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Chromatographic behaviour of the chromium(III) complex of 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone

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Abstract

The separation of the two geometrical isomers of the chromium(III) complex of 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone was carried out using acetonitrile–water (90:10, v/v) and *n*-hexane–tetrahydrofuran (70:30, v/v) as the mobile phases for an ODS column and a silica gel column, respectively. Identification of the isomers was performed using a high-performance liquid chromatography–continuous-flow fast atom bombardment–mass spectrometry system. It was found that the *fac* complex eluted first, followed by the *mer* complex on the ODS column. © 1997 Elsevier Science B.V.

Keywords: 1-Phenyl-3-methyl-4-benzoyl-5-pyrazolone; Metal complexes; Chromium complexes

1. Introduction

The role of high-performance liquid chromatography (HPLC) technology for the analysis of trace inorganic compounds has been well recognized, due to its ability to simultaneously determine multi-elements and to provide information on the speciation of a certain element. Many detection techniques, including UV–Vis and atomic absorption spectrometry (AAS) have been used to determine the desired elements after HPLC separation.

So far, several reviews [1–4] on the separation and determination of metals using chelating agent have been published. We have previously investigated [5,6] the chromatographic behavior of Al, Ga, In and Fe using 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone (PMBP) as a pre-column derivatizing agent. PMBP is an excellent complexing agent of metals due to the high reactivity and molar absorptivity of its complex in the wavelength region of about 240–340 nm. Consequently, PMBP may be quite a good reagent for the chromatographic determination of trace metals.

In this study, we investigated the chromatographic behaviour of the $Cr(PMBP)_3$ complex using ODS and silica gel as the stationary phase. This chelate was easily synthesized by mixing it with PMBP dissolved in methanol and chromium chloride dissolved in water. Although various methods using HPLC have been reported [7–9] for the determination of chromium, we thought that it would be interesting to study the separation mechanism for

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geometrical isomers of the $Cr(PMBP)_3$ complex, using silica gel and ODS columns.

2. Experimental

PMBP was purchased from Hayashi Pure Chemicals, Japan. All other reagents and solvents were of analytical-reagent grade and were obtained from commercial sources. The $Cr(PMBP)_3$ complex was prepared as described below, and the chemical purity of the substance was checked by elemental analysis.

About 1.0 g of chromium chloride was dissolved in 100 ml of water. The solution was adjusted to about pH 4 with sodium acetate. Then the PMBP–methanol solution was added in a molar ratio of 1:3 (metal to PMBP) to the chromium solution. The mixture was allowed to react thoroughly in a water-bath at 80°C, with agitation, for 1 h. The precipitates were washed with water after filtration. The products were then agitated in methanol by stirring, to remove the unreacted PMBP. After separation of the two phases, the refining process was repeated five times. The precipitates were dried at 80°C in vacuo for 2 h and used as our testing chelate. Its composition was determined by elemental analysis to be Cr–PMBP= 1:3; Found: C, 69.07; H, 4.39; N, 9.48; O, 10.92; Cr, 5.49. Calculated: C, 69.30; H, 4.45; N, 9.51; O, 10.86; Cr, 5.88. Elemental analysis for C, H, O and N was performed using a Heraeus CHN–O-Rapid analyzer. Chromium was determined by AAS.

Two different chromatographic systems were used for this study. In system I, the measurements were performed using a JEOL LC–MS system consisting of a Model HP-1090L liquid chromatograph equipped with a 150×4.6 mm I.D. Inertsil ODS-2 column and a Model JMS-AX 505W mass spectrometer. A JMA-DA 7000 data system was used for data acquisition and editing. LC separation were carried out using acetonitrile–water (85:15, v/v) as the mobile phase at a constant flow-rate of 1.0 ml min^{-1} at 40°C. The wavelength control of the spectrometer was set at 250 nm.

After passing through the UV detector, the effluent from the column was mixed with a 3% glycerol–methanol solution and introduced into the interface region at a flow-rate of 0.3 ml/min. The MS conditions were as follow: Accelerating voltage, 3 kV; conversion dynode voltage, −10 kV; resolution, 1000. The mass spectrometer was scanned from 50 to 1500 a.m.u. (positive ion detection). The fast atom bombardment (FAB) gun (JEOL) was operated at 5 kV with xenon.

In system II, the HPLC system consisted of a JASCO Model 880-PU pump and a Model 875 UV spectrometric detector. The recorder was a Model 807IT integrator. The chromatographic columns used were Chemcosorb 5-ODS-UH (150×4.6 mm I.D.) and Fine Pak SIL (250×4.6 mm I.D.).

The $Cr(PMBP)_3$ complex was dissolved in dioxane and the solution was diluted with acetonitrile or *n*-hexane to give the appropriate concentration. Dioxane was used initially as the solvent for dissolution of the solid $Cr(PMBP)_3$ complex as it was better than other solvents, such as acetonitrile or methanol, at dissolving metal–PMBP complexes.

Chromatographic separation of the $Cr(PMBP)_3$ complex was carried out at 40°C, using acetonitrile–water (90:10, v/v) and *n*-hexane–THF (70:30, v/v) as the mobile phases for the ODS column and the silica gel column, respectively. The flow-rate of the mobile phase was fixed at 1.0 ml min^{-1}. A 20-μl volume of the solution was injected, using a loop injection in all cases. The wavelength control of the spectrometer was set at 250 nm.

3. Results and discussion

The $Cr(PMBP)_3$ complex shows a maximum absorption at 250 nm. The molar absorptivity for the $Cr(PMBP)_3$ complex in dioxane at 250 nm, calculated from the spectrum, was found to be $5.2 \cdot 10^4$ dm^3 mol^{-1} cm^{-1}, indicating the sensitive determination of the chromium. On reversed-phase chromatography, the ODS column was investigated using pure acetonitrile, methanol and a mixture of them as the mobile phase. As shown in Fig. 1a, the complex eluted within 25 min with acetonitrile–water (90:10, v/v) solution as the mobile phase. The PMBP peak appeared at 2.5 min and was found to be broad with severe tailing.

Two peaks were observed for $Cr(PMBP)_3$, regardless of its high purity, indicating the presence of two components, however, the separation of these chelates was not sufficient when pure methanol or

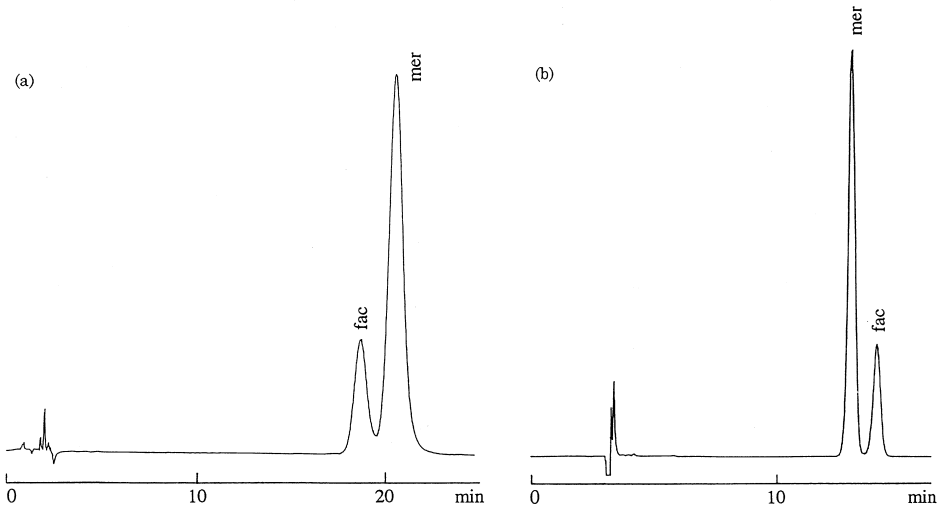

Fig. 1. Separation of the geometrical isomers of the $Cr(PMBP)_3$ complex. (a) Eluent: $CH_3CN–H_2O$ (90:10, v/v); flow-rate: 1.0 ml min^{-1}; column: Chemcosorb 5-ODS-UH (150×4.6 mm I.D.); detector: UV (250 nm); $Cr(PMBP)_3=3.8\cdot10^{-5}$ *M*. (b) Eluent: *n*-hexane–THF (70:30, v/v); flow-rate: 1.0 ml min^{-1}; column: Fine Pak SIL (250×4.6 mm I.D.); detector: UV (250 nm); Cr $(PMBP)_3=3.8\cdot10^{-5}$ *M*).

acetonitrile was used as the mobile phase. The effect of the water content in acetonitrile on the separation of the complex was investigated in the range of 0–15% (v/v). The retention time of the complex increased with increasing water content. Relatively good separation of the two components of the $Cr(PMBP)_3$ complex was obtained with a water content of above 10% (v/v) in the mobile phase.

In previous papers [5,6], we reported that the PMBP complexes of trivalent cations such as Al, Ga and In are partly decomposed during the chromatographic process when a small amount of water is present in the mobile phase. However, the $Cr(PMBP)_3$ and $Fe(PMBP)_3$ complexes did not decompose, even when the mobile phase contained 10% water. The stability of these complexes on chromatographic analysis follows the sequence In< Ga<Al, in accordance with their decreasing ionic radii. This is probably due to the stronger interaction between PMBP and Cr^{3+} ion, which has a shorter ionic radius than the above metals. The two components of the complex were separated and their absorption spectra were measured using the LC–UV system. Differences were not observed for their UV absorption spectra. A straight line was obtained between the concentrations of the $Cr(PMBP)_3$ complex (*mer*) and the peak area over the range studied ($1.1\cdot10^{-6}$–$2.0\cdot10^{-4}$ *M*).

Mass spectra was also measured for these components using a combined liquid chromatograph and mass spectrometer, as shown in Fig. 2. The most prominent ions were *m*/*z* 884, 698, 606, 530, 421, 329, 241, 165, 105 and 76. The fragment ions at *m*/*z* 606 and 329, respectively, correspond to the loss of one and two PMBPs from the molecular ions at *m*/*z* 884, respectively. The ion at *m*/*z* 530 arises from the elimination of C_6H_5 from the $Cr(PMBP)^{2+}$ ion. The fragment ions at *m*/*z* 279 and at 105 correspond to H_2PMBP^+ and $C_6H_5CO^+$, respectively. The molecular ion at *m*/*z* 884 was observed in both chromatographic peaks of component A (former peak) and component B (latter peak), indicating that the molecular weight of component A is the same as that of component B. From these mass spectra and UV absorption spectra, we concluded that the geometrical isomers of $Cr(PMBP)_3$ had been separated. Three bidentate ligands containing the substituent groups OH and C=O coordinate with a metal ion in the manner illustrated in Fig. 3.

It has been reported [10] that the *mer* isomer is eluted first, followed by the *fac* isomer using a silica gel column. When the silica gel column was used for

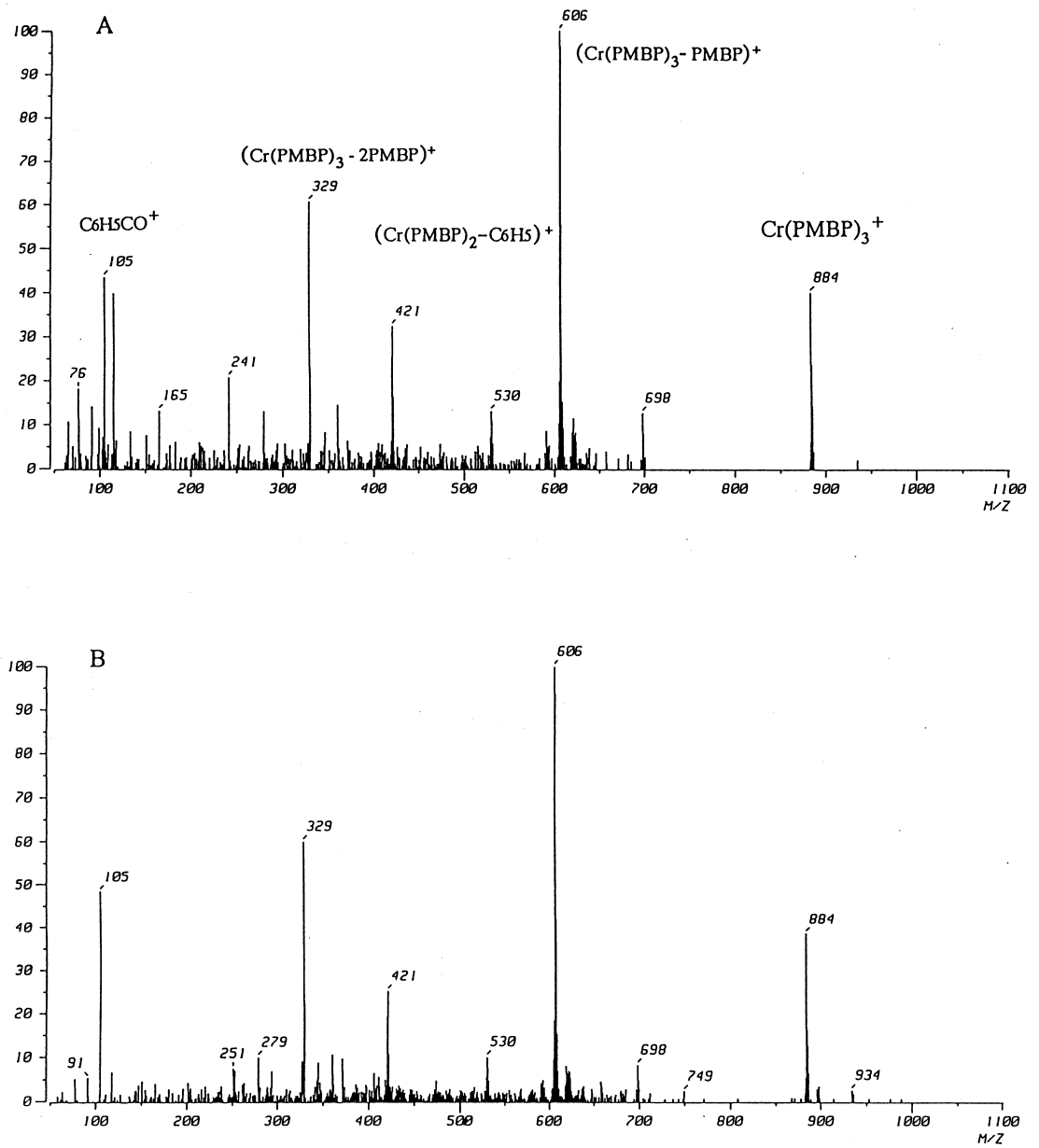

Fig. 2. Mass spectra of the geometrical isomers of the $Cr(PMBP)_3$ complex. (A) the facial isomer and (B) the meridional isomer.

the $Cr(PMBP)_3$ complex, complete separation of the geometrical isomers was also achieved using *n*-hexane–THF (70:30, v/v) as the mobile phase, as shown in Fig. 1b. In both separation systems (ODS and silica gel columns), the complex resolved into two peaks with an area ratio of approximately 3:1 (*mer* to *fac*). Furthermore, the peak elution order is found to be the reverse of that obtained using the ODS column. Palmar et al. [11] suggested that the order of elution depends on the dipole moments of the isomers. Although the geometrical isomers of the $Cr(PMBP)_3$ complex are electrically neutral, the *fac* isomer has a greater dipole moment than the *mer* isomer. Thus, the *fac* isomer is considered to interact

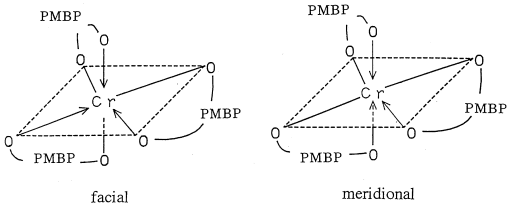

Fig. 3. Configurations of the *fac*- and *mer* isomers of the $Cr(PMBP)_3$ complex.

more strongly with the silanol group of the silica gel than the meridional isomer. Thus, the *mer* isomer elutes before the *fac* isomer.

The fractions containing the first and second peaks were collected separately and concentrated using a rotary evaporator. The residues were dissolved in *n*-hexane and then chromatographic analysis was carried out on the silica gel column using *n*-hexane–THF (70:30, v/v) as the eluent. It was found that the isolated complex showed the two peaks after about 4 h progress with its geometrical isomers.

In conclusion, the good separation and quality of the geometrical isomers for $Cr(PMBP)_3$ could have been achieved using the HPLC continuous flow-FAB–MS system. It was found that the *mer* isomer is eluted first, followed by the *fac* isomer on reversed-phase LC. Furthermore, this study may be used for the sensitive determination of trace chromium, due to the high molar absorptivity of the $Cr(PMBP)_3$ complex.

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