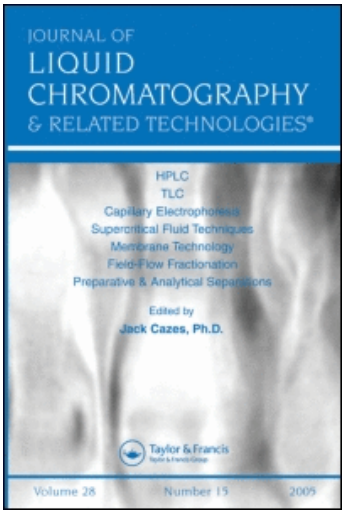
This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Separation of α-Hydroxy Acid Enantiomers by High Performance Liquid Chromatography Using Copper(II)-L-amino Acid Eluent

R. Horikawa[a]; H. Sakamoto[a]; T. Tanimura[a]
[a] Laboratory of Analytical Chemistry, Toyama Medical and Pharmaceutical University, Toyama, Japan

To cite this Article Horikawa, R. , Sakamoto, H. and Tanimura, T.(1986) 'Separation of α-Hydroxy Acid Enantiomers by High Performance Liquid Chromatography Using Copper(II)-L-amino Acid Eluent', Journal of Liquid Chromatography & Related Technologies, 9: 2, 537 — 549
To link to this Article: DOI: 10.1080/01483918608076652
URL: http://dx.doi.org/10.1080/01483918608076652

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(2&3), 537-549 (1986)

SEPARATION OF α-HYDROXY ACID ENANTIOMERS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY USING COPPER(II)-L-AMINO ACID ELUENT

R. Horikawa, H. Sakamoto, and T. Tanimura
Laboratory of Analytical Chemistry
Toyama Medical and Pharmaceutical University
2630 Sugitani, Toyama-shi
Toyama 930-01, Japan

Downloaded At: 16:11 24 January 2011

ABSTRACT

Enantiomers of underivatized α-hydroxy acids including lactic, mandelic, malic, and tartaric acids have been separated by reversed phase liquid chromatography using Cu(II) - L-amino acid complexes as eluents. The hydroxy acids in the column effluent were detected by a postcolumn color reaction with acidic iron(III) solution. Upon change in elution conditions α-hydroxy acids with another hydroxy group and/or carboxyl group in the β-position behaved quite differently from the acids with no functional group in the β-position, which indicated the involvement of more than one resolution mechanism in the present system.

INTRODUCTION

The great success achieved in the separation of underivatized amino acid enantiomers by high-performance liquid chromatographic systems, which are based on in situ mixed-ligand complex formation

Copyright © 1986 by Marcel Dekker, Inc. 0148-3919/86/0902-0537$3.50/0

of the enantiomers with chiral Cu(II) complexes of amino acid or its derivatives, has prompted the application of the systems to the resolution of α-hydroxy acid racemates in recent years. On a reversed-phase column enantiomers of mandelic and hydroxymandelic acids have been separated by using D,L-phenylalanine as a chiral additive to the eluent (1,2) or by coating the column with L-phenylalanine (2). The resolution of aliphatic and aromatic hydroxy acid racemates has been also achieved on a reversed-phase column using Cu(II) - N,N-dialkyl-L-amino acid eluents (3) or on a silica gel column bonded with L-hydroxyproline through 3-glycidoxypropyltrimethoxysilane (4).

We have shown the separation of enantiomers of underivatized α-hydroxy acids including tartaric and glyceric acids on a reversed phase column using Cu(II) complexes of underivatized L-amino acids as eluents (5); the problem of detecting the hydroxy acids in the column effluent containing Cu(II) - amino acid complexes was solved by employing a postcolumn color reaction of the hydroxy acids with iron(III). Details of this work are described in the present paper.

Downloaded At: 16:11 24 January 2011

EXPERIMENTAL

Chemicals

To avoid the ambiguity of D,L-nomenclature for the configurational notation of α-hydroxy acid enantiomers, the (R,S)-system was employed in the text. The S-isomers of the hydroxy acids listed below are designated by asterisks. D(-)- and L(+)*-lactic acids, D- and L*-glyceric acids (hemi-calcium salts), L*- and DL-β-phenyllactic acids, (+)*- and (-)-citramalic acids (sodium salts), and D- and L*-α-hydroxyglutaric acids (sodium salts) were obtained from Sigma (St. Louis, MO, U.S.A.); d*- and l-mandelic acids and d-malic acid from Aldrich (Milwaukee, WI, U.S.A.); L*- and DL-malic acids and DL-lactic acid from Wako Pure Chemical Industries (Osaka, Japan); L(+)-, D(-)*-, and DL-tartaric acids from Kanto Chemical Co. (Tokyo, Japan); L*- and DL-leucic acids and DL_mandelic acid from Tokyo Chemical Industry (Tokyo, Japan).

Iron(III) perchlorate (hexahydrate) was purchased from Kanto Chemical Co., and amino acids and other reagents were from Wako Pure Chemical Industries. N-Methyl-L-proline was prepared by reductive condensation of L-proline with formaldehyde and hydrogen in the presence of palladium - carbon catalyst (6).

Chromatographic Conditions

Stainless-steel columns, 4 mm I.D. x 26, 50, 150, and 250 mm, were packed with Develosil ODS-7 (7μm spherical silica gel chemically bonded with octadecyl group; Nomura Chemical Co., Seto-shi, Japan) by a balanced-density slurry packing technique using Slurry Solvent B conc (Marchery, Nagel & Co., Düren, G.F.R.), and were thermostated at 25±0.1°C by a Model Uni Cool UC-65 circulating thermostat (Tokyo Rikakikai Co., Tokyo, Japan). Five μl of aqueous solutions of α-hydroxy acids were injected into the column by a Model VMD-350V loop injector (Seishin Pharmaceutical Co., Tokyo, Japan). The Cu(II) - amino acid eluents were delivered by a Model PSD-2.5W double plunger pump (Seishin Pharmaceutical Co.) at a flow rate of 0.7 ml/min. The eluent pH was adjusted with 40% sodium hydroxide, 4% sodium hydroxide, and 13% perchloric acid, or with acetate buffer.

Detection System

The hydroxy acids in the column effluent were detected by modifying the method of Lunder and Messori (7) To the effluent the iron(III) reagent, 2.5 mM iron(III) perchlorate in diluted perchloric acid, was added through a mixing T at a flow rate of 0.7 ml/min using the second plunger head of the pump. The resultant solution was mixed in a PTFE tube coil, 0.5 mm I.D. x 5 m, then the absorbance at 420 nm was measured by a Model DV-C filter photometer (Seishin Pharmaceutical Co.). The flow-cell volume and light-path length of the detector were 135 μl and 20 mm, respectively. The recorder used was a Model RW-101 X-Y recorder (Rikadenki Kogyo Co., Tokyo, Japan).

The perchloric acid concentration of the iron(III) reagent was adjusted so that the mixture of the column effluent and the

Downloaded At: 16:11 24 January 2011

reagent solution showed pH 2.1. For instance, when an eluent containing 8 mM Cu(II) acetate and 16 mM L-proline, pH 6.0, was used, the perchloric acid concentration was adjusted to 0.06 N. With thus prepared reagent solutions α-hydroxy acids could be detected, the coloration and the base-line absorbance being little affected by the eluent pH and the eluent composition. Calibration curves were linear through the origin by peak height measurement between 2 and 50 nmol for leucic, mandelic, and malic acid enantiomers, and between 2 and 25 nmol for tartaric acid enantiomers when these pairs of enantiomers were separated on a Develosil ODS column, 4 mm I.D. x 50 mm, using an eluent containing 8 mM Cu(II) acetate and 16 mM L-proline, pH 6.0.

The residence time in the detection system was determined as 0.90 min by injecting the hydroxy acid into the detection system at the column outlet, and the time to elute the column void volume, t_0, was determined by subtracting the residence time in the detection system from the time at which a base-line fluctuation caused by injecting 5 μl of 0.2 M sodium perchlorate into the column emerged.

The resolution of racemic leucine by the same separation system as above was monitored by a postcolumn derivatization using o-phthalaldehyde as described by Hare and Gil-Av (8).

Downloaded At: 16:11 24 January 2011

RESULTS AND DISCUSSION

The detection system based on the coloration of α-hydroxy acids in the presence of iron(III) was not interfered by amino acids, which permitted the use of various L-amino acids as chiral additives in the mobile phase to manipulate the separation of α-hydroxy acid enantiomers. Fig. 1 and 2 illustrate the resolution of aliphatic and aromatic hydroxy acid racemates by the present system using Cu(II) complexes of L-valine and L-proline as eluents. The resolution was also achieved by using other amino acids, such as L-isoleucine and L-phenylalanine, as chiral reagents (TABLE 1).

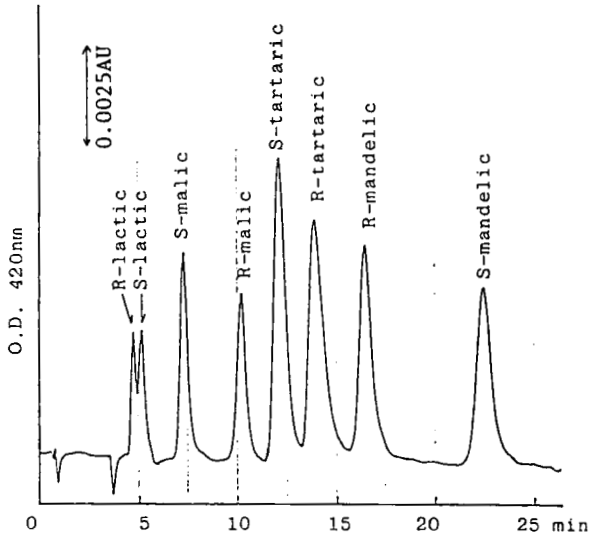


FIGURE 1. Resolution of α-hydroxy acid racemates. column: Develosil ODS-7 (4 mm I.D. x 250 mm), eluent: 8 mM $Cu(OAc)_2$ and 16 mM L-Val in 50 mM sodium acetate buffer (pH 5.0), sample: 20 nmol of each racemic acid.

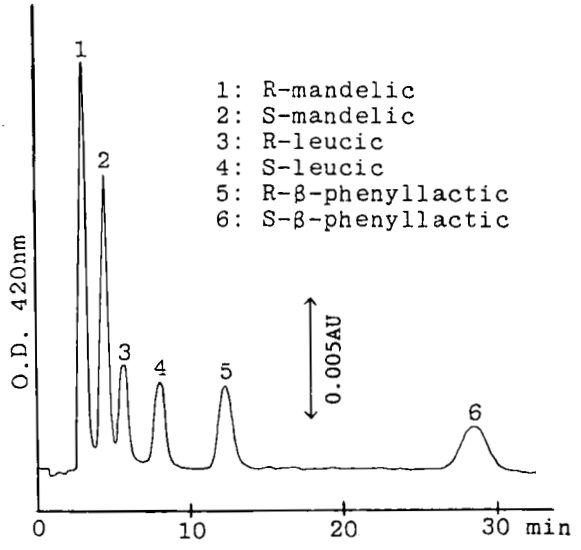


FIGURE 2. Resolution of α-hydroxy acid racemates. column: Develosil ODS-7 (4 mm I.D. x 50 mm), eluent: 8 mM $Cu(OAc)_2$ and 16 mM L-Pro (pH 6.8), sample: 40 nmol of each racemic acid.

Downloaded At: 16:11 24 January 2011

Downloaded At: 16:11 24 January 2011

TABLE 1

Capacity Factors (k') and Separation Factors ($\alpha = k_2'/k_1'$, $k_2' \geq k_1'$) of α-Hydroxy Acid Enantiomers with Cu(II) - L-Amino Acid Eluents

α-hydroxy acid		L-Pro*		L-Val*		L-Ile*		L-Phe**	
		k'	α	k'	α	k'	α	k'	α
lactic	R	0.94	1.38	0.80	1.39	1.30	1.21	1.53	1.12
	S	1.30		1.11		1.57		1.71	
leucic	R	32.1	1.63	20.6	1.67	26.5	1.31	16.7	1.27
	S	52.3		34.5		34.8		21.2	
β-phenyllactic	R	58.4	2.26	57.2	1.76	56.7	1.62	42.2	1.40
	S	131.9		100.7		91.9		59.1	
mandelic	R	8.82	1.61	8.19	1.57	12.1	1.47	6.62	1.47
	S	14.2		12.9		17.8		9.74	
α-hydroxy-glutaric	R	1.25	1.49	1.34	1.16	3.65	1.08	3.90	1.02
	S	1.86		1.55		3.94		3.99	
glyceric	S	0.38	1.82	0.60	1.00	1.57	1.06	1.34	1.07
	R	0.69		0.60		1.67		1.44	
malic	S	1.52	2.53	4.18	2.07	13.3	3.77	19.4	1.88
	R	3.85		8.67		50.2		36.4	
citramalic	S	4.88	2.50	5.79	1.63	16.1	2.55	19.6	2.34
	R	12.2		9.41		41.0		45.9	
tartaric	S	0.95	3.52	15.1	1.33	>200	—	30.4	1.19
	R	3.34		20.1		—		36.2	

* eluent: 8 mM $Cu(OAc)_2$ and 16 mM L-amino acid (pH 5.0).
** eluent: 1 mM $Cu(OAc)_2$ and 2 mM L-Phe (pH 5.0).

By inspecting the data in TABLE 1, α-hydroxy acids examined were found to be classified into two groups. The group A acids, e.g., leucic and mandelic acids, with no functional group on the carbon atom at β-position were resolved, the R-isomers being eluted before the S-isomers. The same elution order of enantiomers was observed when corresponding racemic amino acids were resolved by an identical chromatographic system. The group B acids, e.g., glyceric and tartaric acids, with hydroxyl group and/or carboxyl group at β-position were resolved, the S-isomers being eluted before the R-isomers. Capacity factors (k') and separation factors (α) of the group B acids depended more on the kind of L-amino acid in the eluent than those of the group A acids did.

The k' values of enantiomers of the acids of each group changed characteristically when pH of the eluent comprising 8 mM Cu(II) acetate and 16 mM L-proline was changed from 4.5 to 7.5 (Fig. 3). While leucine, amino acid corresponding to leucic acid, increased its retention gradually as eluent pH rose, k' of R- and S-leucic acids decreased rapidly at high pH, α remaining essentially constant. A similar effect of eluent pH was also observed for mandelic acid, an another acid of the group A. On the other hand, enantiomers, especially the R-isomers, of malic and tartaric acids of the group B were retained more strongly at higher pH, which resulted in marked increase of α values. Resolution of racemic glyceric acid was also enhanced at high pH. However, in this case, k' of the S-isomer decreased slightly as eluent pH rose.

The effect of molar ratio of L-proline to Cu(II) on the retention of enantiomers were examined at pH 6 by varying the concentration of L-proline in the eluent containing 4 mM Cu(II) acetate (Fig. 4). Leucine showed maximum retention at [L-Pro]/[Cu(II)] ratio, ∿2, and, at higher ratios, k' decreased gradually, while α remained unchanged. Enantiomers of tartaric acid were also retained most strongly at [L-Pro]/[Cu(II)] ratio, ∿2. However, the dependence of k' and α values on the concentration ratio of reagents was more pronounced than in the case of leucine. Enantiomers of leucic acid, on the other hand, were retained most strongly at

Downloaded At: 16:11 24 January 2011

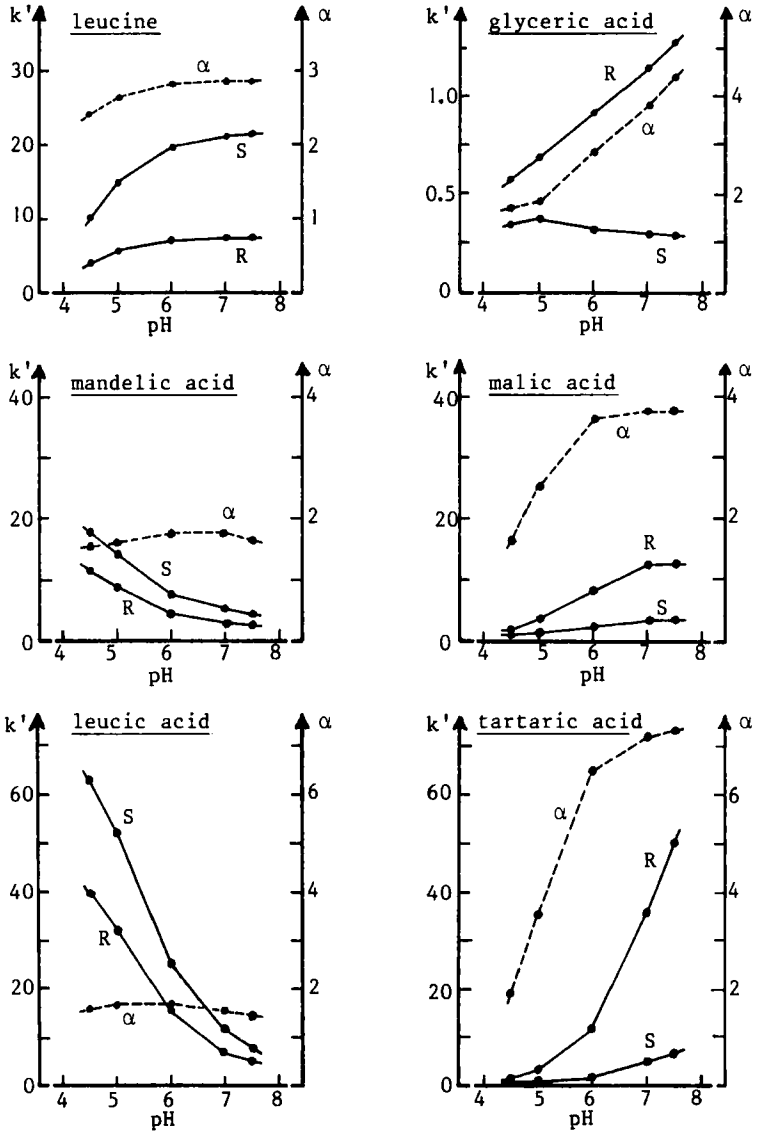


FIGURE 3. Effect of eluent pH on the separation of leucine and α-hydroxy acid enantiomers. eluent: 8 mM $Cu(OAc)_2$ and 16 mM L-Pro.

Downloaded At: 16:11 24 January 2011

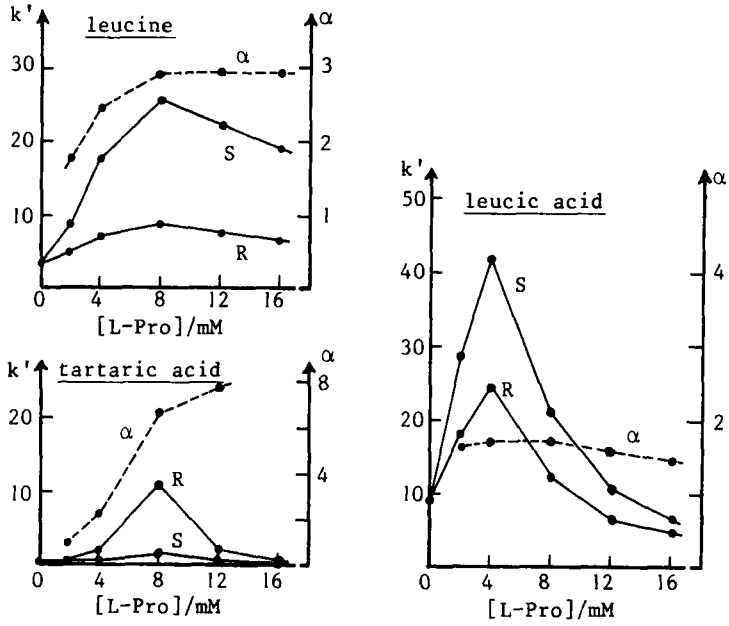


FIGURE 4. Effect of L-proline concentration in the eluent on the separation of leucine and α-hydroxy acid enantiomers. eluent: 4 mM $Cu(OAc)_2$ and L-Pro of varying concentration (pH 6.0).

Downloaded At: 16:11 24 January 2011

[L-Pro]/[Cu(II)] ratio, ∿1, and α was little affected by the L-proline concentration. Malic and mandelic acids changed their retention with change in L-proline concentration in a manner similar to tartaric and leucic acids, respectively.

The basis of the separation of enantiomers on reversed-phase column using labile chiral metal complexes as eluent is generally considered to be the formation of diastereomeric mixed-ligand complexes. The diastereomeric complexes with difference in stability constants or in affinity to the stationary phase can be formed in the mobile phase or in the stationary phase. Though both of these processes could be involved in the separation to produce enantioselectivity of the system, Karger and co-workers (9) showed the primary importance of the mixed-ligand complex formation on or in

the stationary phase in their work on the separation of enantiomers of dansyl amino acids using an eluent containing L-prolyl-n-octylamide - Ni(II). The alkyl-bonded stationary phase may either adsorb mixed-ligand complexes on its surface with spatial arrangement in favor of the stability of one of the diastereomeric complexes (10), or act as "bulk" organic liquid phase to enhance enantioselective complex formation, as a significant difference in the stability of D,L-amino acid - Cu(II) - N-dodecyl-L-proline complexes in n-butanol phase was observed in the water - n-butanol liquid - liquid partition system (6).

A ternary complex reported for glycine-Cu(II)-glycolic acid system (11) may also be formed between L-amino acid, Cu(II), and chiral α-hydroxy acid (Fig. 5-A) in the present chromatographic system, and the diastereomeric ternary complexes would be the major species responsible for the retention and resolution of α-hydroxy acids of the group A as in the resolution of racemic amino acids by the same chromatographic system (12). In the pH range studied the hydroxy acids are practically fully dissociated into anionic form which coordinate to Cu(II). However, the formation of the ternary complex involving α-hydroxy acid may proceed preferentially under the condition where the 1:1 complex, rather than the 1:2 complex, of Cu(II) and L-amino acid is predominant in the system because the coordination of α-hydroxy acid to Cu(II) is much weaker than that of amino acid (13). The elution behavior of the group A acids upon change in eluent pH at [L-Pro]/[Cu(II)] ratio = 2 (Fig. 3) and in L-proline concentration in the eluent at pH 6.0 (Fig. 4) is consistent with this view.

In contrast to the group A acids, the group B acids, especially the R-isomers, were retained strongly on the column under the condition where the 1:2 complex of Cu(II) and L-proline is predominant in the system (Fig. 3 and 4). This observation seemed to indicate the resolution of the group B acid racemates is achieved mainly through an interaction of the acid with $Cu(L\text{-}Pro)_2$. However, though α-hydroxy acids of the group B have another carboxyl group or hydroxyl group, it is difficult to assume their chelating

Downloaded At: 16:11 24 January 2011

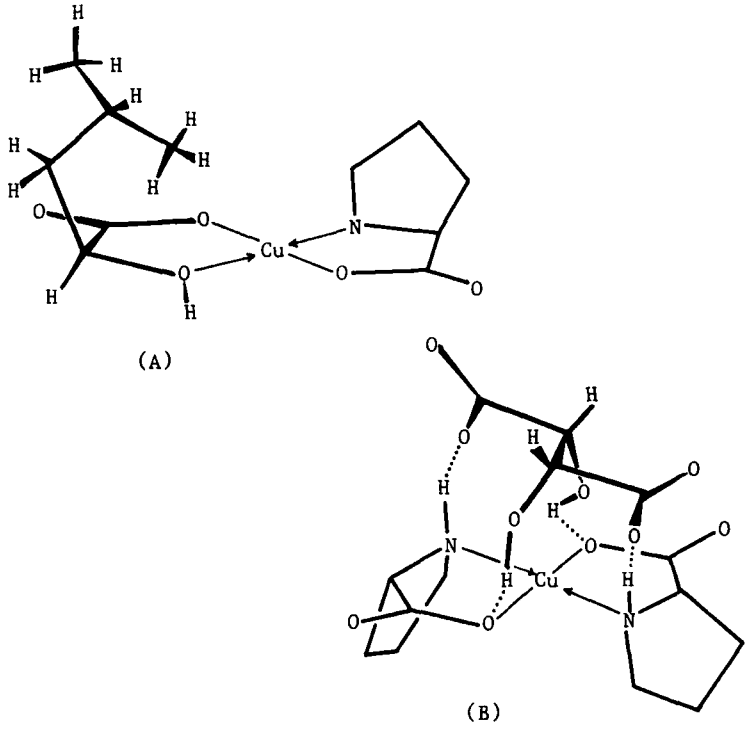


FIGURE 5. Possible structures of complexes responsible for retention of S-leucic acid (A) and R-tartaric acid (B) in Cu(II) - L-proline system.

TABLE 2

Resolution of α-Hydroxy Acid Racemates Using L-Proline and N-Methyl-L-proline as Chiral Additives to the Eluent

eluent: 4 mM $Cu(OAc)_2$ and 8 mM L-Pro or N-methyl-L-Pro in 50 mM sodium acetate buffer (pH 6.0).

α-hydroxy acid		L-Pro		N-methyl-L-Pro	
		k'	α	k'	α
leucic	R	11.8	1.40	30.2	1.57
	S	16.5		47.3	
mandelic	R	3.28	1.43	11.8	2.14
	S	4.70		25 3	
malic	S	1.09	3.39	0.63	1.0
	R	3.70		0.63	
tartaric	S	0.58	4.93	0.40	1.0
	R	2.86		0.40	

Downloaded At: 16:11 24 January 2011

ability to Cu(II) is high enough for the acid to undergo ligand-exchange on $Cu(L\text{-}Pro)_2$ to form ternary complexes. When N-methyl-L-proline was used as a chiral additive in place of L-proline, the resolution of tartaric and malic acid racemates was completely lost, while enantiomers of mandelic and leucic acids were separated with increased k' and α values (TABLE 2). The results imply that the ternary complexes, if any, little contribute to the retention of tartaric and malic acids and that N-methylation of L-proline hinders the enantioselective interaction between $Cu(L\text{-}Pro)_2$ and these acids. The group B acids might associate with $Cu(L\text{-}Pro)_2$ possibly through hydrogen bond formation rather than through coordination to Cu(II) (Fig. 5-B). In the case of glyceric acid this association may not be strong; k' of S-glyceric acid did not increase with rise in eluent pH (Fig. 3), which may indicate a contribution of the ternary complex formation for the retention of this isomer.

Downloaded At: 16:11 24 January 2011

RREFERENCES

1. Oelich, E., Preusch, H., and Wilhelm, E., "Separation of Enantiomers by High Performance Liquid Chromatography Using Chiral Eluents," J. High Resolut. Chromatogr. Chromatogr. Commun., 3, 269 (1980).

2. Klemisch, W., von Hodenberg, A., and Vollmer, K.O., "Resolution of the Enantiomers of m- and p-Hydroxymandelic Acid by High Performance Liquid Chromatography," J. High Resolut. Chromatogr. Chromatogr. Commun., 4, 535 (1981).

3. Benecke, I., "Resolution of Underivatized 2-Hydroxy Acids by High-Performance Liquid Chromatography," J. Chromatogr., 291, 155 (1984).

4. Gübitz, G., and Mihellyes, S., "Direct Separation of 2-Hydroxy Acid Enantiomers by High-Performance Liquid Chromatography on Chemically Bonded Chiral Phases," Chromatographia, 19, 257(1984).

5. Horikawa, R., Sakamoto, H., and Tanimura, T., "Separation of α-Hydroxy Carboxylic Acid Enantiomers by High-Performance Liquid Chromatography Using Chiral Eluent," Advances in Chromatography, 18th International Symposium, Tokyo, 1982, Abstracts, p. 78.

6. Takeuchi, T., Horikawa, R., and Tanimura, T., "Enantioselective Solvent Extraction of Neutral DL-Amino Acids in Two-Phase Systems Containing N-n-Alkyl-L-proline Derivatives and Copper(II) Ion," Anal. Chem., 56, 1152 (1984).

7. Lunder, T.L., and Messori, F., "Determination of Ten Organic Acids by Low Pressure Liquid Chromatography," Chromatographia, 12, 716 (1979).

8. Hare, P.E., and Gil-Av, E., "Separation of D and L Amino Acids by Liquid Chromatography: Use of Chiral Eluants," Science, 204, 1226 (1979).

9. Tapuhi, Y., Miller, N., and Karger, B.L., "Practical Considerations in the Chiral Separation of Dns-Amino Acids by Reversed-Phase Liquid Chromatography Using Metal Chelate Additives," J. Chromatogr., 205, 325 (1981).

10. Davankov, V.A., and Kurganov, A.A., "The Role of Achiral Sorbent Matrix in Chiral Recognition of Amino Acid Enantiomers in Ligand-Exchange Chromatography," Chromatographia, 17, 686(1983).

11. Martin, R.P., and Paris, R.A., "Etude du Systeme Cuivre-Acide Glycolique-Glycine," Bull. Soc. Chim. Fr., 1600 (1963).

12. Gil-Av, E., Tishbee, A., and Hare, P.E., "Resolution of Underivatized Amino Acids by Reversed-Phase Chromatography," J. Am. Chem. Soc., 102, 5115 (1980).

13. Perrin, D.D., "IUPAC Chemical Data Series, No. 22: Stability Constants of Metal-Ion Complexes(Part B) - Organic Ligands," Pergamon, Oxford (1979).

Downloaded At: 16:11 24 January 2011