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LIGAND-EXCHANGE CHROMATOGRAPHY OF RACEMATES

VI. SEPARATION OF OPTICAL ISOMERS OF AMINO ACIDS ON POLYSTYRENE RESINS CONTAINING L-PROLINE OR L-AZETIDINE CARBOXYLIC ACID

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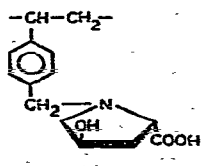
SUMMARY

Optical isomers of amino acids have been separated on the copper form of asymmetric resins containing residues of heterocyclic L-α-amino acids (proline and azetidine carboxylic acid) on a cross-linked polystyrene matrix. The enantioselectivity of the sorbents with respect to the optical isomers of many amino acids is high enough (200–800 cal/mole) to allow their quantitative separation. The separating capacity of the sorbent containing L-proline residues is generally higher than that of the sorbent based on L-azetidine carboxylic acid.

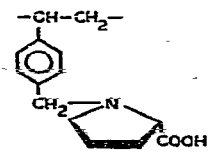
INTRODUCTION

Ligand-exchange chromatographic resolution of racemates of amino acids on a polystyrene resin containing L-hydroxyproline residues (I) as fixed ligands and charged with copper ions has been described in our previous paper[1]. The results are provided evidence that ligand-exchange chromatography (LEC) of enantiomers is a very promising technique for both analysis and preparation.

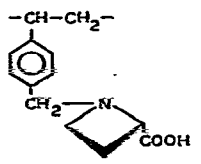
The present paper is the next step in determining the influence of the fixed ligand structure on LEC of racemic amino acids. It describes the resolution of racemates on sorbents containing fixed five- and four-membered heterocyclic ligands that are residues of L-proline (II) and L-azetidine carboxylic acid (III).



I



II



III

EXPERIMENTAL

The asymmetric resins were prepared by aminating the chloromethylated macronet polystyrene matrix containing 11 mol% of cross-links of diphenylmethane structure by methyl esters of proline or azetidine carboxylic acid, as described in ref. 2. The sorbent capacity was 2.78 (for II) and 2.40 (for III) mmol fixed ligands per gram of dry resin in its zwitterionic form. The resin particles were of irregular shape, and their size in the swollen form was *ca.* 100 μm. The resins were charged with copper(II) ions from a copper–ammonia solution, until they contained 80% of the theoretical amount of copper corresponding to the formation of fixed complexes containing two fixed ligands per copper ion. The equilibrium water content in the copper form of the resin was 170% for sorbent II and 140% for sorbent III.

As in the previous study[1], the chromatographic column (14 cm × 7.8 mm I.D.) contained 6.3 ml resin. The detector used was Uvicord III (LKB) with the 206 nm light filter. Elution at the rate 10 ml/h was carried out at room temperature by ammonia solutions of concentration 0.1, 0.3 and 1.5 M containing $1.2 \cdot 10^{-5}$, $3.8 \cdot 10^{-5}$ and $2.0 \cdot 10^{-4}$ M $CuSO_4$ respectively, and by an 0.017 M ammonium phosphate solution (pH 8.8) containing $2.5 \cdot 10^{-5}$ M $CuSO_4$. In each experimental run either 2–3 mg of racemic amino acid or 1.0–1.5 mg of each enantiomer were chromatographed.

RESULTS AND DISCUSSION

Tables I and II give retention parameters for D- and L-amino acid enantiomers in LEC on columns filled with copper complexes of sorbents II and III, respectively. The retention volumes, V_L and V_D, which are expressed in multiples of the void volume of the column (4.7 ml), were used to calculate the column selectivity, α, and the difference in standard free energies, $\delta\Delta G°$, of two diastereomeric sorption complexes formed by coordination of amino acid enantiomers to the chiral resin chelate according to the relation:

$$\delta\Delta G° = -RT \ln \alpha = -RT \ln \frac{V_D}{V_L}$$

Aliphatic amino acids

As indicated by Tables I and II, the larger substituent on the α-carbon atom in the mobile amino acid ligand, the longer the retention time of enantiomers and the better their separation.

The steric structure of the chiral sorption centres of the resin is also very important. Even minor changes in the fixed ligand structure markedly affect the sorption enantioselectivity. For instance, chromatography of D,L-Val on resin I with hydroxyproline residues results in a value of $\delta\Delta G°$ almost twice as high as for sorbent II with proline residues, in spite of the fact that for spatial reasons the hydroxy group of the fixed hydroxyproline ligand cannot directly interact with the chelated copper ion and the mobile ligand attached to it. A decrease in the size of the fixed ligand ring by one methylene group on going from sorbent II to sorbent III leads to a considerable increase in valine sorption enantioselectivity (from 150 to 320 cal/mol).

TABLE I

ELUTION PARAMETERS OF AMINO ACIDS ON THE L-PROLINE RESIN IN THE COPPER(II) FORM

Eluents: 0.1 *M* NH_4OH (N = 1–15); 0.3 *M* NH_4OH (N = 16–24); 1.5 *M* NH_4OH (N = 25–29); 0.017 *M* $(NH_4)_3PO_4$, pH 8.8 (N = 30–32).

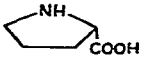
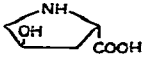
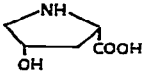
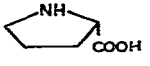
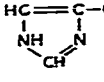
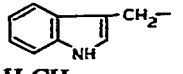
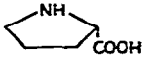
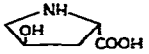
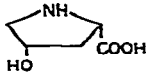
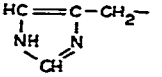
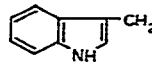
N	*Amino acid*	*α-Radicals or molecular structure*	*V*		*α*	*δΔG°* (cal/mol)	*HEEP (cm)*	
			L	D			L	D
1	Glycine	H–	5.0		—	—	0.66	
2	Alanine	CH_3–	6.75	7.25	1.08	46	0.67	0.64
3	Aminobutyric acid	CH_3CH_2–	7.20	8.50	1.17	92	0.70	0.68
4	Norvaline	$CH_3CH_2CH_2$–	14.2	18.2	1.28	145	0.68	0.70
5	Norleucine	$CH_3CH_2CH_2CH_2$–	25.5	39.4	1.54	225	0.65	0.67
6	Valine	$CH_3CH(CH_3)$–	9.0	11.6	1.29	150	1.00	0.95
7	Serine	$HOCH_2$–	4.0	4.35	1.09	52	0.85	0.90
8	Threonine	$HOCH(CH_3)$–	4.0	5.5	1.38	190	1.00	0.95
9	allo-Threonine	$HOCH(CH_3)$–	3.25	5.0	1.55	260	1.05	1.00
10	Asparagine	H_2NCOCH_2–	4.25	5.0	1.18	97	0.70	0.62
11	Glutamine	$H_2NCOCH_2CH_2$–	3.75	4.5	1.20	110	0.90	0.74
12	Proline		17.0	70	4.10	825	0.62	0.60
13	Hydroxyproline		9.9	38.2	3.85	790	0.42	0.46
14	allo-Hydroxyproline		43.5	18.8	2.32	490	0.96	0.92
15	Phenylglycine	C_6H_5–	11.3	18.8	1.67	300	1.40	1.20
16	Norvaline	$CH_3CH_2CH_2$–	4.10	5.75	1.40	200	0.66	0.68
17	Norleucine	$CH_3CH_2CH_2CH_2$–	12.0	18.5	1.54	250	0.65	0.68
18	Leucine	$CH_3CH(CH_3)CH_2$–	13.0	16.5	1.27	140	0.56	0.58
19	Isoleucine	$CH_3CH_2CH(CH_3)$–	7.0	10.5	1.50	240	0.49	0.48
20	Phenylalanine	$C_6H_5CH_2$–	31.5	51.5	1.63	286	1.05	0.98
21	Tyrosine	$HOC_6H_4CH_2$–	2.65	6.5	2.46	530	1.20	1.06
22	Proline		6.25	25.0	4.00	815	0.67	0.62
23	Hydroxyproline		3.50	13.5	3.85	790	0.37	0.43
24	Methionine	$CH_3SCH_2CH_2$–	6.25	6.5	1.04	24	0.46	0.48
25	Lysine	$H_2NCH_2CH_2CH_2CH_2$–	2.5	2.75	1.10	57	1.80	1.64
26	Ornithine	$H_2NCH_2CH_2CH_2$–	2.5	2.5	1.0	—	2.6	2.2
27	Histidine		15.5	5.75	2.70	365	1.00	0.95
28	Tryptophan		5.5	7.8	1.40	200	1.1	1.03
29	Phenylalanine	$C_6H_5CH_2$–	6.0	9.25	1.54	255	0.95	0.96
30	Aspartic acid	$HOOCCH_2$–	4.25	3.75	1.10	57	1.6	1.4
31	Glutamic acid	$HOOCCH_2CH_2$–	2.0	1.25	1.60	275	1.75	1.6
32	Iminodiacetic acid	$HN(CH_2COOH)_2$	7.5		—	—	1.2	

TABLE II

ELUTION PARAMETERS OF AMINO ACIDS ON THE AZETIDINE-CARBOXYLIC ACID RESIN III IN THE COPPER(II) FORM

Eluents: 0.1 *M* NH_4OH ($N = 1$–6); 0.3 *M* NH_4OH ($N = 7$–17); 1.5 *M* NH_4OH ($N = 18$–23); 0.017 *M* $(NH_4)_3PO_4$, pH 8.8 ($N = 24$–31).

N	*Amino acid*	*α-Radicals or molecular structure*	*V*		*α*	*δΔG°* (cal/mol)	*HEEP (cm)*	
			L	D			L	D
1	Glycine	H–	7.2		—	—	1.72	
2	Alanine	CH_3–	11.2	11.9	1.06	35	1.68	1.70
3	Aminobutyric acid	CH_3CH_2–	15.0	19.2	1.28	145	1.91	1.84
4	Valine	$CH_3CH(CH_3)$–	24.0	41.0	1.78	315	1.87	1.93
5	Norvaline	$CH_3CH_2CH_2$–	52	64	1.23	120	1.66	1.74
6	Tyrosine	$HOC_6H_4CH_2$–	9.6	19.0	1.78	335	2.04	2.01
7	Valine	$CH_3CH(CH_3)$–	3.1	5.4	1.74	320	1.92	1.96
8	Aminobutyric acid	CH_3CH_2–	1.85	2.4	1.30	155	1.97	1.93
9	Methionine	$CH_3SCH_2CH_2$–	7.2	9.3	1.29	150	1.59	1.67
10	Proline		7.5	18.6	2.48	530	1.93	1.84
11	Hydroxyproline		3.6	8.1	2.25	475	1.86	1.85
12	allo-Hydroxyproline		8.3	5.7	1.46	220	1.84	1.93
13	Leucine	$CH_3CH(CH_3)CH_2$–	18.2	22.5	1.24	125	1.62	1.69
14	Isoleucine	$CH_3CH_2CH(CH_3)$–	15.1	25.5	1.68	305	1.57	1.63
15	Norvaline	$CH_3CH_2CH_2$–	9.12	11.4	1.25	130	1.69	1.74
16	Norleucine	$CH_3CH_2CH_2CH_2$–	25.2	35.4	1.41	200	1.63	1.60
17	Phenylglycine	C_6H_5–	4.8	6.6	1.38	190	1.94	1.97
18	Ornithine	$H_2NCH_2CH_2CH_2$–	2.1	2.1	1.0	—	2.4	2.4
19	Lysine	$H_2NCH_2CH_2CH_2CH_2$–	1.8	1.91	1.06	35	2.3	2.25
20	Histidine		27.6	15.3	1.80	340	2.1	2.2
21	Tryptophan		33.2	37.4	1.13	70	2.25	2.16
22	Phenylalanine	$C_6H_5CH_2$–	7.25	13.5	1.86	360	2.24	2.20
23	Norleucine	$CH_3CH_2CH_2CH_2$–	3.10	4.28	1.38	190	1.80	1.73
24	Serine	$HOCH_2$–	6.0	12.9	2.15	445	1.92	1.96
25	Threonine	$HOCH(CH_3)$–	13.7	10.7	1.28	145	1.94	1.92
26	Asparagine	H_2NCOCH_2–	13.8	9.6	1.44	210	1.78	1.83
27	Glutamine	$H_2NCOCH_2CH_2$–	17.4	21.8	1.25	130	1.84	1.96
28	Glycine	H–	36		—	—	1.78	
29	Aspartic acid	$HOOCCH_2$–	3.05	2.7	1.13	70	2.15	2.23
30	Glutamic acid	$HOOCCH_2CH_2$–	9.0	7.2	1.29	150	2.08	2.18
31	Iminodiacetic acid	$HN(CH_2COOH)_2$	2.4		—	—	2.04	

It is also noteworthy that sorbent III is more sensitive to an increase in the size of the hydrocarbon radical of the mobile amino acid ligand. When passing from non-substituted Gly to L-Nva, the retention volume increases on this sorbent by almost 7 times, but by only 3.5 times on sorbent II.

Hydroxyl-containing amino acids

It was found for sorbent I that introduction of a hydroxy group on to the β-C-atom of the mobile amino acid ligands decreases their retention time and increases the sorption enantioselectivity. The same proved true for the sorbents II and III. The most pronounced decrease in retention time is exhibited by the sorbent III with azetidine carboxylic acid residues. In 0.1 *M* NH_4OH, L-Ser very weakly interacts with this sorbent; elution by phosphate buffer solution indicates that retention of this amino acid is about 10 times weaker than that of alanine. Nevertheless, serine sorption enantioselectivity in this system is very high ($\alpha = 2.15$). Retention of threonine on all three sorbents is greater than that of serine. Surprisingly, the order of elution of threonine enantiomers (D, L) on sorbent III is reversed.

Resolution of allo-threonine on sorbent II is better than that of threonine whereas on sorbent I it is somewhat worse.

Aromatic amino acids

The sorbents II and III, with heterocyclic fixed ligands, exhibit high affinity and enantioselectivity towards aromatic amino acids. On these sorbents, the separation factors for Phgl and Phe are lower than on sorbent I. For Tyr, which differs from Phe by a hydroxy group in the *p*-position of the aromatic ring, the separation factor proved to be the highest on sorbent II ($\alpha = 2.46$).

Cyclic amino acids

The highest enantioselectivity of the sorbents studied was observed in resolutions of cyclic amino acids. The retention volumes, the order of enantiomer elution and enantioselectivity values for Pro, Hyp and aHyp on the sorbents I and II (with proline and hydroxyproline residues) proved to be close to each other. The separation factors on the sorbent III (with azetidine carboxylic acid residues) are *ca.* 2 times lower. These results are consistent with the appreciably lower enantioselectivity of sorbent I towards racemic azetidine carboxylic acid than towards racemates of proline or hydroxyproline[1]. The lower stability of mixed-ligand sorption complexes with D-aHyp on all three sorbents, which leads to the inverse order of elution of isomers of aHyp, is due to the fact that contrary L-aHyp D-aHyp cannot act in a tridentate manner in complex formation with fixed ligands of L-configuration. We have already explained the reasons for this behaviour with reference to sorbent I[1].

Basic and acidic amino acids

As basic amino acids display a particularly strong affinity for asymmetric sorbents II and III, their elution was carried out in ammonia solutions of high concentration (1.5 *M*). The sorption enantioselectivity proved somewhat lower than in the case of sorbent I. As typical tridentate ligands, His isomers displayed an inverse order of elution on all three sorbents.

On the contrary, acidic amino acids possess a weak affinity for the sorbents

in alkaline eluents. Thus, their chromatography requires the use of a phosphate buffer (pH 8.8). D-Isomers of glutamic and aspartic acids are eluted first, this being a possible indication that L-enantiomers can participate in the sorption complexes as tridentate ligands. The highest enantioselectivity towards dicarboxylic amino acids is manifested by sorbent II with proline residues.

In the series glutamic, aspartic, iminodiacetic acid the retention time on sorbent III falls, whereas the affinity towards sorbents I and II increases.

General remarks

LEC of racemic amino acids using sorbents II and III, containing L-proline and L-azetidine carboxylic acid residues, is highly enantioselective. Resin II provides better resolution of racemic allo-threonine, proline, hydroxyproline, allo-hydroxyproline, tyrosine and glutamic acid than does sorbent I. The average enantioselectivity of sorbent III is somewhat lower. However, it provides better resolution of aminobutyric acid, valine, methionine, serine, asparagine and aspartic acid. Fig. 1 illustrates the high resolution capacity of asymmetric resins in LEC of racemic amino acids —the capacity that is adequate for preparative separations.

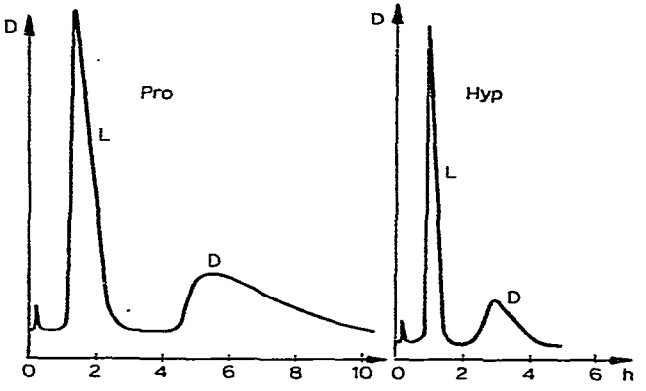


Fig. 1. Chromatography of racemic proline and hydroxyproline. Column 7.8 × 140 mm; 0.3 *M* NH_4OH; 10 ml/h. The degree of saturation of the L-proline resin by copper(II) ions was 80%. Particle size *ca.* 100 μm. Degree of cross-linking 11%.

When copper(II) ions are used as the complex-forming agent, all three resins with fixed ligands of the L-configuration exhibit higher affinity for D-amino acids. However, the tridentate amino acid ligands (histidine, allo-hydroxyproline, aspartic acid and glutamic acid) are an exception. This may be because the D-isomers of these amino acids cannot act as tridentates in the mixed-ligand sorption complexes of the *trans* structure in the coordination square-plane of the copper(II) ion because of steric interactions of their side-groups with the *N*-benzyl radical of the L-fixed ligands[1]. This consideration may also account for the inverse elution order of threonine enantiomers on sorbent III.

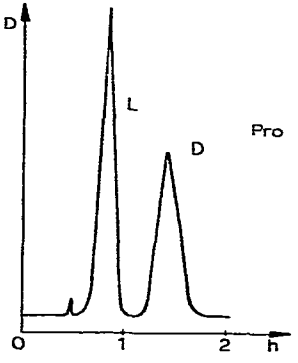
We shall show in a following paper that sorption of amino acids on the copper form of the sorbents obeys gel-phase diffusion kinetics. Therefore the sooner interfacial equilibrium is reached, the greater are the dispersity and swelling ability of

the resin. The latter property sharply increases with the sorbent exchange capacity, thus providing for higher efficiency in LEC.

The swelling ability of sorbent I was 200%, and its capacity was 3.44 mmol L-hydroxyproline residues per gram of dry sorbent (in its zwitterionic form), the copper ion content being 92% of the theoretical value. The heights equivalent to effective plate (HEEP) for aliphatic amino acids separated on this sorbent were 0.3–0.5 cm, so that a number of racemates could be completely resolved on a column of 14 cm[1]. Complete separation of enantiomers at a sorption enantioselectivity of *ca.* 300 cal/mol requires *ca.* 50 theoretical plates.

The capacities of sorbents II and III (2.78 and 2.40 mmol/g), as well as their swelling abilities (170 and 140%), were lower than those of the sorbent I. This apparently was the reason for increased HEEP values (Tables I and II) under identical chromatography conditions. In case of sorbent II, a column 14 cm long corresponded (for aliphatic amino acids) to 25–15 theoretical plates, but in case of sorbent III to just 7–9. Therefore, the ultimate separation of enantiomers on sorbents II and III was poorer than that on similar columns with sorbent I. However, the chromatographic efficiency can be enhanced. We have synthesized a sorbent with L-proline fixed ligands on spherical polystyrene beads containing 1% divinylbenzene and displaying a narrow distribution of particle size (0.64–0.72 μm). The capacity of this sorbent is 3.5 mmol per gram of dry resin. The HEEP values appeared to be 2.5–3 times lower than those given in Table I. Fig. 2 illustrates how this variation in the sorbent performance accelerates and improves the resolution of D,L-proline without decreasing the sorbent enantioselectivity. However, further enhancement of the efficiency is still needed for the complete resolution of aromatic amino acids, which have especially broad elution bands (Fig. 3).

It is of interest that for proline and hydroxyproline resins of equal capacity, swelling ability, and granule size, the efficiency parameters are almost equal.



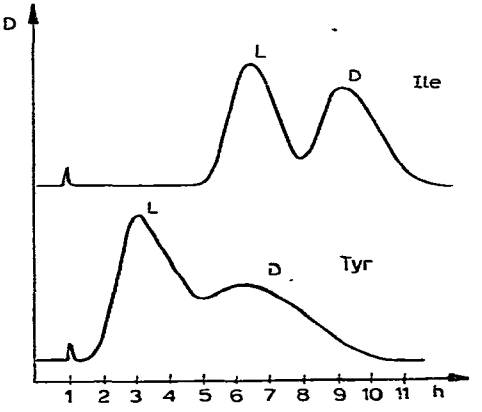


Fig. 2. Chromatography of racemic proline. Column 7.8 × 140 mm; 1.0 *M* NH_4OH; 10 ml/h. The degree of saturation of the L-proline resin by copper(II) ions was 60%. Particle size *ca.* 70 μm. Degree of cross-linking 1%.

Fig. 3. Chromatography of racemic isoleucine and tyrosine. Column 7.8 × 140 mm; 0.1 *M* NH_4OH; 5 ml/h. The degree of saturation of the L-proline resin by copper(II) ions was 70%. Other resin parameters as given in Fig. 2.

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