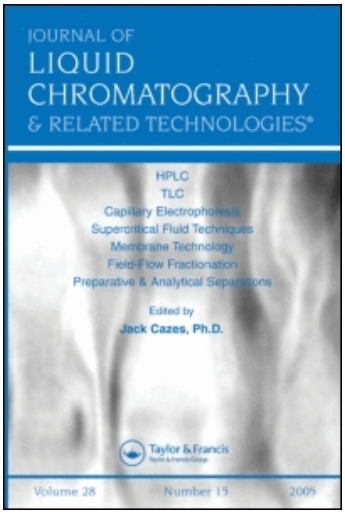

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LIGAND-EXCHANGE CHROMATOGRAPHY OF RACEMATES XI. COMPLETE RESOLUTION OF SOME CHELATING RACEMIC COMPOUNDS AND NATURE OF SORPTION ENANTIOSELECTIVITY

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ABSTRACT

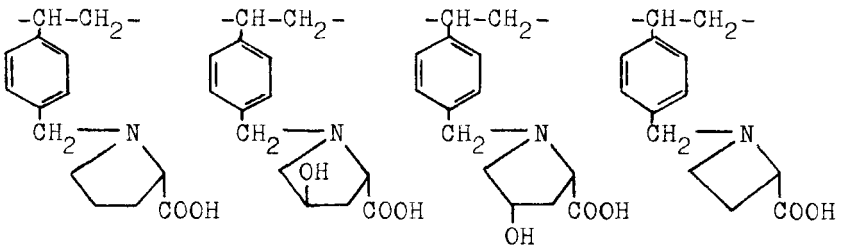
The results of a systematic study are summarized on chromatographic resolutions of racemic amino acids using ligand exchange on copper(II) ion-charged polystyrene type resins containing residues of optically active proline (I), hydroxyproline (II), allo-hydroxyproline (III) and azetidine carboxylic acid (IV). Possible variants of the enantioselective complex formation in the resine phase are discussed.

Several examples of quantitative resolutions of racemic amino acids as well as members of other classes of chelating organic compounds are given.

INTRODUCTION

The enormous possibilities of ligand-exchange chromatography (LEC) in successful separation of compounds with very similar physico-chemical properties such as isomers have recently been reviewed (1). Most convincing are advances in separating optical isomers by LEC on optically active complex-forming resins. Besides the series of communications of the present authors (2), some papers on this subject appeared by Bernauer (3), Angelichi et al. (4), Tsuchida et al. (5), Lefebvre et al. (6), Jozefonvicz et al. (7,8).

Due to the synthesis and thorough investigation of resins containing residues of chiral heterocyclic α-amino acids in a cross-linked polystyrene matrix, a complete resolution of several racemates both in analytical and preparative experiments has become a reality. It is the aim of the present paper (9) to compare the resolving power of the most promising resins based upon L-proline (I), L-hydroxyproline (II), L-allo-hydroxyproline (III) and L-azetidine carboxylic acid (IV) which were described in (10)



I II III IV

and to present new examples of complete resolution of amino acid racemates as well as some members of other classes of organic compounds.

RESULTS

The resins of the above structure of elementary units after being saturated with copper(II) ions to form bis-chelates with two fixed ligands, display a high affinity for mobile amino acid type ligands and a definite discriminating ability between the enantiomers of the latter. Enantioselectivity, α, which is expressed in the form of the ratio of the two enantiomers retention volumes is given in Table 1. As a general regularity, the increase in the value of α can be pointed out with the growing steric requirements of the asymmetric carbon atom substituent, i.e. the ri-

TABLE 1

Enantioselectivity, $\alpha=(V_D-V_o)/(V_L-V_o)$, of Resins I-IV

Racemate	Resin I	II	III	IV
Alanine	1.08	1.04	1.04	1.06
Aminobutyric acid	1.17	1.22	1.18	1.29
Norvaline	1.34	1.65	1.42	1.24
Norleucine	1.54	2.20	1.46	1.40
Valine	1.29	1.61	1.58	1.76
Isovaline		1.25		
Leucine	1.27	1.70	1.54	1.24
Isoleucine	1.50	1.89	1.74	1.68
Serine	1.09	1.29	1.24	2.15
Threonine	1.38	1.52	1.48	1.28[x]
allo-Threonine	1.55	1.45		
Homoserine		1.25		
Methionine	1.04	1.22	1.52	1.29
Asparagine	1.18	1.17	1.20	1.44
Glutamine	1.20	1.50	1.40	1.25
Phenylglycine	1.67	2.22	1.78	1.38
Phenylalanine	1.59	2.89	3.10	1.86
α-Phenyl-α-alanine		1.07		
Tyrosine	2.46	2.23	2.36	1.78
Phenylserine		1.82		
Proline	4.05	3.95	1.84	2.48
Hydroxyproline	3.85	3.17	1.63	2.25
allo-Hydroxyproline	2.32[x]	1.65[x]	1.48	1.46[x]
Azetidine carboxylic acid		2.25		
Ornitine	1.0	1.0	1.20	1.0
Lysine	1.10	1.22	1.33	1.06
Histidine	2.70[x]	2.8-8.0[xx]	1.32	1.80[x]
Tryptophane	1.40	1.8-3.1[xx]	1.10	1.13
Aspartic acid	1.10[x]	1.0	1.23[x]	1.13[x]
Glutamic acid	1.60[x]	1.22[x]	1.45[x]	1.29[x]

[x] D-Enantiomer eluated ahead of the L-isomer.
[xx] The value depends on the NH_4OH conc. in the eluent.

sing length of the side chain or introduction of aromatic systems into it. Branching of the side radical, especially, at its ß-carbon atom enhances enantioselectivity. The same is true for the influence of a hydroxy group attached to the ß-C-atom (compare Ser and Thr with Ala and Abu). Contrary to this, a γ-hydroxy substituent (Hse) or other polar groupings in the γ-position (Met, Asn) cannot effect favourably the separation selectivity. Isovaline and α-phenyl-α-alanine containing two substituents at the asymmetric C-atom are resolved worse than their isomeric amino acids.

The structure of the resin-fixed ligands has a strong impact on its resolving ability. On transition from resin I to resin II, enantioselectivity increases by a factor of 2 in the chromatography of DL-Val (the values of α are 1.29 and 1.61, respectively) inspite of the fact that the γ-OH group in the fixed hydroxyproline ligand for steric reasons is deprived of the ability to interact with the chelated Cu(II) ion and with the mobile ligand (Val) in the sorption complex. Decreasing the ring size of resin-fixed ligand (resin IV) results in a similar unexpected improvement of separation of valine enantiomers (α=1.76).

The resins under investigation display a high enantioselectivity on chromatography of aromatic amino acids (Phgl, Phe, Ser(Ph), Tyr) and only a poor resolution of diamino (Orn, Lys) and dicarboxylic (Asp, Glu) amino acids. It is noteworthy that the basic compounds possess an exceptionally high affinity (large retention volumes), while acid compounds, on the contrary, a very low affinity for resin copper chelates.

Among the best resolutions observed are those of the heterocyclic amino acids, five-membered heterocycles appearing to produce the highest enantioselectivity. Thus, in the chromatography of racemic azetidine carboxylic acid, proline and α-pipecolinic acid on the $\overline{R}$LHyp resin (II) in its copper form, the maximal value of α (2.25, 3.95 and 1.25, respectively) is that shown by proline.

Interesting peculiarities in the elution order of enantiomers of bidentate and tridentate amino acid ligands render some information on the possible structure of the sorption complexes. As a rule, the resin-fixed ligands of the L-configuration prefer forming mixed-ligand sorption complexes with bidentate mobile ligands of D-configuration. Thus, of the two possible diastereomeric sorption complexes produced upon interaction of the L-proline containing resin I with a solution of racemic proline, $\overline{R}$LPro-Cu-LPro and $\overline{R}$LPro-Cu-DPro (Fig. 1), the latter is more stable, the difference in the thermodynamic standard free energy, $\delta\Delta G^{o}$, being about 835 cal/mol. This difference is mainly due to the fact that the α-radical of the L-amino acid has to interfere with the strongly binded water molecule in the upper axial Cu(II) coordination position, whereas the D-amino acid has to displace just the lower water molecule which coordination was weaker anyway, because of its interference with the resin-fixed ligand benzyl radical (11).

Typically tridentate amino acid ligands His, Orn, Asp, allo-Hyp, as well as Lys and Glu are liable to form additional coordination bonds engaging one of the two axial positions of the Cu(II) ion coordination oc-

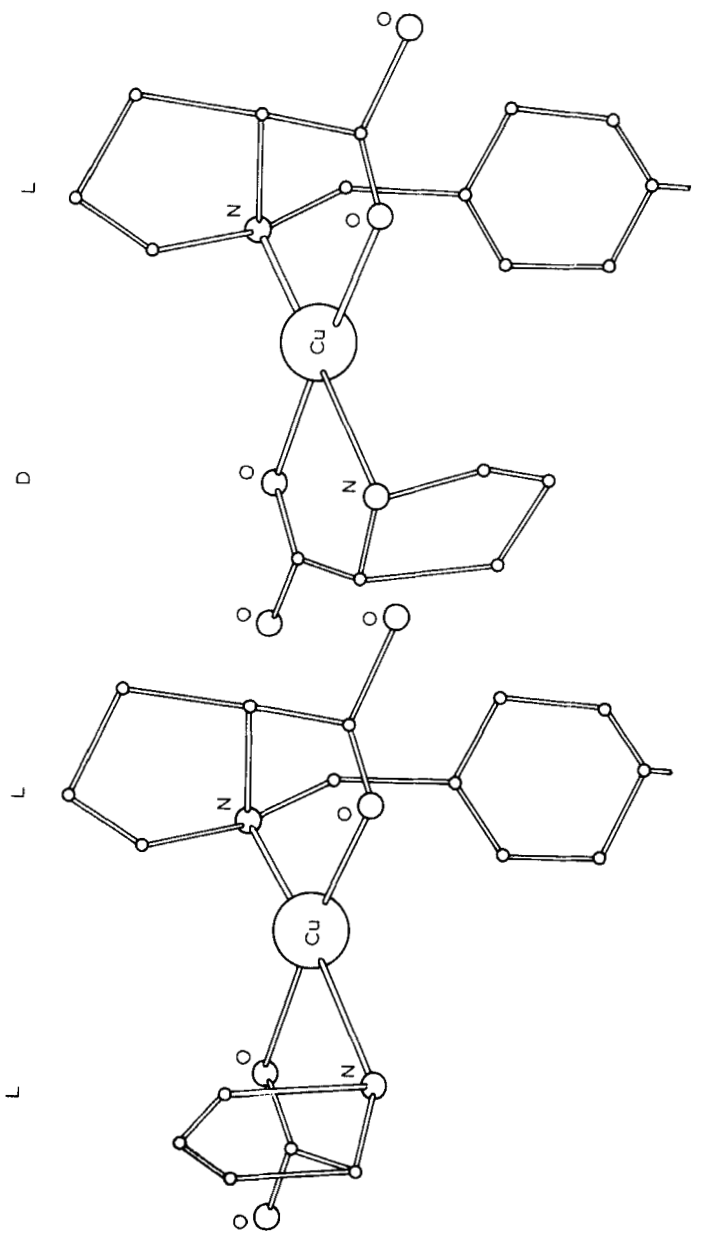


FIGURE 1: Structure of the less stable sorption complex RLPro-Cu-LPro and the more stable sorption complex RLPro-Cu-DPro; $\delta\Delta G^{o}$ = 835 cal/mol.

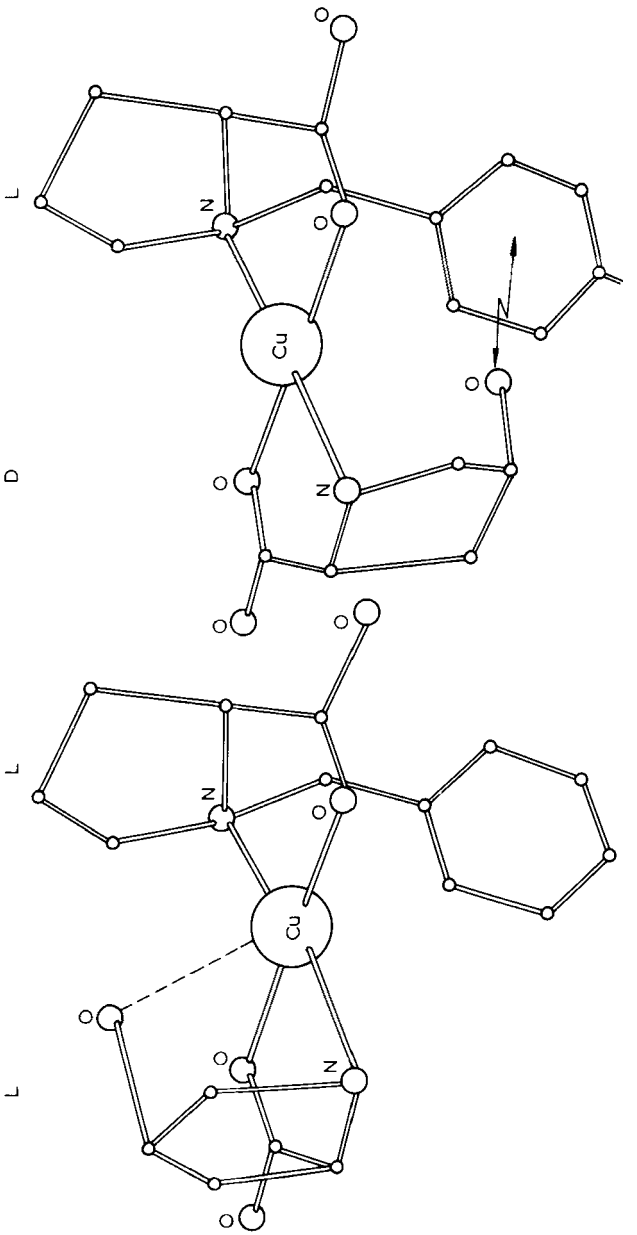


FIGURE 2. Structure of the more stable sorption complex RLPro-Cu-LaHyp and the less stable sorption complex RLPro-Cu-DaHyp; $\delta\Delta G^{o}$ = 490 cal/mol.

tahedron. Howewver, the lower of those two positions is sterically hindered by the N-benzyl radical of the fixed ligand (11). Therefore, only mobile ligands having L-configuration have the privelege to fully reveal their tridentate nature (Fig. 2) when forming sorption complexes with the fixed ligands of L-configuration in resins I, II and IV. In this case D-enantiomers of the tridentate mobile ligands form weaker sorption complexes and are eluated first.

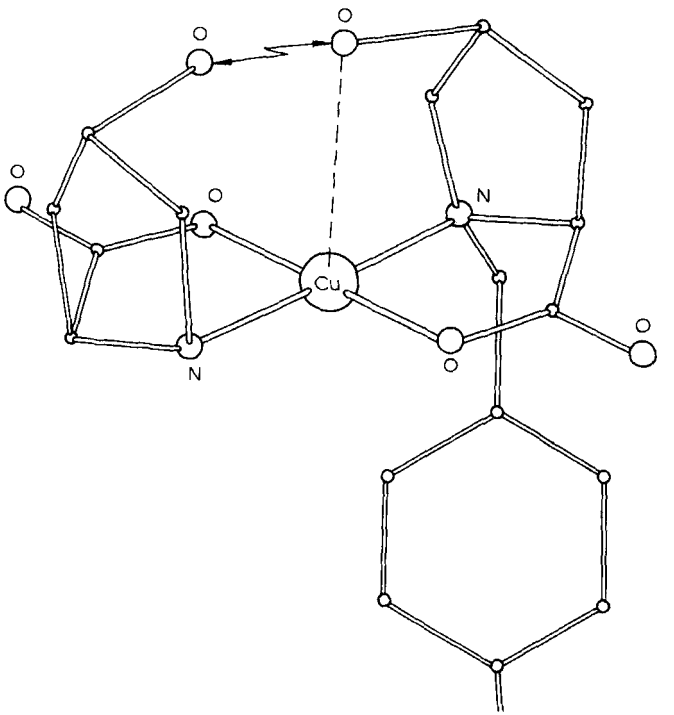


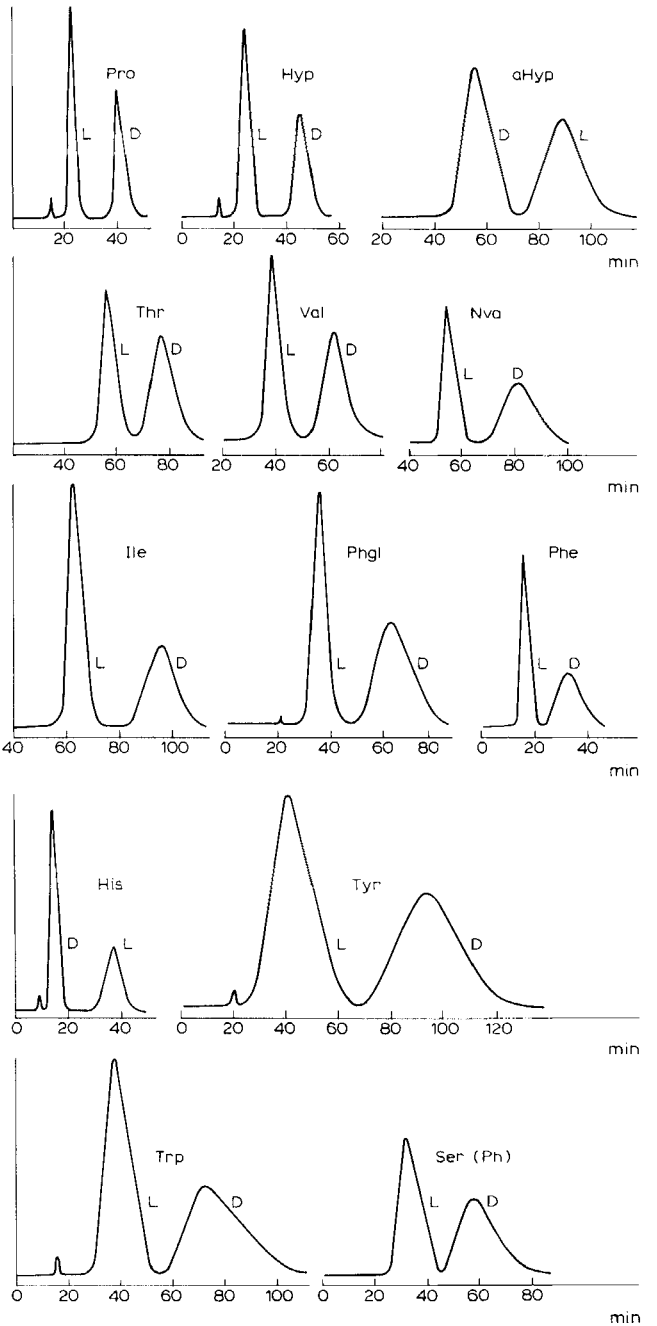
FIGURE 3

Structure of the less stable sorption complex RLaHyp-Cu-LaHyp; $\delta\Delta G^{O}$= 285 cal/mol.

The situation becomes completely different with resin III the fixed $\bar{R}$LaHyp-type ligand of which is tridentate itself and effectively blocks with its γ-hydroxy group the upper axial position of the Cu(II) ion. Here, the mobile L-ligands are deprived of the possibility to form unusually stable sorption complexes (Fig. 3) and are eluated ahead of the D-isomers, just like in the case with all the typically bidentate amino acids. Alone L-aspartic and L-glutamic acids manage to produce stabler sorption complexes than their D-enantiomers do, probably as a result of a hydrogen bond formation between the ω-carboxy group and the fixed ligand hydroxy group coordinated in the axial position.

Retention volumes of different amino acids on the copper form of chiral resins (10) vary in a wide range. (This may be promising in that using ammonia gradient elution it will be possible to analyse mixtures of several amino acids.) However, choosing the proper degree of saturation of the resin with metal ions (12) one can make the first enantiomer leave the chromatographic column within 1.5-3 void volumes. In this case both the separation from accompanying impurities and the resolution of two enantiomers are accomplished in a short period of time. Fig. 4 presents a set of examples of base-line resolutions which can be easily achieved in systems displaying selectivity of $\alpha \geq 1.5$ (Table 1).

It should be emphasized that using ligand-exchange chromatography techniques no preliminary chemical modifications of amino and carboxy groups of the starting racemate are needed, which are indispensable in



resolutions using gas chromatography. Moreover, LEC has doubtless advantages in preparative-scale experiments: a 1 l column containing 300 g of resin II granulae, 0.1-0.3 mm in diameter, is capable of resolving up to 20 g of DL-proline or 6 g of DL-threonine in one elution cycle (values of α 3.95 and 1.52, respectively).

Finally, one has to bear in mind that LEC is a general method for separating compounds which are able to form labile complexes with transition metal ions. Thus the asymmetric resins described in this paper can be employed in resolving many chelating agents, other than α-amino acids. Fig. 5 gives examples of successful resolutions from the following classes of organic compounds: α-hydroxy acids, β-amino acids, 1,2-diamines, 1,2-amino alkohols.

FIGURE 4

Chromatography of ca 1 mg racemic amino acid on a 7.8x140 mm column containing 6.3 ml L-hydroxyproline resin II (capacity 3.86 mmol/g, degree of cross-linking 6 %, particles of irregular shape ca 50 μm). Detector Uvicord I (LKB) without light filter. For each racemate are given: copper content of the resin in % to the theoretical value of 1.93 mmol/g, ammonia concentration in the eluent in mol/l, flow rate in ml/h. Pro: 65, 1.0, 20; Hyp: 65, 0.5, 20; aHyp: 55, 0.05, 20; Thr: 80, 0.05, 20; Val: 65, 0.1, 13; Nva: 65, 0.05, 16; Ile: 65, 0.1, 13; Phgl: 65, 0.1, 13; Phe: 45, 0.1, 20; His: 30, 0.5, 25; Tyr: 65, 0.1, 16; Trp: 30, 0.4, 20; Ser(Ph): 45, 0.05, 14.

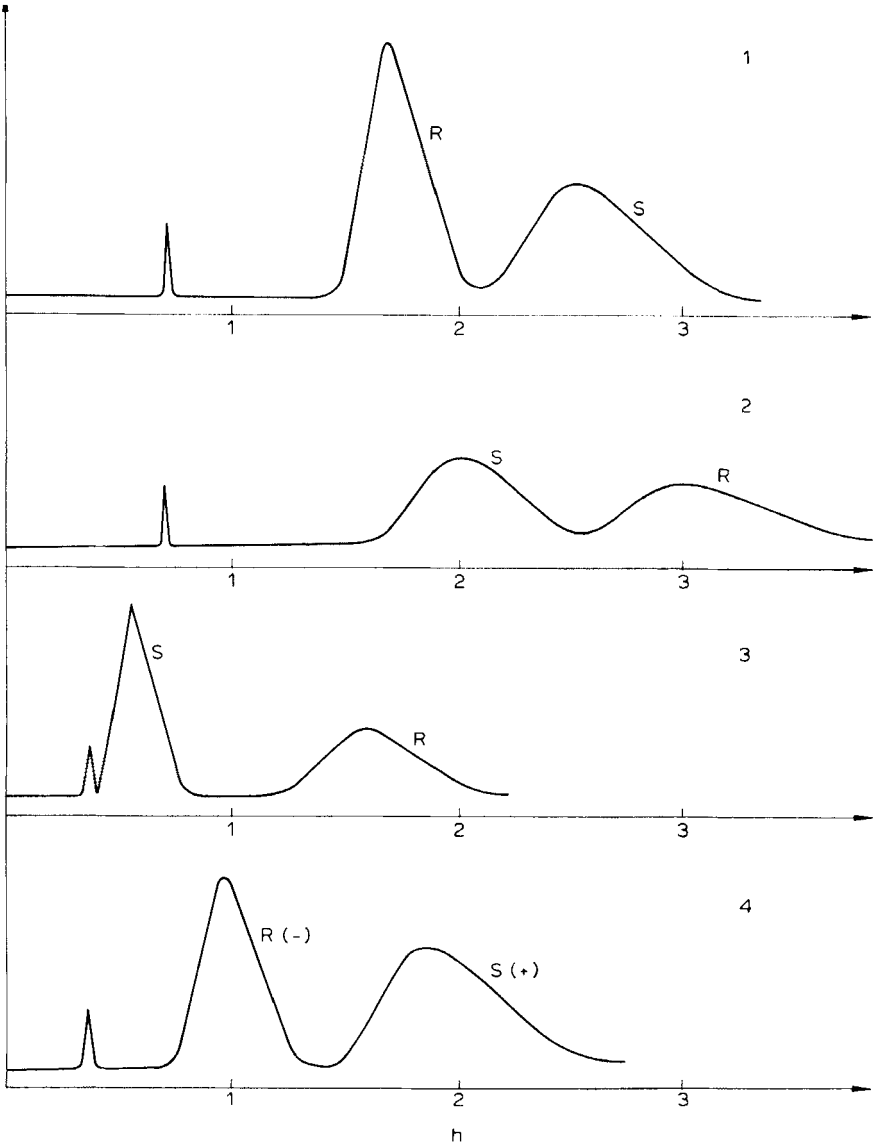


FIGURE 5

Chromatography of different racemates on L-hydroxyproline resin II (capacity 3.86 mmol/g, degree of cross-linking 6 %, particles of irregular shape ca 50 µm). Detector Uvicord III (LKB) at 206 nm.

1: β-Phenyl-β-alanine (α =1.78), column 5x280 mm, 90 % Cu, 0.05 M NH_3, 10 ml/h.

2: Mandelic acid (α =1.65), column 5x280 mm, 100 % Cu, 0.01 M $(NH_4)_3PO_4$, 10 ml/h.

3: 2-Aminopropanol-1 (α =7.0), column 7.8x140 mm, 50 % Cu, 0.05 NH_3, 15 ml/h.

4: N'-Benzyl-propylenediamine-1,2 (α =2.50), column 7.8x140 mm, 50 % Cu, 0.05 NH_3, 15 ml/h.

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