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EFFECT OF THE SKELETAL STRUCTURE OF SORBENTS CONTAINING L-HYDROXYPROLINE GROUPS ON ENANTIOSELECTIVITY IN LIGAND-EXCHANGE CHROMATOGRAPHY OF AMINO ACID RACEMATES

Yu. A. ZOLOTAREV* and N. F. MYASOEDOV
Institute of Molecular Genetics, USSR Academy of Sciences, Kurchatov Sq. 46, Moscow (U.S.S.R.)
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SUMMARY

Asymmetric sorbents containing L-hydroxyproline groups on modified polystyrene skeletons have been synthesized. The sorbents, saturated with copper ions, were used for ligand-exchange chromatography of amino acid racemates. The modification of the polymeric skeleton is shown to be a promising way of increasing the enantioselectivity in racemate chromatography.

INTRODUCTION

Ligand-exchange chromatography is increasingly used for separating amino acid racemates. Polystyrene sorbents containing L-proline and L-hydroxyproline groups have successfully been used for enantiomeric analysis of amino acids[1], for obtaining tritium-labelled optically active amino acids[2,3] and for micropreparative separation of proline and leucine racemates[4,5]. However, these sorbents, are not highly enantioselective with respect to alanine, asparagine, methionine, lysine, ornithine, aspartic acid and glutamic acid, which makes it difficult to use them for separating the racemates of those amino acids.

Polystyrene sorbents containing various amino acid groups, including complex polydentate ones[6], have been synthesized in an attempt to raise the enantioselectivity of the chromatographic process, yet this has not so far yielded any sorbents that might be of practical value.

With a view to raising the efficiency of ligand-exchange chromatography, 5–10 μm sorbents containing proline groups were synthesized on silica gel[7] and on microporous polyacrylamide[8]. These sorbents are of some interest for the analytical separation of racemates; they have successfully been used for a rapid quantitative separation of some racemates, but mostly those that were also readily separated on polystyrene sorbents containing proline and hydroxyproline groups[3].

Compared to silica gel and polyacrylamide-based sorbents, the polystyrene sorbents are superior in capacity, strength and chemical stability, which makes them a promising candidate for the preparative separation of racemates. In the present study we looked at the possibility of raising their enantioselectivity by modifying the poly-

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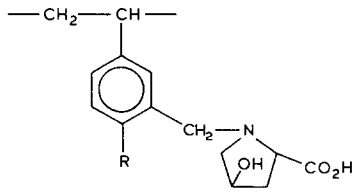
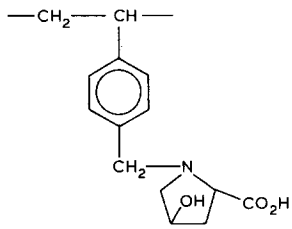
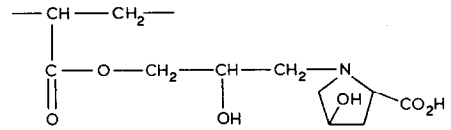
styrene skeleton by means of alkylation and acylation. Subsequent chloromethylation and amination led to the following sorbent structure:



1 R = $(CH_3)_3C$—
2 R =  (cyclohexyl radical)
3 R = CH_3—$(CH_2)_3$—
4 R =  —$(CH_2)_2$—

The chromatographic properties of the modified polystyrene sorbents are compared to those of sorbents based on cross-linked unmodified polystyrene as well as polyacrylamide and polymethcrylate. The repeating units of these sorbents (5–8) can be represented as follows:

5 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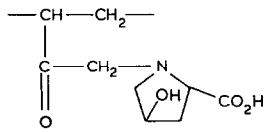
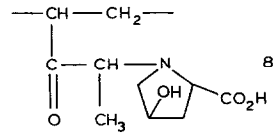
7 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MATERIALS AND METHODS

Cross-linked polystyrene Bio-Beads SX1 (200–400 mesh, Bio-Rad) was used as starting material.

Synthesis of sorbent 1

Alkylation. Bio-Beads SX1 (4.0 g, 38 mmol), 15.0 ml (140 mmol) of *tert.*-butyl chloride 23 ml of dichloroethane and 3 ml (26 mmol) of $SnCl_4$ were placed in a three-necked flask. The reaction mixture was periodically stirred at 20°C for 20 h. The polymer was filtered off, then washed with dichloroethane, acetone, a mixture of acetone and 1 *M* hydrochloric acid, acetone and diethyl ether. As a result, 5.9 g of yellow polymer were obtained.

Chloromethylation. Alkylated polystyrene skeleton (5.9 g) was placed in a three-necked flask. A cold (0°C) mixture comprising 23 ml of dichloroethane, 8.0 ml (105 mmol) of monochlorodimethyl ether and 1.0 ml (8.6 mmol) of $SnCl_4$ was added. The reaction solution was periodically stirred at 20°C for 10 h. The polymer was filtered off and washed with dichloroethane, acetone, a mixture of acetone and 1 *M* hydrochloric acid, acetone and sulphur ether. As a result, 7.3 g of yellow polymer containing 18.6% chloride were obtained.

Amination. Chloromethylated polymer (7.3 g) was aminated with methyl L-hydroxyprolinate hydrochloride in methanol–dioxane (1:4) in the presence of sodium bicarbonate and iodide as described earlier[9]. The capacity of the sorbent obtained is 2.4 mmol/g.

Synthesis of sorbent 2

Alkylation. Bio-Beads SX1 (3.0 g, 29 mmol), 15 ml of nitrobenzene, 3.0 ml (26 mmol) of $SnCl_4$ and 6.0 ml (52 mmol) of cyclohexyl chloride were placed in a three-necked flask. The reaction mixture was periodically stirred at 80°C for 10 h. The polymer was filtered off and washed on the filter, as for sorbent 1. As a result, 5.8 g of yellow polymer were obtained.

Chloromethylation and amination were carried out as for sorbent 1. The capacity of the sorbent is 2.6 mmol/g.

Synthesis of sorbent 3

Bio-Beads SX1 (3.0 g, 29 mmol), 20 ml of nitrobenzene, 6 ml (58 mmol) of butyl chloride, 2.5 ml (21 mmol) of $SnCl_4$ and 1.0 ml (7.1 mmol) of boron trifluoride etherate were placed in a steel autoclave. The reaction mixture was heated for 4 h at 90°C. The polymer was filtered off and washed on the filter. As a result, 4.4 g of yellow polymer were obtained.

Chloromethylation and amination were carried out as for sorbent 1. The capacity of the sorbent is 2.9 mmol/g.

Synthesis of sorbent 4

Acylation. Bio-Beads SX1 (3.0 g, 29 mol), 20 ml of nitrobenzene, 2.5 ml (21 mmol) of $SnCl_4$ and 8.0 g (46 mmol) of cyclohexapropyl chloride were placed in a three-necked flask. The reaction solution was kept at 40°C for 20 h. The polymer was filtered off and washed on the filter. As a result, 6.1 g of brown polymer were obtained.

Chloromethylation and amination were carried out as for sorbent 1. The capacity of the sorbent is 2.2 mmol/g.

Bio-Gel P-4 granules (−400 mesh, Serva) were used as starting material for synthesizing polyacrylamide-based sorbents.

Synthesis of sorbent 5

The synthesis of sorbent 5 containing L-hydroxyproline ligands fixed to a cross-linked polystyrene matrix was described earlier[9].

Synthesis of sorbent 6

The synthesis of sorbent 6 via the reaction of L-hydroxyproline with epoxy groups of poly(2,3-epoxypropyl methacrylate) was as described[10].

Synthesis of sorbent 7

Bio-Gel P-4 (10 g), 100 ml of a 5% aqueous solution of Na_3PO_4 and 10.2 ml (34 mmol) of formaldehyde solution were placed in a three-necked flask. The reaction mixture was heated to 60°C with continuous stirring and kept at this temperature for 1 h. L-Hydroxyproline (6.66 g, 50 mmol) was dissolved in 40 ml of 1 *M* sodium

hydroxide and added to the reaction mixture. The reaction mixture was then kept at 70°C for 30 min. The polymer was filtered off and washed on the filter with water and acetone. As a result, 14.9 g of polymer were obtained having a capacity of 2.5 mmol/g.

Synthesis of sorbent 8

Bio-Gel P-4 (4.0 g), 36 ml of water, 1.2 ml (27.5 mmol) of acetaldehyde and 4.0 g (22 mmol) of L-hydroxyproline methyl ether hydrochloride were placed in a three-necked flask. The solution was heated to 60°C over 1 h and kept at this temperature for 4 h. The polymer was washed with water on a filter and placed in 100 ml of 2 *M* ammonium hydroxide containing 0.1 *M* $[Cu(NH_3)_4]SO_4$. The reaction solution was periodically stirred at 20°C for 20 h. The polymer was washed on a filter with 0.1 *M* hydrochloric acid, water and acetone. As a result, 4.7 g of polymer were obtained having a capacity of 1.0 mmol/g.

Chromatography of racemates

The sorbents were saturated with copper ions to the required degree[1], suspended in 0.1 *M* ammonium hydroxide and placed in a 140 × 8 mm glass column. A 0.5-mg amount of L- and D-amino acids was introduced into the column in the form of a 1% solution. A UV detector was used at a wavelength of 250 nm.

RESULTS AND DISCUSSION

The chromatographic elution order of amino acid isomers depends on the relative stability of the diastereomeric sorption complexes formed between the fixed ligand, Cu^{2+} and mobile ligand. Enantioselectivity may be assessed from the viewpoints of the equilibrium distribution and chromatography.

Fig. 1 shows tentative structures for the more stable sorption complexes formed on sorbents containing L-hydroxyproline ligands during the chromatography of phenylalanine isomers. For polystyrene-based sorbent 5 the value of the free energy difference, $\delta\Delta G$, for is +2630 J/mol, sorption complex formation for glycidyl methacrylate-based sorbent 6 it is −1480 J/mol, for polyacrylamide-based sorbents 7 and 8 the values are −2010 and −240 J/mol.

With Sorbent 5, which contains a hydrophobic benzyl group, the sorption complex formed with D-phenylalanine is the more stable; but with sorbents 6–8 the sorption complex with the L isomer is the more stable. Sorbents 6–8 have the following feature in common: L-hydroxyproline is attached to them by hydrophilic groups which may be involved in coordinative interaction with an axially positioned copper ion.

The sign and magnitude of the enantioselective effect depend not only on the nature of the fixed ligand but largely on the nature of the group binding the ligand to the skeleton. The nature of the skeleton itself does not seem to be crucial. The enantioselective effects observed for sorbent 6 proved to be of about the same magnitude as for a silica gel-based sorbent[7], containing the same fixed ligand and binding group.

Hydrophobic interactions play an important part in the stabilization of sorption complexes. This is especially manifest when one compares ligand-exchange chro-

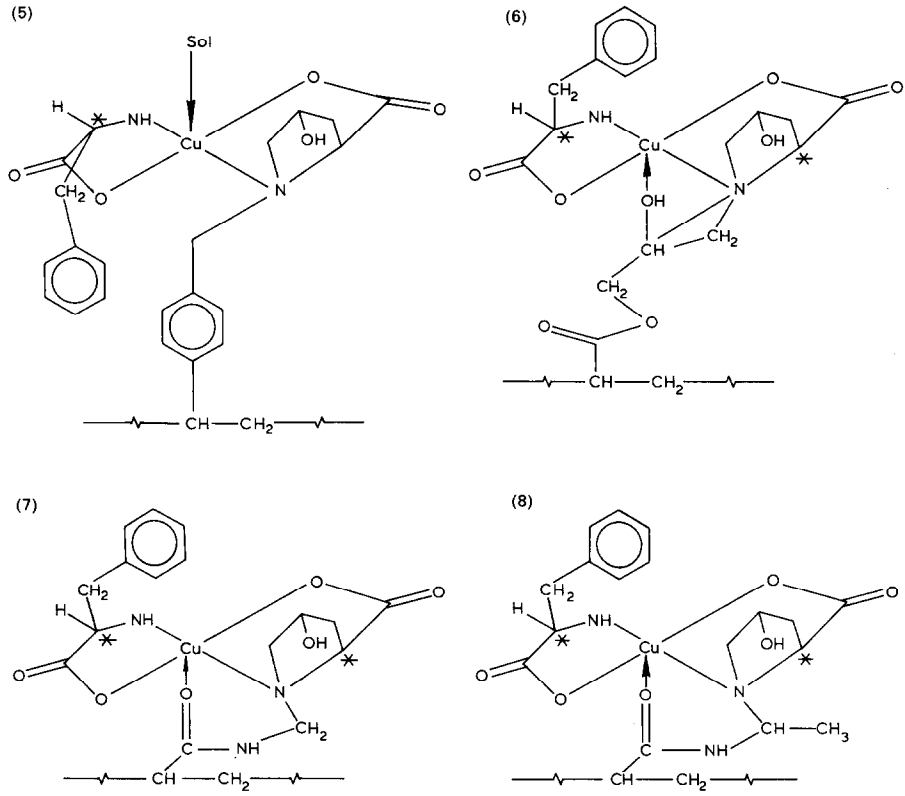


Fig. 1. Structures of more stable mixed-ligand sorption complexes formed by L- or D-phenylalanine on asymmetric sorbents (5-8) containing L-hydroxyproline. k'_D/k'_L values; 5, 2.89; 6, 0.55; 7, 0.44; 8, 0.91.

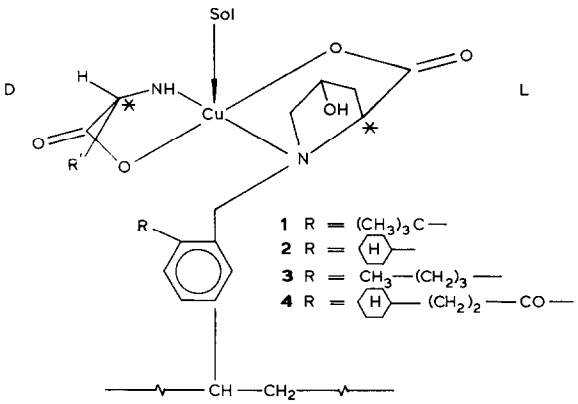


Fig. 2. Structure of mixed-ligand sorption complexes formed by D-amino acids on modified polystyrene sorbents (1–4) containing L-hydroxyproline.

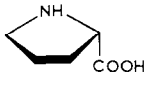
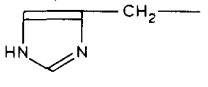
TABLE I

RETENTION PARAMETERS FOR AMINO ACID ENANTIOMERS ON THE POLYSTYRENE RESINS WITH L-HYDROXYPROLINE GROUPS AND SATURATED WITH Cu^{2+}

k' = Capacity factor; $\delta\Delta G$ = enantioselectivity. Eluent: 0.1 *M* ammonium hydroxide (1–14); 1.0 *M* ammonium hydroxide (15, 16).

No.	*Amino acid*	*R'*	*Sorbent*	k'_L	k'_D	$\delta\Delta G$ *J/mol)*
1	Ala	CH_3–	1	5.5	7.4	740
			2	6.0	7.0	370
			3	7.8	8.4	300
			4	7.6	9.2	620
			5	5.8	6.0	150
2	Abu	CH_3CH_2–	1	8.0	12.9	1180
			2	7.5	10.5	830
			3	10.4	12.4	450
			4	8.0	12.0	1010
			5	6.5	8.0	490
3	Val	$(CH_3)_2CH$–	1	9.2	13.1	920
			2	9.0	17.5	1620
			3	9.2	12.0	650
			4	9.0	18.4	1620
			5	7.3	11.8	1180
4	Leu	$(CH_3)_2CHCH_2$–	1	45	50	260
			2	8.5	11	650
			3	20.8	30.0	900
			4	24.8	42.8	1890
			5	14.2	24.2	1320
5	Ile	$CH_3CH_2CH(CH_3)$–	1	32	45	830
			2	8	11	800
			3	15.2	26.4	1380
			4	26.0	62.0	2170
			5	11.1	20.9	1580
6	Nva	$CH_3CH_2CH_2$–	1	53	–	–
			2	8.2	–	–
			3	16.8	–	–
			4	14.4	23.2	1200
			5	11.2	19.9	1290
7	Ser	HO–CH_2–	1	4.4	4.7	170
			2	4.0	4.8	450
			3	5.3	5.7	170
			4	6.0	7.8	650
			5	3.5	4.5	630
8	Thr	$CH_3CH(OH)$–	1	15.0	16.5	240
			2	4.3	5.6	650
			3	6.0	6.2	130
			4	8.2	10.8	690
			5	3.5	5.3	1040

TABLE I *(continued)*

No.	*Amino acid*	*R'*	*Sorbent*	k'_L	k'_D	$\delta\Delta G$ *(J/mol)*
9	Asn	NH_2COCH_2–	1	7.0	7.5	170
			2	4.0	4.8	450
			3	1.4	4.0	1090
			4	6.8	8.4	470
			5	4.6	5.4	390
10	Gln	$NH_2COCH_2CH_2$–	1	7.2	9.0	550
			2	3.9	5.1	650
			3	2.0	2.4	450
			4	8.0	8.8	240
			5	2.5	3.7	1000
11	Met	CH_3–S–CH_2CH_2–	1	2.5	35	830
			2	9.0	10.0	240
			3	18.0	31.2	1360
			4	28.8	42.0	930
			5	11.7	14.3	460
12	Tyr	HO–C_6H_4–CH_2–	1	22	44	1600
			2	17.0	70.0	3500
			3	25	30	450
			4	14.4	32.0	1840
			5	9.0	19.9	2000
13	Phe	C_6H_5–CH_2–	1	112	141	650
			2	110	225	1780
			3	88	110	550
			4	82	278	3040
			5	34.0	98.0	2630
14	Pro		1	13.0	60	3800
			2	41.0	108	1530
			3	8.0	68	5100
			4	39	250	4620
			5	15.2	91.2	4450
15	His		1	40.0	9.0	−3680
			2	66.0	19.0	−3090
			3	35.0	11.3	−2820
			4	43.5	10.6	−3500
			5	27.3	7.8	−3100
16	Lys	$NH_2(CH_2)_3CH_2$–	1	9.0	10.0	240
			2	21	23	240
			3	12.6	15.1	450
			4	12.3	16.0	650
			5	4.0	5.0	550

matography on ODS silica gels in the presence of bis(L-prolinato)copper[11] and its N-alkylhydroxyproline derivative[12], large enantioselective effects with different signs being observed. The more stable sorption complexes are those that favour interaction

between the hydrocarbon α-radical of the amino acid concerned and a hydrophobic ODS phase. It seems that the N-benzyl radical in sorbent 5 can serve as such a hydrophobic phase. The hydrophobic interaction must be most pronounced for bulky alkyl and aryl radicals of amino acids. A change in $\delta\Delta G$ does in fact occur in the series alanine, α-aminobutyric acid, valine and leucine. It would seem that the hydrophobic interactions in respect of D-alanine must be enhanced if additional hydrophobic groups are introduced at the *ortho* position of the N-benzyl group. Such an increase in enantioselectivity was indeed observed will sorbents 1–4 comparing to the resolving power of sorbent 5 which is based on the unsubstituted polystyrene matrix.

Fig. 2 shows suggested structures of the more stable sorption complexes formed on the sorbents synthesized. These complexes contain mobile ligands of the D configuration (for sorbents based on modified or unmodified polystyrene). Table I shows the chromatographic data for those sorbents.

In many cases the enantioselectivity of sorption is considerably higher for hydroxyproline sorbents on modified polystyrene skeletons than for sorbent 5 which has an unmodified skeleton. The value of $\delta\Delta G$ with respect to alanine and aminobutyric acid is especially different in the case of a skeleton containing a tertiary butyl group. The enantioselectivity is considerably increased in the chromatography of tyrosine on a sorbent containing an *n*-butyl group and in the chromatography of methionine on a sorbent containing a cyclohexyl group.

An increased enantioselectivity with respect to most amino acids was observed for sorbent 4 containing a cyclohexyl propionic acid residue. This radical allows interaction between the carbonyl oxygen and the copper ion at the axial position, which in turn must cause the cyclohexyl group to take up a position near the α-hydrocarbon radical of the amino acid in question.

For all the modified sorbents, the chromatographic elution order of the amino acid isomers is preserved, but the $\delta\Delta G$ values are somewhat lower for amino acids containing hydrophilic groups, such as serine, threonine and glutamine, as compared to values on the unmodified sorbent 5.

Thus, modification of the polymeric skeletons used for synthesizing asymmetric sorbents may increase the enantioselectivity in racemate chromatography, as is the case with amino acids containing hydrophobic α-radicals.

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