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LIGAND-EXCHANGE CHROMATOGRAPHY OF RACEMATES

XVI. MICROBORE COLUMN CHROMATOGRAPHY OF AMINO ACID RACEMATES USING N,N,N′,N′-TETRAMETHYL-(*R*)-PROPANEDIAMINE-1,2-COPPER(II) COMPLEXES AS CHIRAL ADDITIVES TO THE ELUENT

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SUMMARY

A $5 \cdot 10^{-4}$ *M* solution of $Cu_2(tmpn)_2(OH)_2(ClO_4)_2$ in water–acetonitrile (5:1) permits the direct resolution of racemates of aromatic amino acids on reversed-phase silica gel. D-Isomers are eluted after the corresponding L-antipodes. Almost all common racemic amino acids can be resolved in a normal-phase system with water–acetonitrile (1:9) containing the above complex and additional free (*R*)-tmpn as the eluent. In this instance the order of elution of the enantiomers is reversed (with the exception of Asp and Glu).

INTRODUCTION

In the chromatographic separation of optical isomers, there has recently been great interest in the proposal[1] to add the necessary resolving chiral agent to the eluent instead of grafting it to the stationary phase. Any chiral compound can be used as the resolving reagent if it forms two labile diastereomeric adducts with the racemate under resolution (with adducts that appear to be kinetically inert, their separation becomes trivial, like that of diastereomeric compounds).

As enantioselectivity in the formation of labile coordination compounds has proved to be a common phenomenon, thus providing a general base for ligand-exchange resolutions of complexing racemic compounds (for a review, see ref. 2), chiral complexes have been introduced as the resolving additives in to the eluent in high-performance liquid chromatography (HPLC)[3–8]. The use of a "chiral eluent" has the advantage over the classical "chiral stationary phase" method of making possible the use of commercially available HPLC columns. In most instances reversed-phase systems were used[4–7], although ion-exchange resins[3] and unmodified silica gel packings[8] have also been tested. As far as the chiral resolving agent is concerned, much remains to be learned about the resolving power and efficiency of different chiral complexes.

This paper describes the use of copper(II)–N,N,N′,N′-tetramethyl-(*R*)-propanediamine-1,2 (tmpn) complexes as chiral additives to the eluent for the resolu-

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tion of unmodified α-amino acids on reversed-phase and normal-phase silica gel packings.

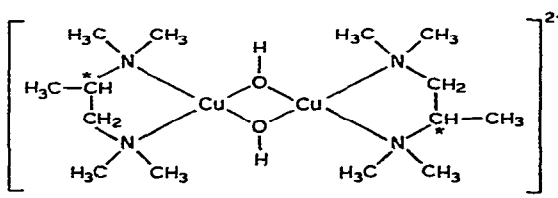
EXPERIMENTAL

N,N,N′,N′-Tetramethyl-(*R*)-propanediamine-1,2 was prepared by methylation of (*R*)-propanediamine-1,2 according to Leuckart-Wallach[9], $[\alpha]_D^{20} = +39.25°$ (benzene; *c* 1.7). The complex $Cu_2(tmpn)_2(OH)_2(ClO_4)_2$ was obtained by mixing ethanolic solutions of (*R*)-tmpn and copper(II) perchlorate and crystallization from aqueous ethanol. For $Cu_2C_{14}H_{38}N_4Cl_2O_{10}$: calculated, Cu 20.48, N 9.03%; found, Cu 20.49, 20.19, N 8.53, 8.84%. Amino acids were purchased from Reanal (Budapest, Hungary), Merck (Darmstadt, G.F.R.) or Serva (Heidelberg, G.F.R.). LiChrosorb RP-18 (5 μm) and Si 100 (10 μm) were obtained from Merck.

The instrument used was a Model 1305 liquid chromatograph (PO "Nauchpribor", U.S.S.R.) equipped with a spectrophotometric detector operated at 254 nm. The detector cell volume was 1 μl. Home-made microbore glass columns (200 × 1.3 and 100 × 1.3 mm I.D.) were packed by the balanced-density slurry technique at 600 bar. The volume of the sample solution was 0.5–1.0 μl. The columns were operated at room temperature and a flow-rate of 1–8 ml/h.

RESULTS AND DISCUSSION

Tetramethylpropanediamine-1,2 reacts with Cu(II) ions to form binuclear hydroxo complexes with the structure[10]



These complexes are not very stable and are readily transformed into ternary mixed ligand structures if an appropriate amino acid ligand is present in the system:

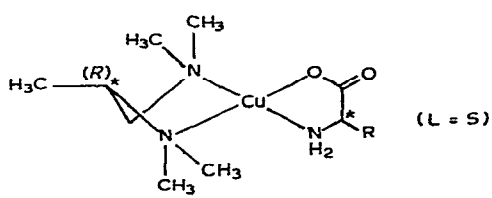


Table I shows the resolutions of a series of racemic amino acids using reversed-phase LiChrosorb RP-18 as the stationary phase and a solution of $Cu_2(tmpn)_2(OH)_2^{2+}$ in acetonitrile–water (15:85) as the chiral eluent. These results imply that a hydrophobic α-substituent in the amino acid molecule is an important prerequisite for the solute to be retained and chirally recognized in the above system. Hydrophilic amino acids display very low capacity factors, *k′*, which reflects the hydrophilic character of

TABLE I

RETENTION AND ENANTIOSELECTIVITY OF SEPARATION OF RACEMIC AMINO ACIDS AND MANDELIC ACID ON A LICHROSORB RP-18 (5 μm) COLUMN (200 × 1.3 mm I.D.) IN A $5 \cdot 10^{-4}$ *M* SOLUTION OF $Cu_2(tmpn)_2(OH)_2(ClO_4)_2$ IN ACETONITRILE–WATER (1:5)

Racemate	k'_D	k'_L	$\alpha = k'_D/k'_L$
Aspartic acid	0.18		1
Alanine	0.35		1
Isovaline	0.42		1
Valine	0.85		1
Norvaline	0.80		1
Leucine	1.10		1
Norleucine	1.70		1
Methionine	1.67		1
Serine	0.37		1
N,N-Dibenzylserine	3.90		1
Phenylserine*	7.33	6.81	1.08
Phenylglycine	2.00		1
Phenylalanine	5.96	5.75	1.04
Tyrosine	1.87	1.67	1.12
Dihydroxyphenylalanine	2.40	2.25	1.07
Tryptophan	10.30	9.00	1.14
Mandelic acid*	1.33	1.12	1.20

* Arbitrary assignment of enantiomers.

the resolving chiral reagent, $Cu_2(tmpn)_2(OH)_2^{2+}$, which is eluted within the void volume of the reversed-phase column.

Obviously, the amino acid (AA) itself has to be sufficiently hydrophobic in order to be retained on the sorbent surface and to promote adsorption of the ternary complex Cu(AA) $(tmpn)^+$. This particular case fits into the second or third of the three previously discussed[11] extreme situations in the "chiral eluent" technique, where the solute retention is governed by adsorption of (i) the resolving chiral ligand, (ii) the ternary complex or (iii) the racemate itself (the first and, occasionally, the third situations are accompanied by the ternary complex adsorption).

Two requirements should be met in order to achieve resolution of a racemic solute according to the "chiral eluent" method: (i) the solute should be retained on the sorbent (by one of the above retention mechanisms or their combination) and (ii) there should be enantioselectivity in the formation of ternary solute–metal–resolving ligand complexes. Apparently, there is no significant enantioselectivity in the case of Val, Norval, Leu, Norleu, Met, Ph-Gly and Bz_2-Ser which are retained but are not resolved. We cannot be certain about the absence of enantioselectivity in ternary complexes of Ala, Asp and Ser because these solutes are hardly retained in our system. Enantioselectivity, although relatively small, is certainly present in ternary tmpn–copper complexes of Tyr, DOPA, Phe, Ph-Ser and Trp, the L-enantiomers of which elute ahead of the D-isomers.

The nature of these enantioselectivity effects remains largely unknown. It is important to be aware that in the system discussed ternary complexes are formed both in the bulk solution and on the surface of the sorbent and that the stability and

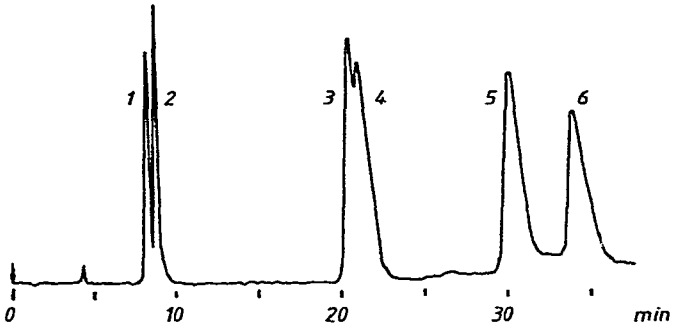


Fig. 1. Separation of three racemic amino acids on a LiChrosorb RP-18 (5 μm) column (200 × 1.3 mm I.D.). Eluent: acetonitrile–water (1:5) containing $5 \cdot 10^{-4}$ *M* $Cu_2(tmpn)_2(OH)_2(ClO_4)_2$. Flow-rate, 1 ml/h. Detection, 254 nm. 1 = L-Tyr; 2 = D-Tyr; 3 = L-Phe; 4 = D-Phe; 5 = L-Trp; 6 = D-Trp.

enantioselectivity of these two species can be entirely different. We can assume that sorbent-bond complexes ("sorption complexes") are responsible for the resolution in our work, because only the more strongly retained amino acids are resolved in the reversed-phase system and because the k' values of these amino acids are significantly lower in the absence of Cu(II) or tmpn.

The fact that all of the solutes resolved happen to be aromatic amino acids (Fig. 1) suggests that the ternary sorption complexes of these solutes possess an unusual conformation, probably with the aromatic group axial to the copper ion. This conformation may result in an enhanced interaction of the diamine chelate ring with the hydrophobic surface of the sorbent, thus increasing the enantioselectivity of the ternary sorption complex.

Contrary to LiChrosorb RP-18, unmodified LiChrosorb Si 100 silica gel strongly retains the chiral resolving complex from its solution in aqueous acetonitrile, which is also shown by the intense blue colour of the sorbent. The interaction of Cu(II) ions or positively charged copper–diamine complexes with the silica gel surface is primarily of an electrostatic nature, so that decreasing the pH value of the eluent

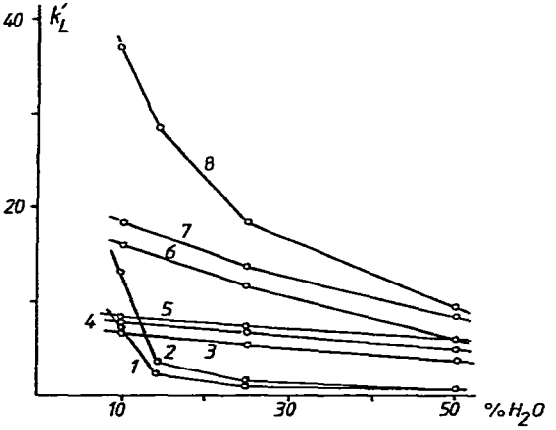


Fig. 2. Retention of L-amino acids as a function of water to acetonitrile ratio in the eluent. LiChrosorb Si 100 (10 μm) column. Chiral additive to the eluent: $5 \cdot 10^{-4}$ *M* $Cu_2(tmpn)_2(OH)_2(ClO_4)_2$ and $5 \cdot 10^{-3}$ *M* (*R*)-tmpn. 1 = Asp; 2 = Glu; 3 = Ile; 4 = Met; 5 = Tyr; 6 = Pro; 7 = Thr; 8 = Ser.

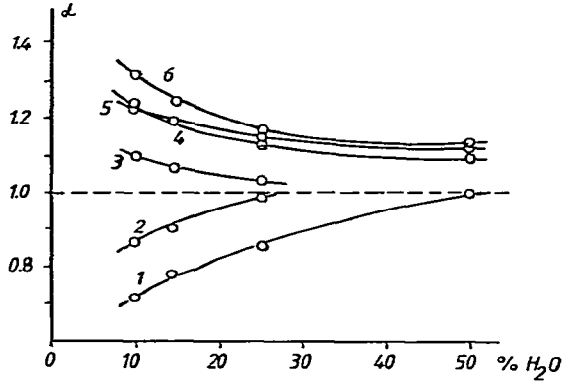


Fig. 3. Enantioselectivity as a function of water to acetonitrile ratio in the eluent. LiChrosorb Si 100 (10 μm) column. Chiral additive to the eluent: $5 \cdot 10^{-4}$ *M* $Cu_2(tmpn)_2(OH)_2(ClO_4)_2$ and $5 \cdot 10^{-3}$ *M* (*R*)-tmpn. 1 = Asp; 2 = Glu; 3 = Val; 4 = Phe; 5 = Tyr; 6 = Trp.

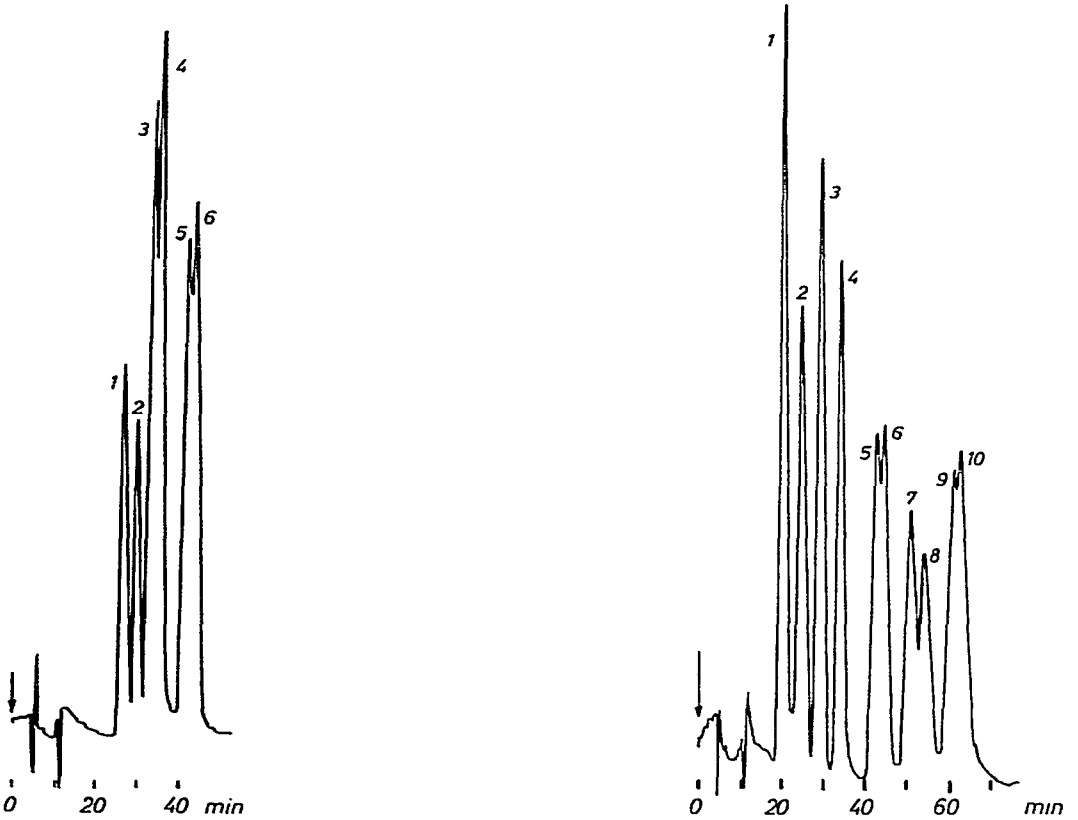


Fig. 4. Separation of three racemic amino acids on a LiChrosorb Si 100 (10 μm) column (100 × 1.3 mm I.D.). Eluent: acetonitrile–water (85:15) containing $5 \cdot 10^{-4}$ *M* $Cu_2(tmpn)_2(OH)_2(ClO_4)_2$ and $5 \cdot 10^{-3}$ *M* (*R*)-tmpn. Flow-rate, 2 ml/h. Detection, 254 nm. 1 = D-Phe; 2 = L-Phe; 3 = D-Val; 4 = L-Val; 5 = D-Abu; 6 = L-Abu.

Fig. 5. Separation of five racemic amino acids on LiChrosorb Si 100. Conditions as in Fig. 4. 1 = L-Asp; 2 = D-Asp; 3 = D-Trp; 4 = L-Trp; 5 = D-Abu; 6 = L-Abu; 7 = D-Pro; 8 = L-Pro; 9 = D-Thr; 10 = L-Thr.

TABLE II

RETENTION AND ENANTIOSELECTIVITY OF SEPARATION OF RACEMIC AMINO ACIDS ON A LICHROSORB Si 100 (10 μm) COLUMN (100 × 1.3 mm I.D.) IN A SOLUTION OF $5 \cdot 10^{-4}$ *M* $Cu_2(tmpn)_2(OH)_2(ClO_4)_2$ AND $5 \cdot 10^{-3}$ *M* (*R*)-tmpn IN DIFFERENT ACETONITRILE–WATER MIXTURES

Amino acid	*90:10*			*85:15*			*75:25*			*50:50*		
	k'_L	k'_D	α*	k'_L	k'_D	α*	k'_L	k'_D	α*	k'_L	k'_D	α*
Aspartic acid	7.44	10.5	0.72	3.40	4.30	0.79	1.28	1.50	0.85	0.55		1
Glutamic acid	15.1	17.4	0.87	6.66		1	1.72		1	0.55		1
Alanine	16.8		1	15.8		1	13.2		1	8.05		1
Aminobutyric acid (Abu)	12.1	11.2	1.07	8.77	8.33	1.05	7.33		1	6.77		1
Isovaline	13.7		1				9.83		1	5.89		1
Valine	8.83	8.05	1.10	7.87	7.37	1.07	7.00	6.80	1.03	5.78		1
Norvaline	8.16	7.61	1.07				6.72		1	5.00		1
Isoleucine	8.05	7.39	1.09	7.50	7.00	1.07	6.75	6.50	1.03	4.83		1
Leucine	6.78		1				5.83		1	4.61		1
Norleucine	7.23		1	6.25		1	5.00		1	4.05		1
Proline	16.1	14.4	1.12	11.0	10.2	1.08	9.30		1	6.11		1
Serine	37.3	35.4	1.05	25.7	24.7	1.04	18.3		1	9.61		1
Phenylserine**	6.10	5.15	1.12	4.89	4.55	1.07	4.28	3.89	1.10	3.94	3.78	1.04
Threonine	18.6	17.6	1.06	12.8	12.4	1.03	10.4		1	8.17		1
Methionine	7.89	7.22	1.09	6.11	5.77	1.06	5.43	5.17	1.05	4.94		1
Phenylglycine	7.17		1				4.66		1	4.28		1
Phenylalanine	6.11	5.00	1.22	5.67	4.89	1.20	5.14	4.66	1.10	4.77	4.38	1.09
Thyrosine	8.55	6.94	1.23	9.11	7.67	1.19	7.17	6.22	1.15	6.05	5.39	1.12
Tryptophan	6.80	5.40	1.31	6.33	5.33	1.16	5.50	4.85	1.13	4.88	4.33	1.13

* $\alpha = k'_L/k'_D$.

** Arbitrary assignment of enantiomers.

or increasing its ionic strength leads to rapid desorption of copper(II) ions. For this reason, it is impossible to keep the column capacity constant if the eluent (acetonitrile–water, 1:1) contains only $5 \cdot 10^{-4}$ *M* $Cu_2(tmpn)_2(OH)_2(ClO_4)_2$. To stabilize the system, an excess of chiral diamine has to be added to the eluent. In addition to stabilization of the column parameters, the excess of diamine ligand results in a decrease in the retention of amino acids.

It can be seen from Table II that the retention of amino acids in the normal-phase system differs considerably from that in the reversed-phase system: hydrophobic amino acids elute first, followed by hydrophilic amino acids. This observation agrees with results of Foucault *et al.*[12] for amino acid analysis using copper-modified silica gel columns. Aspartic acid and glutamic acid, although strongly hydrophilic, are negatively charged in the alkaline eluent and are expelled from the silica surface. However, they are strongly retained with a high content of acetonitrile in the eluent. Generally, the lower the water concentration in the eluent, the higher is the retention of hydrophilic amino acids (Fig. 2). Hence the retention of hydrophobic solutes increases insignificantly.

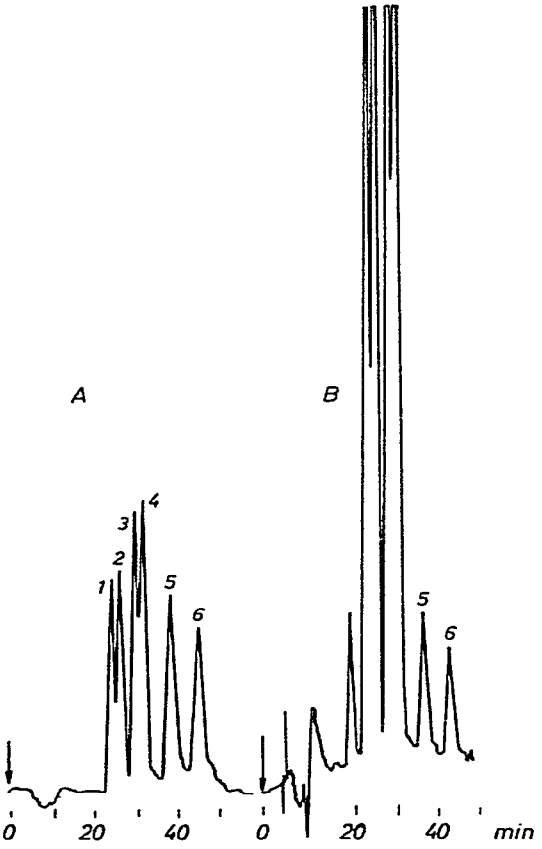


Fig. 6. Separation of three racemic amino acids on LiChrosorb Si 100. Conditions as in Fig. 4. 1 and 2 = Ph-Ser; 3 = D-Met; 4 = L-Met; 5 = D-Tyr; 6 = L-Tyr. A, Fresh solution of amino acids in the eluent; B, the same solution after 4 h.

Simultaneously, a decrease in the water content in the eluent leads to an increase in the separation enantioselectivity (Fig. 3), probably due to enhancement of the interaction of the ternary sorption complex with the silica surface. The mode of this interaction seems to be different from that on the reversed-phase sorbent, as the order of elution of the enantiomers is reversed, with the D-isomers eluting ahead of the L-isomers, except for aspartic acid and glutamic acid isomers.

The opposite directions of the net enantioselectivity effects in normal- and reversed-phase systems clearly demonstrate the difference in the properties of ternary sorption complexes that exist on the sorbent surface and those of the corresponding ternary complexes that exist in the bulk solution.

In both the reversed-phase and normal-phase systems, the enantioselectivity values (α) are not very high, but are still sufficient for the resolution of many amino acids and their mixtures (Figs. 4–6). The normal-phase system is especially useful for the rapid enantiomeric analysis of aromatic amino acids. Phenylalanine and tryptophan can be analysed within a few minutes (Fig. 7). An unexpected peculiarity of the eluent containing an excess of basic (*R*)-tmpn is the oxidation of phenolic groups: DOPA is rapidly oxidized and thyrosine disappears within 4 h (Fig. 6). It is interesting that a neutral solution of $Cu_2(tmpn)_2(OH)_2(ClO_4)_2$ is devoid of oxidizing properties (Fig. 1).

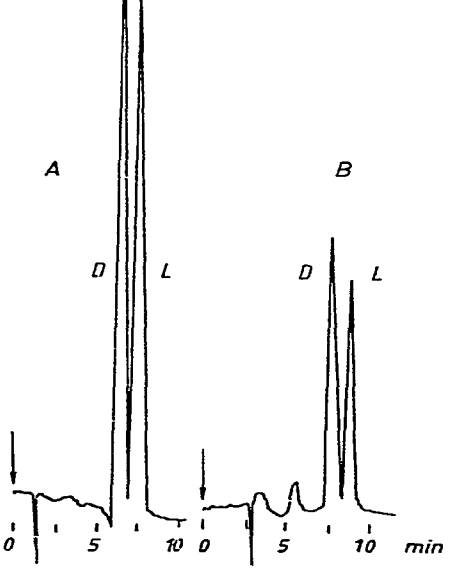


Fig. 7. Rapid analysis of racemic phenylalanine (A) and tryptophan (B) on a LiChrosorb Si 100 (10 μm) column (100 × 1.3 mm I.D.). Eluent: acetonitrile–water (9:1) containing $5 \cdot 10^{-4}$ *M* $Cu_2(tmpn)_2(OH)_2(ClO_4)_2$ and $5 \cdot 10^{-3}$ *M* (*R*)-tmpn. Flow-rate, 8 ml/h. Detection, 254 nm.

REFERENCES

1 L. R. Sousa, D. H. Hoffman, L. Kaplan and D. J. Cram, *J. Amer. Chem. Soc.*, 96 (1974) 7100.
2 V. A. Davankov, *Advan. Chromatogr.*, 18 (1980) 139.
3 P. E. Hare and E. Gil-Av, *Science*, 204 (1979) 1226.
4 Y. Tapuhi, N. Miller and B. L. Karger, *J. Chromatogr.*, 205 (1981) 325.
5 C. Gilon, R. Leshem and E. Grushka, *J. Chromatogr.*, 203 (1981) 365.
6 S. Lam, F. Chow and A. Karmen, *J. Chromatogr.*, 199 (1980) 295.
7 E. Gil-Av, A. Tishbee and P. E. Hare, *J. Amer. Chem. Soc.*, 102 (1980) 5115.
8 E. Oelrich, H. Preusch and E. Wilhelm, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 269.
9 G. Hilgetag and A. Martini, *Weigand-Hilgetag, Organisch-chemische Experimentierkunst*, J. A. Barth Verlag, Leipzig, 3rd ed., 1964.
10 C. Abcus, K. P. Fivizzani and S. Pavkovic, *J. Inorg. Nucl. Chem.*, 39 (1977) 285.
11 V. A. Davankov and A. A. Kurganov, *Chromatographia*, 13 (1980) 339.
12 A. Foucault, M. Caude and L. Oliveros, *J. Chromatogr.*, 185 (1979) 345.