Ligand Exchange Chromatography of Amino Alcohols. Use of Schiff Bases in Enantiomer Resolution

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Abstract: A new method for enantiomeric resolution of primary amino alcohols by HPLC is described involving derivatization to the salicylaldehyde Schiff base followed by ligand exchange chromatography (LEC) with chiral L-proline-bonded stationary phases. All of the α-amino alcohols with a hydroxyl substituent on the asymmetric carbon examined were resolved, including catecholamines and other β-hydroxyphenethylamines. The copper(II) ion employed in LEC serves to stabilize the Schiff base, whereas the derivative permits complexation in a manner favorable for resolution and enhanced detection. A possible structure for the mixed complex responsible for separation is suggested. From this structure, a correlation between elution order and configuration is proposed.

Ligand exchange chromatography (LEC), as shown by Davankov and other workers, has proven to be a very powerful tool for resolving enantiomers, achieving high selectivity and specificity.[2] Its use in this application has, however, been mainly limited to α-amino or α-hydroxy carboxylic acids.[3] Nevertheless, because of its potential for significant chiral resolution, extension of this technique to other classes of compounds would be valuable in both the analytical- and preparative-scale separation of optical isomers. This extension can most easily be accomplished through the formation of derivatives with appropriate chelating properties.

This paper reports on the successful chiral separation of primary α-amino alcohols, including the biologically important β-hydroxyphenethylamines and catecholamines by LEC using a chiral L-proline chelate bonded phase. The resolution is accomplished through derivatization of the racemic amino alcohols to a Schiff base with salicylaldehyde. Such Schiff bases are well-known to form stable copper complexes, even at acidic pH.[4] While these derivatives undergo rapid hydrolysis in aqueous media, we have found that the presence of copper(II) stabilizes the compounds, analogously to amino acid Schiff bases.[5] A possible structure of the complex responsible for separation and elution order is discussed.

Enantiomeric resolutions of some α-amino alcohols by HPLC have been accomplished using other derivatives and chiral bonded stationary phases.[6] However, these procedures have not been used to resolve the more polar and unstable catecholamines. In addition, the methods suffer from either complicated derivatization using toxic reagents or low chiral recognition. In the present work, simple derivatization with good separation using LEC is accomplished.

Experimental Section

Materials. *dl*-Phenylethanolamine [*dl*-2-amino-1-phenylethan-1-ol], *dl*-phenylpropanolamine hydrochloride [*dl-erythro*-2-amino-1-phenylpropan-1-ol hydrochloride], (+)-phenylpropanolamine hydrochloride, (−)-pseudophenylpropanolamine hydrochloride [(−)-*threo*-2-amino-1-phenylpropan-1-ol hydrochloride], *dl*-norepinephrine [*dl*-2-amino-1-(3,4-dihydroxyphenyl)ethan-1-ol], (−)norepinephrine, *dl*-nordefrin hydrochloride [*dl-erythro*-2-amino-1-(3,4-dihydroxyphenyl)propan-1-ol hydrochloride], (−)-nordefrin, *dl*-normethanephrine [*dl*-1-(4-hydroxy-3-methoxyphenyl)-2-aminoethan-1-ol], and 1-eicocene were obtained from Sigma Chemical Co. *dl*-1-Aminopropan-2-ol, (*R*)-(−)-1-aminopropan-2-ol, *dl*-alaninol [*dl*-2-aminopropan-1-ol], L-alaninol, *dl*-valinol [*dl*-2-amino-3-methyl-1-butanol], L-valinol, 10-undecenal, and hydrogen hexachloroplatinate(IV) were obtained from Aldrich Chemical Co. *dl*-Nordefrin was also provided by Sterling Winthrop. Salicylaldehyde was Fisher purified grade and was used as supplied. L-Proline *tert*-butyl ester was obtained from Chemical Dynamics Corp. and dimethylchlorosilane from Petrarch Systems Inc. The mixture of *dl*-phenylpropanolamine and *dl*-pseudophenylpropanolamine was kindly supplied by J. D. Freilich, W. R. Grace and Co. (Nashua, NH). All solvents were HPLC grade. Other chemicals were ACS reagent grade. Elemental analysis was performed by Galbraith Laboratories Inc., Knoxville, TN.

Instrumentation. ^{1}H NMR spectra were taken with a Varian T-60 spectrometer, EI mass spectra with a Nuclide 12-90-G 70 eV mass spectrometer, infrared spectra with a Perkin-Elmer Model 467 spectrophotometer, melting points (uncorrected) with a Thomas-Hoover Uni-Melt, and UV–visible spectra with a Varian Cary 118C. The chromatograph consisted of either a Waters M6000A or a Spectra Physics dual-piston pump, a Du Pont column compartment fitted with a Rheodyne Model 7125 sample injection valve and an LDC Spectromonitor III or a Du Pont model 837 variable-wavelength detector.

Bonded Phases. Preparation of *N*-ω-Undecenyl-L-proline *tert*-Butyl Ester (I). A solution of 30 mmol of L-proline *tert*-butyl ester in 40 mL of methanol was prepared and cooled on an ice bath; 21 mmol of sodium cyanoborohydride was added, followed by dropwise addition (20 min) of a solution of 33 mmol of ω-undecenal in 20 mL of methanol. The solution was kept cooled for an additional 30 min and allowed to stir overnight at room temperature. The reaction mixture was acidified with 20% hydrochloric acid to pH 2 (caution: hydrogen cyanide gas evolves) and filtered and the precipitate washed with methanol. The combined filtrates were concentrated under reduced pressure, dissolved in water, and extracted twice with ether. The aqueous layer was cooled, 10% potassium hydroxide solution added to alkaline pH, and the solution extracted twice with methylene chloride. The combined organic layers were extracted with 5% sodium bicarbonate, dried over sodium sulfate, filtered, and stripped of solvent at reduced pressure. The residue was purified by elution through 90 g of silica gel starting with toluene and increasing eluent polarity with ethyl acetate. The appropriate fractions were treated with charcoal in an alcohol solution and filtered, and the alcohol was removed under reduced pressure to yield a colorless oil. Yield was 50%. ^{1}H NMR δ 1.33 [m, $W_{1/2}$ 6 Hz, $(CH_2)_7$], 1.47 [s, $(CH_3)C$], 2.7–3.3 [m, $(CH_2)_3CHNCH_2$, CH_2 allyl], 4.7–5.2 [m, CH_2 vinyl],

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5.7–6.1 [m, CH vinyl]. Anal. Calcd for $C_{20}H_{37}NO_2$: C, 74.25%; H, 11.53%; N, 4.33%. Found: C, 74.17%; H, 11.20%; N, 4.16%.

N-ω-(Dimethylchlorosilyl)undecyl-L-proline *tert*-Butyl Ester (II). To a solution of 4 mmol of I in 2.5 mL of chloroform was added 1.5 mL (15 mmol) of dimethylchlorosilane and a small crystal of chloroplatinic acid. The mixture was heated to reflux for 30 min and then allowed to cool. The volatiles were stripped off under high vacuum for 4 h to yield a slightly yellowish oil. ^1H NMR ($CDCl_3$) δ 0.38 [s, $Si(CH_3)_2$], 0.65–1.05 [m, CH_2Si], all other protons as in I except olefinic protons absent.

N-ω-(Dimethylsiloxyl)undecyl-L-proline *tert*-Butyl Ester Bonded Phase (III). A solution of II in 20 mL of dry purified pyridine was prepared and filtered through a 0.45-μm Teflon Millipore filter onto 3.0 g of dried (200 °C, high vacuum, overnight) silica gel (Supelcosil; particle size 5 μm, 100-Å pore size). The suspension was rotated slowly for 5 h at room temperature. The mixture was then filtered and washed thoroughly with methylene chloride, followed by methanol. The bonded material was dried at 60 °C for 4 h at atmospheric pressure and then overnight under high vacuum. Anal. Batch 1: C, 9.41%; specific surface area 156.6 m^2/g. Batch 2: C, 9.54%; specific surface area 159 m^2/g. The surface density of the ligand was calculated as 2.63 and 2.64 $\mu mol/m^2$ for batch 1 and 2, respectively.

The bonded phase was then endcapped under similar conditions with a solution of tetramethyldisilazane and trimethylchlorosilane in pyridine. After it was washed and dried as before, the bonded material was suspended for 2 h in trifluoroacetic acid to cleave the *tert*-butyl ester and then filtered, washed thoroughly with methanol, and dried as before.

Preparation of the Diluted Bonded Phase (C_{20}-Proline Phase). A solution of 1 mmol of I and 10 mmol of 1-eicocene in 2.5 mL of chloroform was prepared and hydrosilylated as before. NMR of the product showed no olefinic protons and the appropriate $(CH_3)_2SiCl$ group. It was dissolved in dry cyclohexane, converted to its dimethylamino derivative using dimethylamine (gas), and filtered in situ onto 3.0 g dry silica gel in an ampule. After cooling and degassing, the ampule was sealed, and the mixture was heated with an oil bath to 115 °C, to reflux the sample for 24 h.[7] Subsequent washing, endcapping, and cleavage were as above.

During cleavage of the *tert*-butyl ester group with trifluoroacetic acid, a sample of the mother liquor was analyzed for the amount of isobutylene produced, as described previously.[8] From the amounts of carbon (total bonded ligands) and the isobutylene formed (active ligands) the coverage of the active site, 0.207 $\mu mol/m^2$, and the C_{20} diluent, 3.13 $\mu mol/m^2$, were calculated, as described elsewhere.[8]

Packing of Columns. The packing of the bonded phases into 15 cm × 4.6 mm i.d. stainless-steel columns followed standard slurry procedures using a solution of methanol in carbon tetrachloride as the slurry medium and a Model DSTV 122 air-driven Haskel pump.

Preparation of Schiff Bases. The amino alcohols were dissolved in methanol, and an equimolar amount of salicylaldehyde was added causing a rapid formation of the derivative, as indicated by the development of an intense yellow color. When hydrochloride salts of the amino alcohols were used, an equimolar amount of sodium bicarbonate was added to the above solution and the reaction mixtures were warmed in a 60 °C water bath for 10–20 min.

In order to demonstrate the formation of the Schiff base, 2-([(2-hydroxy-2-phenylethyl)imino]methyl)phenol (IV) was recrystallized from absolute ethanol: mp 89–91 °C; IR (KBr) 1625 (C=N), 1445 (Ar C=C) 750 (CH wag); ^1H NMR ($CDCl_3$) δ 3.8 (m, CH_2), 4.9 (dd, CHO), 6.7–7.5 (m, 10 H, Ar and OH), 8.2 (s, 1H, CH=N); mass spectrum, *m/e* 241, 135, 134, 107, 79, 77; UV (methanol) 402, 315, 276, 254. These results are consistent with spectra of similar compounds.[9] Anal. Calcd for $C_{15}H_{15}NO_2$: C, 74.67; H, 6.27; N, 5.81. Found: C, 74.90; H, 6.37; N, 5.81. As confirmation of the structure of the other Schiff bases, the C=N stretch in the IR spectra was measured and found to fall between 1625 and 1644 cm^{-1}.

Kinetic studies of the hydrolysis of IV were performed as previously described[10] except that the cell compartment was not thermostated and the analytical solutions were maintained in a thermostated water bath, from which samples were withdrawn at appropriate times for immediate UV scan. The wavelengths, λ, and molar absorptivities, ε, used were the following: compound IV in the presence of 5×10^{-4} Cu(II), λ_{max} = 360 nm, ε = 5400 L mol^{-1} cm^{-1}, λ = 325 nm, ε = 2100 L mol^{-1} cm^{-1}; compound IV in the absence of copper, λ_{max} = 400 nm, ε = 3700 L mol^{-1} cm^{-1}; salicylaldehyde, λ_{max} = 325 nm, ε = 2740 L mol^{-1} cm^{-1}, λ = 360 nm, ε = 750 L mol^{-1} cm^{-1}, λ = 400 nm, ε = 0.

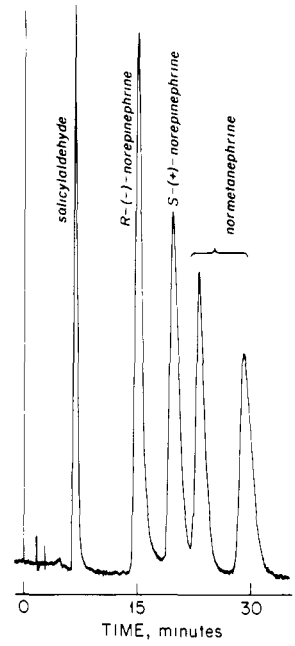

Figure 1. Resolution of (±)-norepinephrine and (±)-normetanephrine as salicylaldehyde Schiff bases by LEC. Column: C_{20}-Proline phase. Mobile phase: 5×10^{-4} M Cu(II), 0.125 M ammonium acetate, pH 5.0, 50% methanol. Flow, 1 mL/min; temperature, 35 °C; detector wavelength, 350 nm; approximately 2 μg injected.

Results and Discussion

An example of the separation of amino alcohols as Schiff bases by LEC is shown in Figure 1. Good resolution and sharp peaks are observed. In the following sections we will discuss the rationale for bonded-phase selection, our observations on Schiff base stability, and our chromatographic results for all amino alcohols studied to date. Finally, we will describe a possible structure of the mixed Schiff base-bonded ligand copper complex responsible for the separation and some general rules for elution order.

Solute Derivatization and Bonded-Phase Strategy. We considered direct resolution of racemic amino alcohols by LEC to be impracticable. The basic pH required for complexation[11] would be unfavorable for column stability. Moreover, catecholamines under basic conditions are readily oxidized to adrenochrome, and this reaction is accelerated by copper.[12] At acidic pH, amino alcohols do not form copper(II) complexes, while catecholamines coordinate at the catechol site.[11a,12a,13] This site is too far removed from the chiral centers, and thus the complex would not be expected to yield enantioselectivity. Therefore, our approach involved the formation of Schiff bases which coordinate to the metal around the asymmetric center even at acidic pH.

With respect to the chromatography, there are two general methods for achieving separation of enantiomers by LEC. In the first, a chiral chelating agent is added to the mobile phase, whereas in the second, such a chiral ligand is bonded to the stationary phase. In this study, the second approach has been selected in order to allow a wider range of chromatographic mobile-phase conditions for separation and the potential for preparative-scale operation. Moreover, bonded phases permit manipulation of the environment and composition of the active site, which can affect retention and separation.[8]

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Table I. Stability of 2-([(2-Hydroxy-2-phenylethyl)imino]methyl)phenol (IV) in Mobile Phase[a]

temp, °C	[Cu(II)], M × 10^4	half-life, min
35	0	2–3[b]
35	1	26
35	5	176
50	0	1–2[b]
50	1	7.0
50	5	55

[a] Reaction medium: 0.125 M ammonium acetate, pH 5.0, 50% methanol. Schiff base concentration: 1.5 × 10^{-4} M. [b] Estimated value.

(L-Proline)copper(II) was selected as the chiral stationary phase on the basis of its successful use in the resolution of DL-amino acids.[2c,e,f] While similar L-proline phases bonded to silica gel via extensive modification of bonded ligands have been described,[14] we prepared the stationary phase in a different manner. The silylating agents were first synthesized, purified, and characterized and then attached to the silica gel surface. The only reaction conducted after bonding was the quantitative cleavage of the reactive *tert*-butyl ester to yield the free acid. By this approach, well-characterized bonded phases are possible. In addition, we chose to bond the active site to the surface through an 11-carbon leash. As previously discussed[8] it was reasoned that a long leash would make the active site more accessible to the solutes and minimize the effect of residual silanols on the silica surface.

Two bonded L-proline stationary phases were synthesized, differing in the surface density of the bonded chelate. The first was a concentrated proline phase, with 2.6 μmol/m^2 of active ligands, while the second was a diluted phase, where 0.2 μmol/m^2 of active ligand was cobonded with an inactive eicosyl (C_{20}) diluent. In a previous study[8] it was found that significant differences in retention and separation of chiral species existed between such phases.

Stability of Schiff Bases under Chromatographic Conditions. Schiff bases are known to be unstable in aqueous media, rapidly hydrolyzing back to the starting aldehyde and amine.[10] For this reason we have investigated the stability of the derivatives in the chromatographic mobile phase. It should be noted that in the case of amino acid Schiff bases, copper complexation has been found to reduce the susceptibility of these compounds to hydrolysis.[5] The Schiff base IV was found to undergo first-order hydrolysis with half-lives under various conditions shown in Table I. It can be seen that hydrolysis is fairly rapid in the absence of copper(II). Upon the addition of roughly stoichiometric amounts of copper(II), a significant decrease in hydrolysis rate can be noted. For example, at 35 °C the half-life of a solution of 1.5 × 10^{-4} M compound IV is about 2–3 min without copper and 176 min in the presence of 5 × 10^{-4} M copper. These results suggest that these Schiff bases hydrolyze only when not coordinated to copper.

The retention time of compound IV is roughly 1 h in the chromatographic systems examined. It is important to note that when the Schiff base is retarded by the stationary phase via complexation, it will be coordinated to copper and thus be protected from hydrolysis. The time spent by a solute in the mobile phase is that required to elute the void volume of the system, in this case 1.5 min. Since this is much smaller than the half-lives observed in the presence of copper (Table I), the stability of the Schiff base is quite adequate for successful chromatography. Stabilization of hydrolytically susceptible species can be an important advantage of LEC. Moreover, this approach can be used for preparative-scale operation, in that by removing copper from a collected fraction, a rapid regeneration of the starting material would be possible.

Chromatography. Table II presents data on all substances examined on both the concentrated and the diluted phases. All enantiomeric pairs have been successfully resolved except alaninol. In most cases, relatively high α values have been obtained.

Comparison of diluted and concentrated phases reveals some interesting trends. First, it can be seen that the diluted proline phase yielded higher retention than the concentrated proline phase for all substances, except the catecholamines in footnotes *d* and *e* of Table II, even though the copper loading on the former phase was 8-fold less. The relative ratio of bonded chelates to copper uptake for the concentrated phase approaches twice that for the diluted phase, suggesting the tendency to form predominantly a bischelate–copper(II) complex in the concentrated phase and mainly a monochelate–copper(II) complex in the diluted phase. The rationale for the higher activity of the mono complex in LEC was the subject of a previous study.[8] In short, the Schiff base has to displace weakly coordinated mobile-phase ligands from a monocomplex, while the solute has to displace a more tightly bound bidentate ligand from a biscomplex. Even though the copper loading of the diluted phase is about 8-fold less, retention on this column is found to be greater, except for the catecholamines. The higher retention of the polar catecholamines in the more polar concentrated phase may be due to nonselective interactions.

Table II also shows that chiral recognition is, in general, higher on the diluted phase, especially for the polar Schiff bases, e.g., those formed by the catecholamines norepinephrine and nordefrin. Indeed, since the free energy differences of distribution for the two optical isomers and thus the resolution are proportional to $\alpha - 1$,[15] the differences between the two phases for certain compounds are thermodynamically and practically quite significant. For example, for nordefrin, the $\alpha - 1$ value increases by almost a factor of 3 from the concentrated to the diluted phase.

Enantiorecognition is possible only via the formation of the mixed complex, i.e., the bonded proline–copper(II)–solute complex. Presumably, when formation of this mixed complex is the only contribution to the retention of both enantiomers, the highest selectivity is achieved. However, other achiral contributions to retention may play a role and reduce separation. Evidently, these nonenantioselective interactions are reduced for the less polar C_{20} diluted phase, and increases in α values are thus observed. Alternatively, the environment around the mixed solute–Cu(II)–ligand complex may be different in the diluted phase. The dependence of enantioselectivity on such factors as pH, temperature, copper concentration, and buffer concentration will be discussed elsewhere.

Possible Mixed Complex Structure. The enantioselectivity observed in Table II must involve a mixed ligand–copper(II) complex, where one ligand is the N-alkylated proline of the bonded phase and the other is the Schiff base. Mixed ligand complexes of this or similar type have not been reported in the literature. However, pure copper complexes of amino acids, peptides and Schiff bases have been thoroughly characterized. In the case of bis(*N*-benzylprolinato)copper(II) or bis(*N*-benzylvalinato)copper(II) complexes it has been shown, for the L or D,L combination, that the amino acids are coordinated in the planar positions of the copper coordination sphere, trans to each other.[16] This structure is similar to that found for underivatized amino acids. N-Alkylated prolinato–copper(II) complexes would be expected to show the same characteristic copper(II) complex structure as the underivatized amino acids. However, those amino acids that are tridentate ligands can show a different complex structure. In many cases where the planar coordination positions are already occupied, the carboxylic group will coordinate to an available axial position of the metal. For example, in (L-histidinato)(L-threoninato)copper(II) hydrate, the threonine coordinates with the amino and carboxylic groups in the equatorial planar position (as usual for an amino acid), while the histidine coordinates with its imidazole and amino nitrogens in the plane and its carboxylic group in an irregular axial position (angle, N(amino)–Cu–O(carboxyl)

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Table II. Enantiomeric Resolution of Amino Alcohol Schiff Bases, 2-(OH)PhCH=NCHR$_2$CH(OH)R$_1$, on Fully Covered and Diluted Chiral Stationary Phases[l]

			concd			dil		
bonded chelate/m^2, μmol			2.64			0.21		
copper loading/column, μmol			140			17		
			concd			dil		
R_1	R_2	F[j]	k'_1	k'_2	α	k'_1	k'_2	α
Ph[a]	H		22.9	24.7	1.08	35.4	46.1	1.30
Ph[b]	Me	1R,2S[k]	8.0	11.2	1.39	12.3	16.2	1.32
Ph[c]	Me	1S,2S[k]				8.4	16.0	1.85
(OH)$_2$Ph[d]	H	R	15.0	17.2	1.14	8.6	11.0	1.28
(OH)$_2$Ph[e]	Me	1R,2S	4.7	5.4	1.15	2.4	3.4	1.43
(OH)(OMe)Ph[f]	H		12.9	14.0	1.08	14.1	17.6	1.25
Me[g]	H	R	2.8	3.2	1.14	4.7	6.0	1.27
H[h]	Me		1.4	1.4	1.00	1.9	1.9	1.00
H[i]	iPr	R	2.2	2.6	1.18	3.5	4.2	1.19

[a] Phenylethanolamine. [b] Phenylpropanolamine. [c] Pseudophenylpropanolamine. [d] Norepinephrine. [e] Nordefrin. [f] Normetanephrine. [g] 1-Aminopropan-2-ol. [h] Alaninol. [i] Valinol. [j] F = first eluting enantiomer. [k] Reference 21. [l] Mobile phase: 5×10^{-4} M Cu(II), 0.125 M ammonium acetate, pH 5.0, 50% methanol, 50 °C.

= 68.3°).[17] A water molecule coordinates to the opposite axial position.

Studies of amino alcohol Schiff base copper(II) complexes have determined that the phenolate oxygen and the imino nitrogen are coordinated in the plane of the copper(II) ion. The alcohol side chain is found either free or coordinated to copper. For example, bis[2-([(2-hydroxyethyl)imino]methyl)phenolato]copper(II) and similar compounds were shown to have the hydroxyl of the side-chain hydrogen bonded to a neighboring hydroxyl group and not coordinated to the copper(II).[18] On the other hand, other amino alcohol Schiff base complexes were found to have the side-chain hydroxyl group coordinated to the copper(II) ion, forming a tridentate planar ligand.[4e,19] Moreover, [2-([(3-hydroxypropyl)imino]methyl)phenolato]chlorocopper(II) in the solid, anhydrous state, was shown[4c] to be a bipyramidal dimer with the chlorine atom in the axial position. Upon hydrolysis to the monomeric complex in polar solvents, a planar tridentate ligand structure was found, consisting of the imino nitrogen and the phenolate and the hydroxyl oxygens.[4e] Thus, from these studies it is clear that when the amino alcohol Schiff base coordinates as a tridentate ligand to the copper in a mixed complex, it does so in the equatorial positions. This result is in part a consequence of the imino nitrogen possessing sp^2 hybridization.

On the basis of these previous studies, we describe two possible structures for the mixed copper(II) complex involved in enantiomer resolution. In one (Figure 2a) the proline and the imino and phenolate moieties of the Schiff base are in the trans planar configuration; two mobile-phase molecules are in the axial positions, while the alcohol side chain is free. In the other (Figure 2b), the Schiff base is a tridentate ligand, coordinated in the plane of the complex, with the proline nitrogen in the remaining equatorial position, while the carboxyl group of the proline is in an axial position. There are two available axial positions and thus two possible mixed complexes of this type, one as shown in Figure 2b or its analogue where the hydroxyl and the phenolate oxygens interchange.

The more likely structure of the complex can be determined by an examination of the data in Table II. If the structure in Figure 2a were responsible for the enantioselectivity observed, the extent of separation of enantiomers would be expected to decrease with increasing distance of the asymmetric center from the imino nitrogen (assuming that achiral contributions to retention were equal). Thus, the Schiff base of alaninol (2-aminopropan-1-ol)

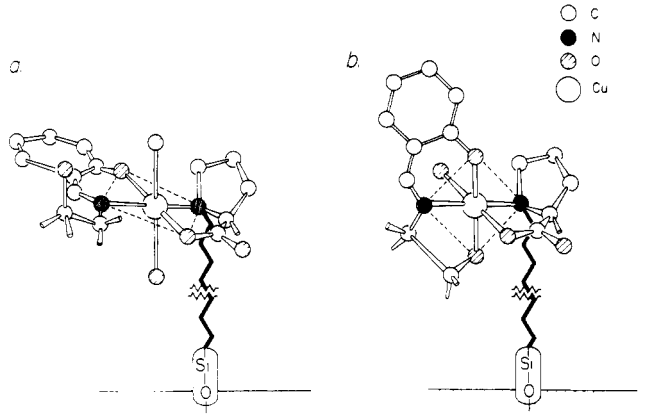

Figure 2. Two possible structures for the *N*-alkylated (L-prolinato)[2-([(2-hydroxyethyl)imino]methyl)phenolato]copper(II) complex involved in enantiomer resolution. (a) Hydroxyl of the side chain free. (b) Hydroxyl of the imino alcohol coordinated.

would be expected to exhibit greater resolution than that of 1-aminopropan-2-ol, since the asymmetric center for the latter is one carbon further removed from the imino nitrogen than the former. Derivatives of compounds such as phenethylamine, which contain no alcoholate group, should likewise be separated. However, the Schiff bases of phenethylamine and alaninol are not resolved, while that of 1-aminopropan-2-ol resolves with an α value of up to 1.27 (Table II).

The above results can be rationalized only if the structure in Figure 2b (or its analogous structure), where the hydroxyl group is coordinated to the metal, is the one responsible for the enantioselectivity. For this structure, when the asymmetric center is at the carbon bearing the hydroxyl group, closer to the chiral center of the proline, resolution should be increased.

The structure of Figure 2b, and/or its analogous form, can be supported by considering the retention of various amino alcohols as shown in Table II. When one examines pairs of compounds that differ only in the nature of R_2, the group attached to the carbon adjacent to the imino nitrogen, i.e., phenylethanolamine–phenylpropanolamine, where R_1 = Ph and R_2 = H or Me, and norepinephrine–nordefrin, where R_1 = (OH)$_2$Ph and again R_2 = H or Me, it can be determined that when R_2 is methyl a much lower retention is observed in both cases. In this latter case, greater steric hindrance to coordination of the alcohol hydroxyl group can be postulated due to more extensive substitution of the heterocyclic ring. This steric effect would be expected to decrease the stability of the complex, leading to the lower retention times observed.

Order of Elution. On the basis of the structure in Figure 2b, it is possible to correlate the absolute configuration of the several Schiff base amino alcohols studied with the observed order of elution. Each enantiomer has two staggered conformations, where

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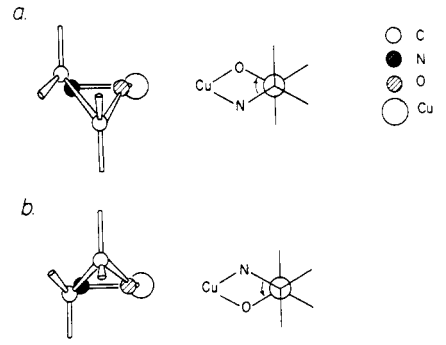

Figure 3. Two possible staggered conformations of the five-membered heterocyclic ring formed by the coordinated imino alcohol of Figure 2b.

the amino and hydroxyl groups are gauche to each other. One of these conformations is more favorable, based on steric considerations. A relationship between the spatial arrangement of the groups in the more favorable conformation and the order of elution from the column has been observed in this work.

In Figure 2b, the coordinated imino alcohol forms a five-membered heterocyclic ring, with an O-Cu-N bond of approximately 90°, which is substantially smaller than the 108° of cyclopentane. As a result, the carbon atom bearing the hydroxyl group in this ring is forced out of the plane and exists in two possible staggered conformations, depicted in Figure 3. For the conformer shown in Figure 3a, the carbon attached to the sp^2 imino nitrogen is slightly above the plane of the complex while the carbon attached to the oxygen is below. The Newman projection of these two carbons for this conformer has a clockwise orientation from the nitrogen atom to the oxygen atom (Figure 3a). The conformer depicted in Figure 3b has the opposite arrangement and thus a counterclockwise orientation.

Figure 4 illustrates the Newman projection formulas for all the compounds that were separated for which the elution order was determined. The compounds are viewed along the C(N)–C(OH) bond. Each enantiomer is shown in the two possible staggered conformations, Figure 3, parts a and b, which place the nitrogen atom and the hydroxyl group in the required conformation, gauche to each other. The conformation having less steric hindrance, the more favorable form, is underlined.

All the enantiomers having the clockwise orientation in the predominant conformer emerge last from the chiral column. For example, for norepinephrine the preferred conformer of the *S*-(+) enantiomer has the clockwise orientation, and this enantiomer elutes from the chiral column after the *R*-(−) enantiomer. Even though further studies are required to elucidate detailed steric and electronic factors involved in the chiral recognition, it is postulated that the conformer represented by Figure 3a is energetically preferred, due to the stereochemistry of the attached L-proline moiety. The enantiomer with the appropriate spatial (clockwise) handedness in its preferred conformation will form a more stable mixed ligand complex and will emerge last from the column. Further development of this concept using other amino alcohols could lead to a method for determining absolute configuration of unknown compounds on the basis of their elution order.

Conclusions

The results of this study demonstrate the application of LEC to separate broad classes of substances via derivatization. In the examples cited, interaction of amino alcohol Schiff bases with the added metal serves the dual role of complexing and stabilizing the hydrolytically susceptible species. Besides salicylaldehyde, we have successfully resolved the amino alcohols shown in this paper using other Schiff base derivatizing agents such as *o*-hydroxyacetophenone and *o*-hydroxybenzophenone. It is also clear that other derivatizing agents could be employed for resolution of substances besides amino alcohols.

Finally, it should be noted that the separation of the chiral compounds in this study, β-hydroxyphenethylamines, is significant from a pharmacological point of view. It is usually only one of the optical isomers in such amines that is biologically active.

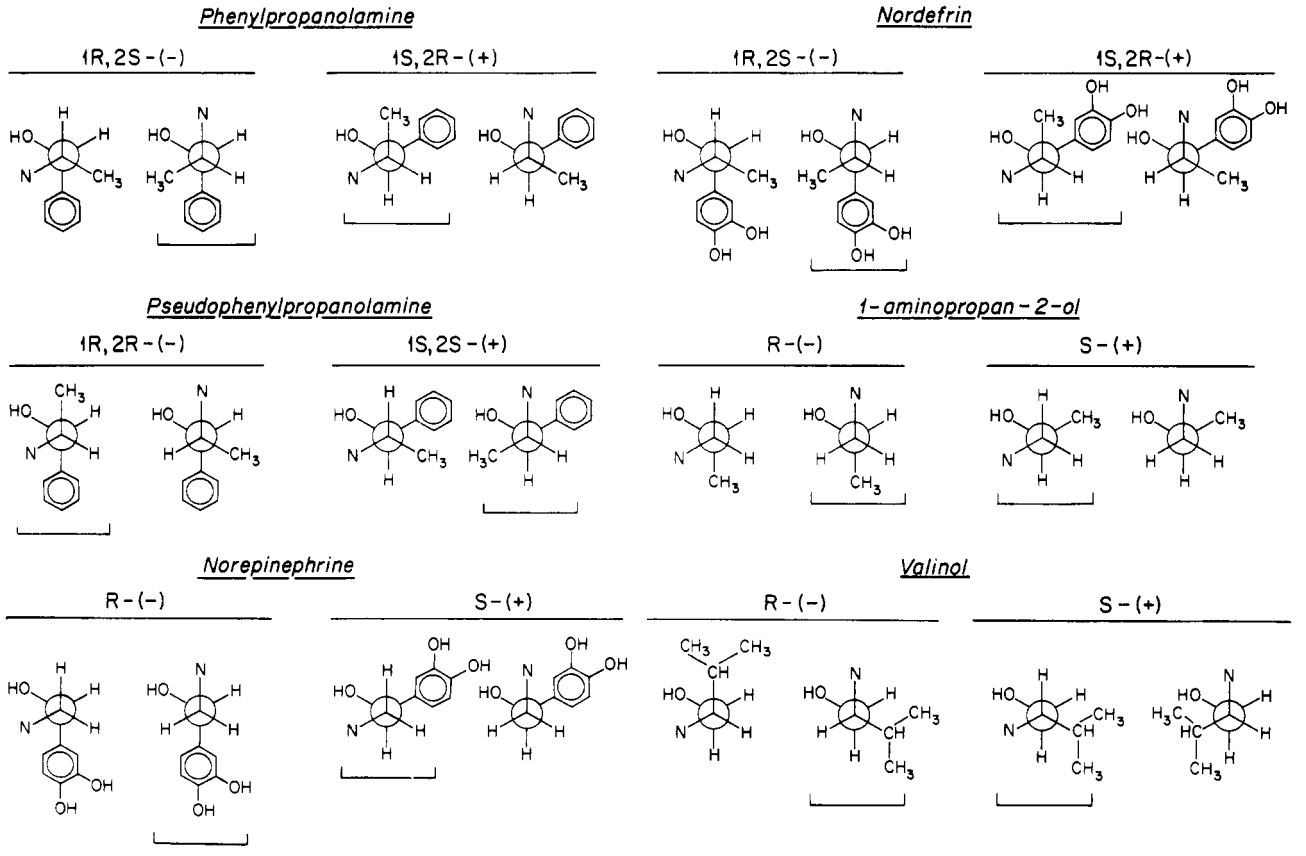

Figure 4. Newman projection formulas of two staggered conformations, Figure 3, parts a and b, for all compounds for which elution order was determined. The more favorable conformer is underlined.

Indeed, in certain instances the less active enantiomer may even possess undesirable properties.[20] Thus, enantiomeric purity is an important criterion for establishing the pharmaceutical acceptability of such drugs.

Acknowledgment. This work was supported in part by the National Science Foundation (B.L.K.). L.R.G. gratefully acknowledges the support of a United States Pharmacopeia Fellowship and the Food and Drug Administration Science Advisor Research Associate Program. The authors thank M. J. Cohen for analysis of the stationary phases and H. Maksoud for mass spectral analysis.

Registry No. I, 92984-07-1; II, 92984-08-2; IV, 67492-08-4; *dl*-phenylethanolamine, 1936-63-6; (*R*)-phenylethanolamine, 2549-14-6; (*S*)-phenylethanolamine, 56613-81-1; *dl*-phenylpropanolamine, 14838-15-4; 1(*R*),2(*S*)-(−)-phenylpropanolamine, 492-41-1; 1(*S*),2(*R*)-(+)-phenylpropanolamine, 37577-28-9; *dl*-pseudophenylpropanolamine, 54680-46-5; 1(*R*),2(*R*)-(−)-pseudophenylpropanolamine, 37577-07-4; 1(*S*),2(*S*)-(+)-pseudophenylpropanolamine, 492-39-7; *dl*-norepinephrine, 138-65-8; (*R*)-(−)-norepinephrine, 51-41-2; (*S*)-(+)-norepinephrine, 149-95-1; *dl*-nordefrin, 74812-63-8; 1(*R*),2(*S*)-(−)-nordefrin, 829-74-3; 1(*S*),2(*R*)-(+)-nordefrin, 829-75-4; *dl*-normetanephrine, 709-52-4; (*R*)-normetanephrine, 2282-53-3; (*S*)-normetanephrine, 35778-41-7; *dl*-1-amino-2-propanol, 1674-56-2; (*R*)-(−)-1-amino-2-propanol, 2799-16-8; (*S*)-(+)-1-amino-2-propanol, 2799-17-9; *dl*-valinol, 16369-05-4; (*R*)-(−)-valinol, 4276-09-9; (*S*)-(−)-valinol, 2026-48-4; *dl*-alaninol, 6168-72-5; L-proline, 2812-46-6; ω-undecenal, 112-45-8; dimethylchlorosilane, 1066-35-9; copper, 7440-50-8.

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Photoelectron Spectroscopy of the Allylic Anion

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Abstract: We have examined the photodetachment spectra of the allylic anion ($CH_2CHCH_2^-$) and two of its deuterated isotopes, $CH_2CDCH_2^-$ and $CD_2CDCD_2^-$. The electron affinities (EA) of the corresponding allyl radicals are EA(CH_2CHCH_2) = 0.362 ± 0.020 eV, EA(CH_2CDCH_2) = 0.373 ± 0.020 eV, and EA(CD_2CDCD_2) = 0.381 ± 0.026 eV. The photoelectron spectra of these ions show only one active vibronic mode which we assign as the in-plane C-C-C bend. This leads us to conclude that the anion has a planar, symmetric C_{2v} geometry. Computational modeling yields a geometry for the $CH_2CHCH_2^-$ ion with a C-C-C bond angle 16° greater than that of the allyl radical.

Almost since radicals have been known to exist, the allyl radical ($CH_2CH{=}CH_2$) has been of fundamental interest to chemists because it is the simplest of conjugated hydrocarbon radicals. For this reason much work has been done to study its thermochemistry and spectroscopy, in addition to a great deal of theoretical effort directed at understanding its electronic structure. The geometry of the allyl radical is qualitatively understood from electron spin resonance (ESR) studies[1-3] and matrix infrared absorption spectra of this important hydrocarbon. All experimental and theoretical studies of allyl radical find it to be a planar molecule with C_{2v} symmetry. A detailed computational study of its geometry and vibrational spectrum has been published recently.[4] The electron affinity has been reported[5] for the allyl radical in an ion cyclotron resonance (ICR) threshold photodetachment experiment, but this is in slight disagreement with flowing afterglow results.[6-8]

The corresponding anion ($CH_2{=}CHCH_2^-$) has also generated much study. Of fundamental interest is the question of whether the geometry of the allylic anion still has C_{2v} symmetry. Earlier studies[9] have focused on a related simple organic radical which is a flat, symmetric, resonance stabilized molecule, the propargyl radical ($CH_2{-}C{\equiv}CH$). It is found that the corresponding ion, $CH{=}_2C{=}CH^-$, is a nonplanar molecule and not conjugatively stabilized. Rather little is known about the structure of the allylic anion. There is some chemical evidence that the two ends of the negative ion behave similarly in the gas-phase experiments.[10] This would lead one to suspect it still has a symmetric, C_{2v} structure. Theoretical structural studies have predicted both planar and nonplanar structures. A multiphoton infrared photodetachment study has recently been made of this ion as well.[11]

Using negative ion photoelectron spectroscopy,[12,13] we have studied the allylic anion (m/z 41) using both propene and 1,5-hexadiene as ion precursors. We have also studied two deuterium-substituted isomers, $CH_2CDCH_2^-$ (m/z 42) and $CD_2CDCD_2^-$ (m/z 46), from the corresponding propenes. From the appearance of the photoelectron spectrum of the allylic ion and by comparison to those of the two deuterated isomers, we conclude that the allylic anion retains its planar, C_{2v} structure. The major structural change observed is the opening of the skeletal C-C-C bond angle. Our analysis quantifies this geometry change and also yields a refined electron affinity of $CH_2{=}CH{-}CH_2$.

Experimental Section

The negative ion photoelectron spectrum is obtained by intersecting a mass-selected ion beam with a laser of fixed frequency ($\hbar\omega_0$) and determining the distribution of kinetic energy (KE) for the detached electrons $M^- + \hbar\omega_0 \rightarrow M + e^-$ (KE). Although the photoelectron

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