram of the HPLC separation of the R and S enantiomers of the EE isomer of a substituted pentadienyl carboxamide on a chiracel OF column using a 70:30 hexane-isopropanol mobile phase.

isopropanol as mobile phases was unable to provide satisfactory separation of the four geometric isomers. However, when the aminopropyl column was coupled in tandem with a silica precolumn, all of the geometric isomers were well-resolved. A thorough examination of different mobile phase compositions of hexane and isopropanol

containing 1 mM diethylamine finally resulted in baseline resolution of the four isomers. By varying the diethylamine concentration, silanol interactions with each analyte were controlled such that excellent resolution was obtained. Increasing the diethylamine concentration from 0.5 to 1.0 mM significantly improved the peak asymmetry. Therefore, the final composition of the mobile phase used for the achiral separation of the four geometric isomers was 92:8 hexane-isopropanol containing 1 mM diethyl amine. Thus, it was now possible to transfer the EE isomer via column switching from achiral System #1 to the chiral System #2 for resolution of the R and S enantiomers of the EE isomer (see Fig. 3a and 3b). An R_s of 1.65 was obtained for the separation of the EE enantiomers on the Chiralcel OF column.

In summary, an HPLC achiral/chiral column switching method was developed for the achiral separation of the geometric isomers of of a substituted pentadienyl carboxamide including transfer and chiral resolution of the R and S enantiomers of the EE isomer. This multidimensional chromatographic approach to the analysis of geometric isomers and enantiomers permits two HPLC systems to be linked together to solve a complex analytical problem.

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