nocarboxylate segment)/K(electrostatic) and k_n is the rate constant for the nickel–nitrogen or nickel–water bond-breaking process corrected for rotation barrier involved in the particular case.[4] By using eq 9 for calculation of k and comparing it with k(exptl), we could reach the conclusion arrived at by Rorabacher and Margerum[4] that, in all probability, the rate-determining step is where four nitrogens of Tetren and one acetate of aminocarboxylate is coordinated to Ni^{2+}. The rate-determining step thus appears to be the loss of a water molecule followed by coordination of the first nitrogen of TMDTA or HEEDTA.

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Preparative High-Pressure Liquid Chromatographic Separation of Cobalt(III) Coordination Complexes

Brian D. Warner* and J. Ivan Legg*

We have previously reported[1] a versatile solvent system that has proven extremely useful for the analytical and preparative thin-layer chromatographic (TLC) separations of a variety of cobalt(III) complexes and azodye ligands.[1,2] By extending the previous results to include high-pressure liquid chromatographic techniques (HPLC), it was assumed that *any* type of separation problem could be approached in a systematic way by using a combination of TLC and HPLC methods. Analytical TLC would be used to rapidly define reaction conditions favorable to product formation. The TLC results could then be directly applied to preparative isolation procedures, since all of the chromatography would be performed on the same type of stationary phase with the same solvent system. This is in direct contrast to the way most complexes have been isolated in our laboratory. Even when a suitable TLC separation can be performed on a reaction mixture, preparative TLC has not always provided sufficient material for subsequent manipulations. In most cases ion exchange chromatography on polystyrene or dextran stationary phases must be performed. In these separations the elution order of the various compounds, and the resolution exhibited, does not necessarily follow the TLC results. The colors of the metal complexes on different stationary phases are not always comparable, complicating the isolation procedure. Furthermore, for complexes with a charge greater than 1, or for the case where aromatic groups are present in the ligands, high salt concentrations (>1 M) are required for elution. The use of these high ionic strengths often degrades resolution and lengthens the isolation procedure due to the desalting that is required.

Though cobalt(III) complexes have been separated by HPLC,[3,4] a general preparative method that would be useful to synthetic inorganic chemists has not appeared. This may be due in part to the theoretical nature of the publications

dealing with the preparative separations of nonpolar comounds that appear in the chromatography literature or to the chromatographer's preoccupation with expensive, complicated equipment.[5,6]

This study describes a "bare bones" liquid chromatograph and a "new" sample introduction method. The results of three distinctly different types of separations are presented: different comounds, geometrical isomers, and diastereomers with and without aromatic functionality. From these data some conclusions can be drawn concerning the present method's speed, resolution, and capacity.

Experimental Section

Reagents. All reagents used in this study were at least of reagent grade. The preparation of the 70/30 isopropyl alcohol–2 M pH 9 triethylammonium bicarbonate buffer system (abbreviated 70/30 IS–TEA) has been described previously.[1]

Separations. The majority of the separations were first performed on a programmable gradient liquid chromatograph described elsewhere.[7] A 1 × 100 cm column was constructed from 0.500-in. o.d. × 0.049-in. wall, 304 stainless steel hydraulic tubing (purchased locally), and Swagelok SS-810-6-1-SR-15 $^1/_{16}$–$^1/_2$ in. reducing unions which come fitted with 10-μ frits. A 500-mL packing reservoir (a Whitey gas sampling cylinder, part number 304-HDF4-500) was adapted to the column and filled with a slurry consisting of 200 g of Whatman LP-1, 10–20 μ of silica, and 500 mL of 70/30 IS–TEA. This slurry was packed at approximately 20 mL/min (3500–4500 psi) for 30 min. After the reservoir was removed, the column was connected to an ISCO UA-5 monitor fitted with filters for operation at 510 nm.

Samples from 1–3 mL (solute concentration 50–100 mg/mL) were applied to the column by placing the sample loop of the sample injection valve in the solvent flow stream with the pump's flow rate control set to 0. The flow rate was then gradually increased to the final value, usually 4–8 mL/min, over a 10–20-min period with use of a flow program.[7]

Strongly retained compounds ($R_f \approx 0$ on TLC) were removed from samples prior to introduction to the liquid chromatograph by prechromatography on 60–200 mesh silica (Baker) packed in 30-mL disposable syringe bodies with the use of 70/30 IS–TEA as eluant. Eluant was pulled across the precolumn by vacuum into a filter flask. Samples were then concentrated on a rotovap at 30–40 °C. Prior to injection all samples were filtered to 0.45 μ with a Millipore inline filter unit, as previously described.

A considerably simpler, less expensive, liquid-chromatography system capable of reproducing the separations developed above, but on a larger scale, was also constructed. It consisted of a Milton Roy instrument minipump connected with a 0.0625-in. o.d. Teflon tubing and miniature tubing fittings (both Altex and Unimetrics brands have been used) to a Merk 4 × 40 cm "Lo-Bar" column body (similar to Michel-Miller glass columns manufactured by Ace Glass, Vineland, NJ).

The column was slurry packed with Whatmen LP-1 silica and 70/30 IS–TEA (100 g silica plus 100 mL eluant) at approximately 8 mL/min (200–250 psi). Since a packing reservoir was not available for this column, packing was accomplished in two or three steps, stopping where the column body began to taper to its upper flow adapter. Although contrary to normal packing procedure, excellent results were obtained, with no discontinuities in the bed observed, as compounds were eluted across regions where the packing had been interrupted. (Packing reservoirs are available for the Michel-Miller type columns allowing them to be packed in a single step.)

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(3) P. Heizmann and K. Ballschmiter, *J. Chromatogr.*, **137**, 153 (1977).
(4) G. G. Strazza and C. M. Polcaro, *J. Chromatogr.*, **147**, 516 (1978).
(5) D. R. Baker, R. A. Henry, R. C. Williams, D. R. Hudson, and N. A. Parris, *J. Chromatogr.*, **83**, 233 (1973); E. Godbille and P. Devaux, *J. Chromatogr. Sci.*, **12**, 564 (1974); H. C. Beachell and J. J. DeStefano, *ibid.*, **10**, 481 (1972); R. P. W. Scott and P. Kucera, *J. Chromatogr.*, **119**, 467 (1976); E. Godbille and P. Devaux, *ibid.*, **122**, 317 (1976); B. Coq, G. Cretier, C. Gonnet, and J. L. Rocca, *Chromatographia*, **12**, 139 (1979); H. Loibner and G. Seidl, *ibid.*, **12**, 600 (1979).
(6) B. Coq, G. Cretier, and J. L. Rocca, *J. Chromatogr.*, **186**, 457 (1979).
(7) B. D. Warner, G. H. Boehme, and J. I. Legg, *J. Chromatogr.*, in press.

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Samples, treated as described above, were layered directly onto the column bed in the void created during packing. Up to 10 mL of 100–500 mg/mL solutions were applied in this way. So that the flow program sample application procedure described above could be approximated, the pump's flow rate was increased to its final value (usually 2–4 mL/min) in several discrete steps over approximately a 10-min period. Visual inspection and TLC[1] were used to ascertain the purity of bands eluted from this column.

Some chromatography on the diastereomers was also performed with a Waters Associates Prep 500 liquid chromatograph. Essentially identical results were obtained with this unit as with the aforementioned equipment.

Complex Ion Syntheses. The complex ions [Co(EDDA)(en)]$^+$ and [Co(en)$_2$(Asp)]$^{2+}$ were synthesized by the methods of Halloren[8] and Keyes,[9] respectively. Since one of the goals of this study was to purify compounds directly from reaction mixtures, no attempt was made to isolate these complexes prior to chromatography. The presence of the complex ions in their respective mixtures was confirmed by TLC against known standards.

The method employed by Keyes[9] for the synthesis of many other amino acid (aa) complexes of the type, [Co(en)$_2$(aa)]$^{2+}$, was modified due to the poor solubility of tyrosine in water. It was found that a mixture (1:1) of 1-formyl-N-methylpiperidine and water could be substituted into the original procedure, producing a majority of [Co(en)$_2$(Tyr)]$^{2+}$, as evidenced by its orange spot on TLC, $R_f \approx 0.15$. Chemical analysis of the pure compound, isolated by rotovapping the orange band eluted from the HPLC to dryness, indicated that the phenolic proton on the tyrosine had been replaced with a triethylammonium cation and that the remaining counterions were bicarbonate. Anal. Calcd for CoC$_{21}$H$_{43}$N$_6$O$_9$: C, 43.31; H, 7.39; N, 14.44. Found: C, 42.84; H, 8.19; N, 14.63. The absorption spectrum for this complex (ϵ_{486} 126, ϵ_{344} 322) was very siimilar to those reported for analogous amino acid complexes.[9]

Results and Discussion

Initial attempts to apply TLC results to liquid chromatographic separations revealed several problems. Central among these were sample preparation and application. A method employing flow programming to introduce samples to LC columns reproducibly without solute bleed-through or precipitation, originally developed for azodye reaction mixtures ranging in size from 50 to 100 mg,[7] was not entirely successful when applying 1–5 g quantities of Co(III) complex mixtures. This problem seemed to be due to uneven sample application with the flat-flow adapters present in many commonly available column bodies. It was thought that a column body with conical top and bottom regions might produce better sample loading. But when the sample was applied with a flow program to such a column, even worse results were obtained. This dilemma was rectified by removing the top few centimeters of the column bed and layering the sample directly onto the packing material with a disposable pipet, as is often done in conventional open-column liquid chromatography. The sample was then eluted into the packing material with a flow program consisting of a few discrete changes in flow rate (see Experimental Section). Subsequent to this discovery it was found that another group studying sample loading in preparative HPLC had evaluated a variety of sample injection schemes.[6] Their data suggested that application of the sample into a void above the column bed was one of the most efficient methods.

One of the primary goals of this study, the rapid resolution of diastereomers, has only been partially successful. A representative separation (Figure 1) showing the two diastereomeric forms of [Co(en)$_2$(Tyr)]$^{2+}$ completely resolved at analytical loadings (~1 mg), but less retained and poorly resolved when applied in quantity (100–200 mg), was also typical of the chromatographic behavior of [Co(en)$_2$(Asp)]$^{2+}$. These results were not improved by repeating the separation on a
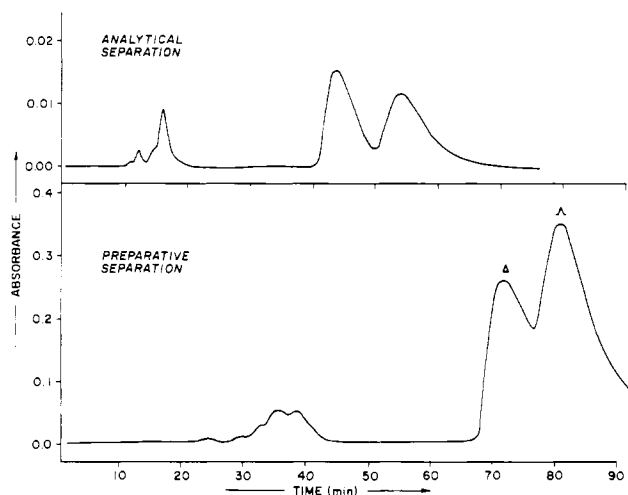

Figure 1. Analytical and preparative separations of [Co(en)$_2$(Tyr)]$^{2+}$ diastereomers. Analytical separation conditions: flow rate was 6 mL/min; approximately 2 mg of mixture was applied in a 20-μL sample volume; elution monitored at 510 nm. Preparative separation conditions: flow rate was 3 mL/min, approximately 100 mg of mixture was applied in a 500-μL sample volume with a 20-min flow program, elution monitored at 510 nm. Both samples were applied to the 1 cm × 1 m stainless steel column.
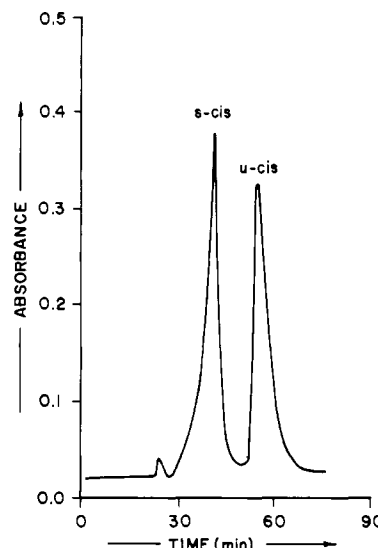

Figure 2. Preparative separation of [Co(EDDA)(en)]$^+$ geometrical isomers. Separation conditions: flow rate was 3 mL/min, approximately 150 mg of mixture was applied in a 1-mL sample volume with a 10-min flow program, elution monitored at 510 nm. Identities of the two isomers were verified by comparison with known isomers on TLC.

Waters Associates Prep 500 HPLC.

Even though complete resolution was not accomplished for either pair of diastereomers, the compounds were rapidly separated from several other components in the mixture, and mirror image circular dichromism (CD) spectra were generated. In each case the first band has a negative CD spectrum corresponding to the Δ isomer (λ_{max} 508–510 nm), while the second band has a positive CD peak, each about half the expected intensity.[9] This is a marked improvement over preparative TLC which could only produce material comparable in purity to the first band.[1] The trailing edge of the preparative TLC band never generated the characteristic positive CD band observed here. It should also be noted that conventional ion exchange chromatography with 1 M NaClO$_4$ solution of [Co(en)$_2$(Tyr)]$^{2+}$ gave no detectable isomer resolution.

(8) L. J. Halloran and J. I. Legg, *Inorg. Chem.*, **13**, 2193 (1974).
(9) W. E. Keyes and J. I. Legg, *J. Am. Chem. Soc.*, **98**, 4970 (1976).

The remaining goal of separating geometrical isomers has been successfully achieved (Figure 2). The sym-cis and uns-cis isomers of [Co(EDDA)(en)]⁺ were rapidly eluted completely resolved from one another and in the same order as on TLC.

The loss of retention and resolution which was seen in the diastereomer separations upon increased loading occurs when the sample's adsorption isotherm is nonlinear.[10] The isotherm describes sample partitioning between the mobile and stationary phases as a function of the amount of sample applied. It is always advisable to operate in the linear portion of the isotherm so that retention (and hence resolution) are invariant with sample loading. Isotherm nonlinearity is an inherent property of each sample, solvent system, and stationary phase combination. Therefore a change in the solvent system is usually necessary to modify the isotherm so that larger samples can be accomodated. Lowering the polarity of the IS–TEA solvent system does not improve resolution and in many cases

causes the sample to precipitate out of solution on contact with the mobile phase. We have yet to find another solvent system with comparable resolving power. Unless one can be found, 5–10-μ silica packing materials will be required to improve resolution greatly, increasing operating expenses and difficulties.

Registry No. [Co(en)₂(Tyr)](HCO₃)₂·NEt₃, 76705-33-4; Λ-[Co(en)₂(Tyr)]²⁺, 76740-68-6; Δ-[Co(en)₂(Tyr)]²⁺, 76740-69-7; *sym-cis*-[Co(EDDA)(en)]⁺, 20084-73-5; *uns-cis*-[Co(EDDA)(en)]⁺, 28459-65-6; [Co(en)₂(Asp)]²⁺, 76705-34-5.

(10) Lloyd R. Snyder, "Principles of Adsorption Chromatography", Marcel Dekker, New York, 1968, pp 22–52.

Correspondence

## Nature of the Highest Occupied Molecular Orbital in R₂C₂Co₂(CO)₆

Sir:

Efforts have been made to rationalize the behavior of compounds containing metal–metal (M–M) bonds in terms of properties associated with the M–M interaction.[1] As numerous examples of bridged dinuclear complexes exist, these compounds have played a prominent role in the investigations of systems containing a M–M bond. Experimental[2,3] studies as well as theoretical work[4–7] indicate that the LUMO in these systems is a M–M antibonding orbital of b₂ symmetry. On the other hand, we have pointed out that for S₂Fe₂(CO)₆, at least, there is no ionization associated with a M–M bonding orbital that is strongly favored in an energetic sense over electron removal from the other metal orbitals.[8] Recent SCF-Xα-SW calculations[9] support this conclusion and the previous suggestion that the HOMO is an orbital having M–M bonding character and little contribution from the bridging ligand. At face value, this would agree with the model for the redox behavior of compounds containing a M–M bond in which the primary effect of oxidation is a reduction in the M–M bond strength.[1] However, the latest calculations[9] also demonstrated an intimate role for the bridging ligand in the net M–M interaction, thereby suggesting that orbital ordering might well be significantly different with ligands of lower electronegativity. In the following, we demonstrate experimentally that the HOMO in R₂C₂Co₂(CO)₆ is a bridging ligand–metal orbital rather than a "simple" M–M bonding orbital. This shows that the nature of the highest filled metal orbitals in bridged dinuclear metal complexes is a sensitive function of the bridging atoms and oxidative behavior in these compounds cannot be simply described in terms of a perturbation of the M–M interaction.

The nature of the HOMO in (R₂C₂)Co₂(CO)₆ is revealed by shifts in ionizations associated with electron removal from bridging ligand–metal orbitals caused by changing R. The two bands in the gas-phase UV photoelectron spectrum of pertinent interest here are shown in Figure 1.[10] These bands correspond to the ionization of MO's with large Co 3d character and in the compound with R = H band 1 contains one metal–metal bonding (a₁), two bridging ligand–metal bonding (a₂, b₁), and six metal "nonbonding" (2a₁, 2b₂, a₂, b₁) ionizations while band 2 contains two bridging ligand–metal bonding (a₁, b₂) ionizations.[11] This assignment is based on the relative intensity of all observed bands, relative intensity measurements as a function of photon energy, and the spectra of model compounds, e.g., Co₂(CO)₈, as well as simple orbital arguments. General band character as well as ordering has been verified with the use of nonparameterized calculations.[12] The a₁ metal–metal bonding and a₂, b₁, a₁, b₂ bridging ligand–metal orbitals constitute five of the six orbitals providing the primary bonding of the C₂Co₂ core, the last four correlating with the π* and π orbitals of free acetylene.[7] The ionizations corresponding to these last four orbitals are expected to be sensitive to π substituents, i.e., substituents with filled orbitals having π symmetry with respect to the C–R axis.

Substituent effects in photoelectron spectra are described in a particularly simple fashion by using a filled orbital model (LCBO–MO),[13] essential features of which appear in I. (The

I

$$-\beta \cdot \left[\Delta E^2 + E \Delta E\right]^{1/2}$$
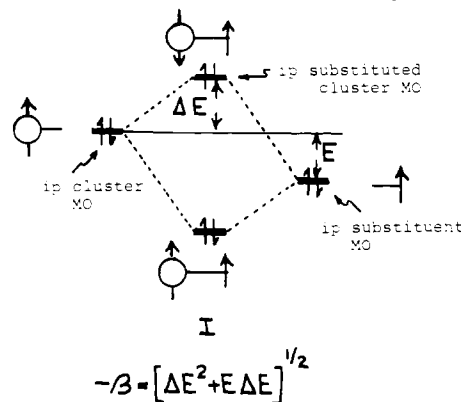
(1) See for example: Meyer, T. J. *Prog. Inorg. Chem.* **1975**, *19*, 1. Vahenkamp, H. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 379.
(2) Ginsburg, R. E.; Rothrock, R. K.; Finke, R. G.; Collman, J. P.; Dahl, L. F. *J. Am. Chem. Soc.* **1979**, *101*, 6550.
(3) Peake, B. M.; Rieger, P. H.; Robinson, B. H.; Simpson, J. *J. Am. Chem. Soc.* **1980**, *102*, 156.
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(7) (a) Thorn, D. L.; Hoffmann, R. *Inorg. Chem.* **1978**, *17*, 126. (b) Anderson, A. B. *Ibid.* **1976**, *15*, 2598.
(8) Andersen, E. L.; Fehlner, T. P. *Inorg. Chem.* **1979**, *18*, 2325.
(9) Andersen, E. L.; Fehlner, T. P.; Foti, A. E.; Salahub, D. R. *J. Am. Chem. Soc.* **1980**, *102*, 7422.

(10) These spectra were obtained with He(I) radiation at a sample temperature of 25 °C.
(11) This nomenclature is drawn from Thorn and Hoffmann (ref 7) and constitutes oversimplification of the situation; e.g., in S₂Fe₂(CO)₆ there is a distinguishable a₁ M–M bonding orbital whereas in the isoelectronic B₂H₆Fe₂(CO)₆ there is not. However, for the purposes of this discussion it is retained.