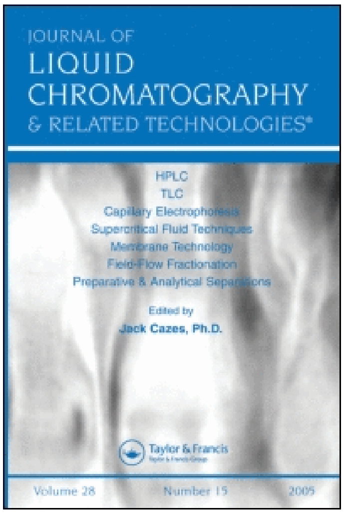

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY SEPARATION AND ANALYSIS OF METALLOTETRA(PENTAFLUORO-PHENYL)PORPHOLACTONE

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ABSTRACT

The analytical separation and analysis of silver, nickel, zinc and palladium complexes of tetra(pentafluorophenyl)porpholactone (TFPL) from free bases is described. An isocratic mobile phase of n-hexane and diethyl ether was developed to separate these complexes using a normal phase μPorasil HPLC column (150 x 3.9 mm I. D.) at a flow rate of 1 mL/min. In addition, two porphodilactone isomers were also isolated from the reaction products, a result which we were unable to achieve with flash chromatography nor with a reversed phase C18 Ultrasphere HPLC column (250 x 4.6 mm I. D.). The optical absorption spectra of the metal derivatives show two bands in the visible region, compared to the four bands shown by the free base. The retention of these metal complexes on a normal phase column was found to be increasing in the order Pd(TFPL) < Ag(TFPL) < Zn(TFPL) < Ni(TFPL).

INTRODUCTION

Porphyrin and metalloporphyrins are a class of important biologically important molecules. Much work have been reported on HPLC separation of these biological porphyrins (1-8). Reversed phase columns were usually used to separate these porphyrins due to their hydrophillic nature. In some cases, when satisfactory separation can not be achieved on reversed phase column, these biologically active porphyrins can be derivatized to water insoluble derivatives so that normal phase column can be deployed to give better separation (9-12).

However, relatively few studies have been reported on HPLC separation of water insoluble free base porphyrins, and even less work has been carried out on metalloporphyrins, despite extensive study on almost every other aspects of porphyrins and metalloporphyrin (13). Eglinton et al. (14) used a silica column to separate some free base porphyrins in crude oil. Rowlands and coworkers (15) separated some metalloporphyrins successfully on a Hypersil column, using 9:1 hexane and ethyl acetate as mobile phase. Suzuki et al (16) developed a 40:60 acetone-acetonitrile mobile phase to separate Mg(II), V(IV), Fe(III), Ni(II), Cu(II), Zn(II), Pd(II) and Cd(II) bonded *meso*-tetraphenylporphyrin on a reversed phase C18 column. Freeman et al. (4) successfully separated a mixture of the octaethylporphyrins of zinc(II), nickel(II), copper(II) and cobalt(II) derivatives also on a reversed phase column, using acetonitrile-ethanol amine (10:1) as mobile phase.

Recently, a new type of porphyrin compound called tetra(pentafluorophenyl)porpholactone (H_2TFPL) was synthesized from tetra(pentafluorophenyl)porphyrin (H_2TFPP) via a simple reaction in our lab (17) (Steps 1 and 2, Figure 1.). This new compound has an unusually intense farthest red band is usually intense (Figure 2). We further synthesized some of its metal derivatives and studied their spectra (17, 18).

However, this seemingly simple one step reaction not only gave complicated products but the products also varies from batch to batch, as shown by our own work and others (17, 19). As a result, we were unable to resolve all the components with flash chromatography. According to our calculation, tetra(pentafluorophenyl)porphodilactone should be a

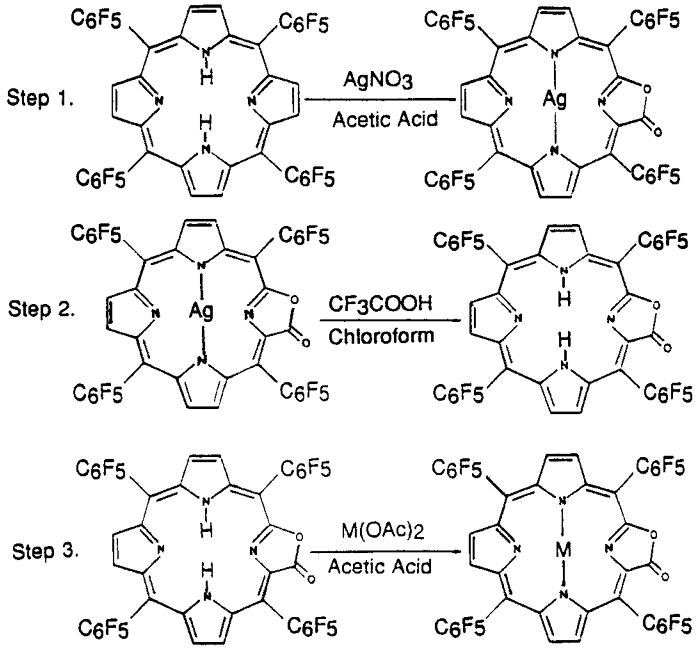

FIGURE 1. Schematic of the synthesis of metallotetra(pentafluorophenyl)porpholactone and its metal derivatives.

stable compound, and we predicated that this molecule may be produced during the reaction (17). Yet so far, we haven't isolated this species. In order to fully characterize the products and understand the mechanism, we need to resolve all the components in the products. Therefore, we decided to pursue the separation of these complexes on HPLC. Since porphyrins have very characteristic absorption in the range from 350nm-700nm, and furthermore, the metal derivatives show a two banded spectra while free bases show a four banded spectra (Figure 2), a UV/VIS detector can be used for both easy detection and analysis of free base porphyrins and metalloporphyrins.

In this paper, we wish to report the HPLC separation and analysis of these metal complexes from their free bases, using a μPorasil column with a hexane and diethyl ether mobile phase.

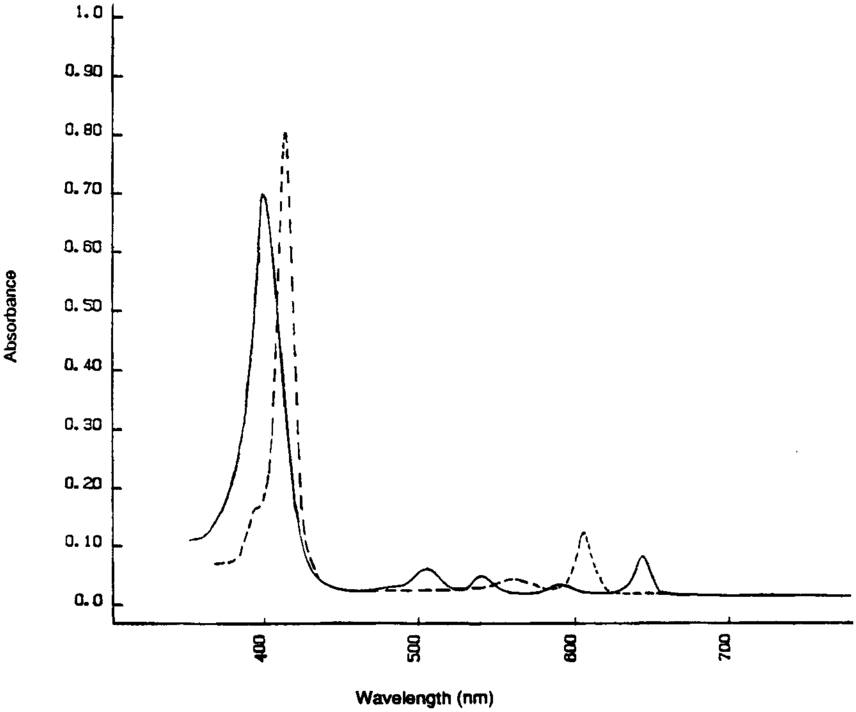

FIGURE 2. Optical absorption spectra of free base H_2TFPL (solid line) and Zn(TFPL) (dotted line) in methylene chloride.

EXPERIMENTAL

Materials

All solvents but diethyl ether were HPLC reagent grade from J. T. Baker and used without further purification. Anhydrous diethyl ether was supplied by J. T. Baker as reagent grade. Flash chromatography grade Silica Gel (100-200 mesh or 70-140 μm with average pore diameter 25 Å) was purchased from Sigma Chemical Company. Tetra(pentafluorophenyl)porphyrin was purchased from Aldrich Chemical Company and purified according to our procedure (18). Silver, zinc, nickel and palladium derivatives of tetrakis(pentafluorophenyl)porpholactone were synthesized according to a procedure described elsewhere (18).

HPLC systems

Metalloporpholactones were separated on a system consisting of two Altex Model 100A pumps with a solvent programmer, a Varian VARI-CHROM variable UV/VIS detector with a plotter and a Hewlett Packard 8452A diode array spectrophotometer. The diode array detector was controlled by an IBM Nec/Multisync 2A computer using a HP-UV program for data acquisition and a coloPro plotter for plotting spectra.

The second system was from Hitachi, which included a Hitachi single L-6200A intelligent pump, a Hitachi L-4250 UV-VIS detector (170 nm - 700 nm) and a Hitachi D-2500 CHROMATO-Integrator. The UV-VIS detector has a stopped flow scanning function.

Columns

The normal phase column was a μPorasil column (150 x 3.9 mm) purchased from Waters. The reversed phase column was a Ultrasphere ODS 5 micro (250 x 4.6 mm) supplied by Beckman. A Direct-Connect TM cartridge guard column system consisting of a direct connect universal guard column cartridge holder and a guard column cartridge was from Alltech Associates. Adsorbsphere Silica 5μ and adsorbsphere C18 5μ guard column cartridges were connected to μPorasil column and Ultrasphere ODS column respectively.

Procedure

Mobile phase was obtained by volume to volume mixing of hexane and diethyl ether. Samples were dissolved in a small amount of mobile phase whenever possible. If the solubility of the sample is poor in the mobile phase, methylene chloride was added to enhance its solubility. All samples were prepurified by flash chromatography using Silica Gel (Sigma) as packing and 9:1 ratio of hexane and methylene chloride as eluents. Sample solution was filtered through a 0.4 μm microfilter. Detector was set at 400nm. A 5 μL sample loop was used for sample injection. Flow rate was set at 1 mL/min.

RESULTS AND DISCUSSION

Silver tetra(pentafluorophenyl)porpholactone (Ag(TFPL)) was synthesized in a procedure as shown in Step 1 of Figure 1 (18). After demetallation, Ag(TFPL) was converted to H_2TFPL (Step 2). Zinc, nickel and palladium were subsequently inserted into the porpholactone ring (Step 3).

HPLC separation of these metalloporpholactones was first tried on reversed phase C18 Ultrasphere ODS column, using 40:60 acetone-acetonitrile as mobile phase according to a procedure described by Suzuki et al (1). However, we were unable to separate these complexes with this mobile phase, not even with a gradient of acetone and acetonitrile. Many other mobile phases have also been tried on this column without good separation.

We then investigated the separation on a normal phase μPorasil (3.9 x 150 mm I. D) column. We eventually came up with an isocratic mobile phase of diethyl ether and hexane. Silver, nickel, palladium and zinc complexes of tetra(pentafluorophenyl)porpholactone were successfully separated from other components with this mobile phase.

HPLC Separation of Ag(TFPL)

A HPLC chromatogram of Ag(TFPL) is shown in Figure 3. The chromatogram showed a total of eight components in the reaction (Step 1). The two major components were identified as free base H_2TFPL and Ag(TFPL). Identifications of peaks were based on two features: (a) optical spectra in each case and (b) FAB-mass spectra in some cases (17, 18). In each of the chromatograms, boldface labels were used if both (a) and (b) are available and standard labels were used if just (a) is available. The optical absorption spectra of Ag(TFPL) is not shown here since its two banded spectra is very similar to that of Zn(TFPL) (Figure 2). The optical absorption spectra of Zn(TFPL) is shown in Figure 2 as an example for all the metal complexes of H_2TFPL. The two minor peaks in the chromatogram were identified as silver tetra(pentafluorophenyl)-porphyrin (Ag(TFPP)) and a tetra(pentafluorophenyl)porphodilactone

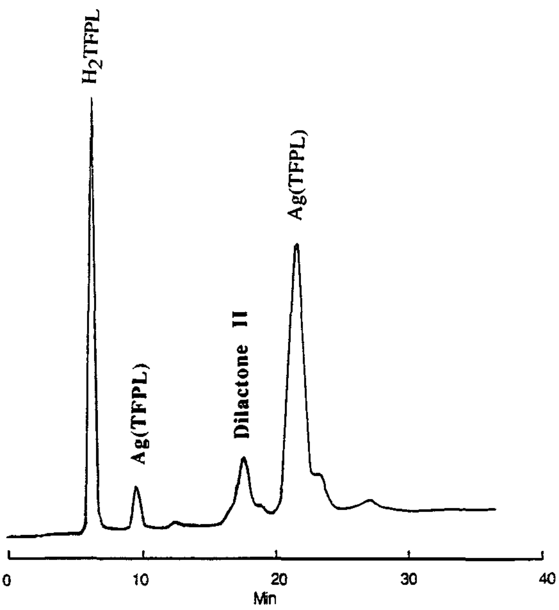

FIGURE 3. HPLC separation of Ag(TFPL) at ambient temperature. Principal peaks are Ag(TFPP), Dilactone II and Ag(TFPL) (see text). Peaks labelled boldface are identified by both optical absorption spectra and FAB-mass spectra.
Mobile Phase: Diethyl ether - hexane 1.5:98.5v/v. Column: Waters µPorasil (3.9 x 150mm I. D.). Detection wavelength: 400nm. Sample loop: 5µl. Flow rate: 1ml/min. * : Not identified.

isomer. We shall call this isomer Dilactone II since we were unable to determine which one of the five isomers it is (17). The other four minor peaks were not identified, although a Soret band at about 400nm for each of them indicates that they are still porphyrin species. The retention time of Ag(TFPL) is 22 minutes with a mobile phase of 98.5% hexane and 1.5% diethyl ether.

HPLC chromatogram of Pd(TFPL)

A HPLC chromatogram of Pd(TFPL) is shown in Figure 4. The chromatogram showed four principal peaks, two of which were identified

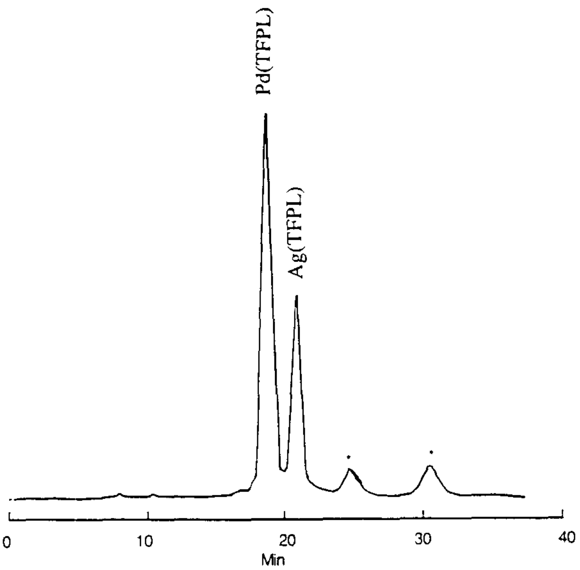

FIGURE 4. HPLC separation of Pd(TFPL) at ambient temperature. All conditions but mobile phase were same as described in Figure 3. Principal peaks are Pd(TFPL), Ag(TFPL) (see text). Mobile Phase: Diethyl ether - Hexane 1:99 v/v. • : Not identified.

as Pd(TFPL) and Ag(TFPL). The two minor peaks were not identified, although they are believed to be porphyrin species as indicated by their Soret bands. No H_2TFPL was detected from this sample, which may indicate that H_2TFPL was totally converted to Pd(TFPL) during the reaction. Again, Pd(TFPL) showed a two banded spectra, similar to that of Zn(TFPL). Pd(TFPL) has a retention time of 18 minutes with a mobile phase of 99% n-hexane and 1% diethyl ether.

HPLC chromatography of Ni(TFPL)

The HPLC chromatogram of Ni(TFPL) showed very complicated products (Figure 5). A total of ten principal peaks and seven minor peaks were detected. Among the ten principal components, four were identified

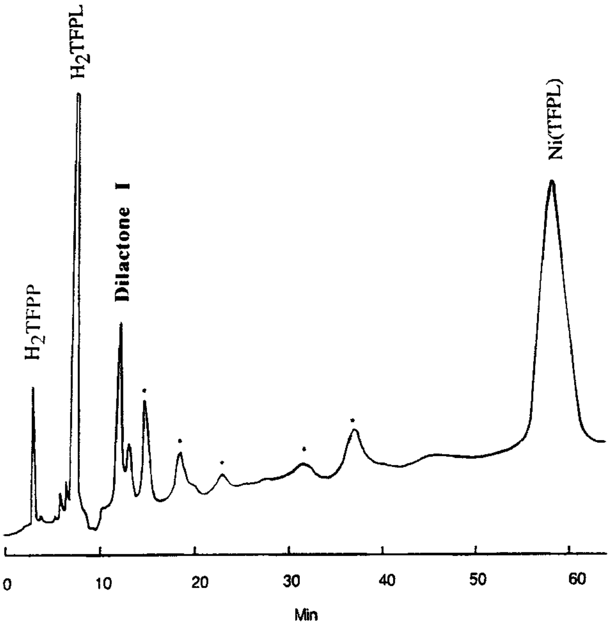

FIGURE 5. HPLC separation of Ni(TFPL) at ambient temperature. All conditions were same as described in Figure 3. Principal peaks are H_2TFPP, H_2TFPL, Dilactone I and Ni(TFPL) (see text).
* : Not identified.

as H_2TFPP, H_2TFPL, a porphodilactone isomer and Ni(TFPL). The other principal peaks were not identified. Again, their optical absorption spectra indicated that all were porphyrin compounds. We were unable to obtain spectra for other minor peaks. The porphodilactone isomer isolated has different absorption spectra from Dilcatone II, and we shall call this isomer Dilactone I. Both isomers were confirmed by their excitation spectra and FAB-mass spectra (18). A fairly large peak of H_2TFPL appeared in the chromatogrm, which indicates that only part of H_2TFPL was converted to Ni(TFPL) in the metal insertion reaction (Step 3, Figure 1). Again, Ni(TFPL) showed a typical two banded spectra, similar to that of Zn(TFPL). The retention time of Ni(TFPL) is very long at 58 min, using 98.5% n-hexane and 1.5% diethyl ether as mobile phase.

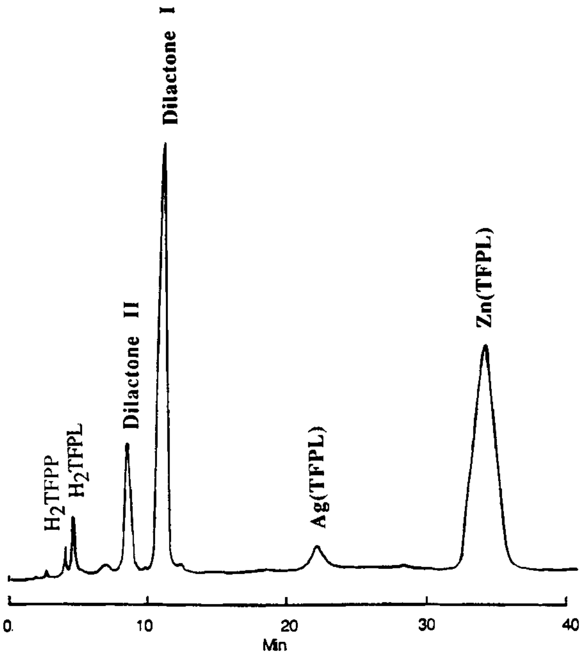

FIGURE 6. HPLC separation of Zn(TFPL) at ambient temperature. All conditions but mobile phase were same as described in Figure 3. Principal peaks are H_2TFPP, H_2TFPL, Dilactone II, Dilactone I, Ag(TFPL) and Zn(TFPL) (see text).
Mobile phase: Diethyl ether - Hexane 2.5:97.5 v/v.

HPLC chromatography of Zn(TFPL)

The HPLC chromatogram of Zn(TFPL) is shown in Figure 6. The chromatogram showed a total of six principal peaks, and all of which were identified as H_2TFPP, H_2TFPL, Dilactone II, Dilactone 1, Ag(TFPL) and Zn(TFPL). Identification of Zn(TFPL) was also confirmed by its FAB-mass spectrum (18). Both dilactone isomers isolated from Ag(TFPL) and Ni(TFPL) samples respectively were found in Zn(TFPL) sample. Only a very small peak of H_2TFPL was detected, which indicates that most free base H_2TFPL was metallated during the reaction (Step 3, Figure 1).

Zn(TFPL) has a retention time of 33 minutes, using a mobile phase of 97.5% n-hexane and 2.5% diethyl ether.

The mobile phases we developed has enabled us to separate Zn(TFPL), Ag(TFPL), Ni(TFPL) and Pd(TFPL) from their free bases successfully. In addition, we also isolated two porphodilactone isomers, a result we were unable to achieve with flash chromatography. This demonstrated the power of HPLC to the separation and characterization of these complicated compounds. Unfortunately, we could not obtain enough sample for the dilactone isomers on a analytical HPLC column to study their NMR.

SAFETY NOTES

Diethyl ether is potentially explosive, and therefore should be handled with great caution. HPLC instrument should be operated in a cool and well ventillated room. Waste mobile phase should be properly disposed.

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