Journal of Chromatography, 147 (1978) 177–183

CHROM. 10,292

PREPARATIVE RESOLUTION OF DL-PROLINE BY LIQUID CHROMATOGRAPHY ON A POLYSTYRENE RESIN CONTAINING THE L-PROLINE COPPER(II) COMPLEX

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(First received March 7th, 1977; revised manuscript received June 6th, 1977)

SUMMARY

Preparative resolution of DL-proline by ligand-exchange chromatography has been achieved by using polystyrene resins containing the L-proline–copper(II) complex as stationary phases and ammonia solutions as eluents. The effects of the following parameters on the resolution are discussed: properties of the resins, flow-rate and ammonia concentration of the eluent, and weight of racemic proline to be resolved.

INTRODUCTION

Recently, various methods have been used for the direct resolution of racemates. Particularly, α-amino acids and some of their derivatives have been resolved partially or completely by gas chromatography[1], by stereo-selective transport across organic liquid membranes[2] and by liquid chromatography. In this last procedure, resolution of α-amino acids can be achieved through several types of interaction between solute and stationary phase. High chiral recognition has been observed in complex formation between α-amino acids and a resin containing chiral cyclic polyether units[3,4]. Enantiomer differentiation has been achieved by the inclusion of racemates in the asymmetric sections of triacetylcellulose[5], and chiral charge-transfer complexing agents have been used for successful resolution of helicenes[6]. The antipodes of some racemic sulphoxides are expected to be separated on chiral fluoroalcohol units covalently bonded to an inert support, since it has been shown that the two enantiomers interact differently with fluoroalcohol molecules, leading to diastereomeric solvates having three or two bonds[7]. Stereo-selectivity has been reported in interactions between chiral bifunctional ligands such as α-amino acids and resins with metal-complexed units of L-α-amino acids[8–15]. Such packing materials have been used successfully by Davankov and co-workers to separate the two enantiomers of proline. According to these workers, the optical resolution of DL-proline is achieved by ligand-exchange chromatography on polystyrene resins with copper(II) complexed L-proline units. Total separations have been reported on such stationary phases cross-

linked with divinylbenzene (DVB)[8] or 4,4'-bis-(chloromethyl)biphenyl, with aqueous solutions of ammonia[9,10] or pyridine[11] or sodium acetate buffers[12] as eluent.

This paper describes the effects of several parameters on the preparative chromatographic resolution of DL-proline under conditions similar to those used by Davankov. Polystyrene resins with various degrees of cross-linking have been used, since interaction between DL-proline and the copper-complexed L-proline units of the resin depends on accessibility to the active sites. The influence of the nature of the eluent on the efficiency and yield of optical resolution has been studied by using aqueous eluents with various concentrations of ammonia. Some other parameters, such as flow-rate of eluent and amount of DL-proline to be resolved, have also been investigated.

EXPERIMENTAL

The starting materials were cross-linked polystyrene resins (200–400 mesh) from Fluka (Buchs, Switzerland) with 2 moles % of DVB and from Bio-Rad Labs. (Richmond, Calif. U.S.A.) with 1, 2, 3, 4 and 8 moles % of DVB. The synthesis of stationary phases through (a) chloromethylation of polystyrene resins, (b) attachment of L-proline and (c) copper(II) complexation of L-proline units has already been reported[16].

The liquid chromatography system consisted of a 6000 A pump and a U6K injector (Waters Assoc., Milford, Mass., U.S.A.). The de-gassed eluents were directed to two stainless-steel columns (80 × 0.75 cm I.D., and 20 × 0.75 cm I.D.) connected in series. The first column was packed with copper(II) complexed resin, whereas the second contained uncomplexed resin in order to trap any copper(II) ions passing into the eluent. The concentration of optical isomers in the eluate from the second column was determined with an R401 differential refractometer (Waters Associates). Fractions (10 ml) of the eluate were collected and their optical rotations were measured at 589 nm using a Type 71 polarimeter (Roussel-Jouan); fractions were then combined according to Fig. 1 and evaporated, the residues were weighed and dissolved in 4*M* hydrochloric acid, and the specific rotations of these solutions were measured. The optical purities were calculated taking $[\alpha]_{589}^{20} = \pm 53.0°$ for the pure enantiomers.

RESULTS

Properties of the resins

The properties of the resins, determined by potentiometric titration, are summarised in Table I. *Ca.* 20% of the benzene rings are substituted by a L-proline unit, except for the most highly cross-linked resins (with 4 moles % and 8 moles % of DVB) in which 15% and 5%, respectively, of the aromatic rings carry one L-proline unit. As can be seen from Table I, there is good agreement between the experimental values for the copper(II) capacity of the lightly cross-linked resins with the theoretical amounts calculated on the assumption that two L-proline ligands bind one copper(II) ion (2:1 complex). At high degrees of cross-linking, diffusion of L-proline molecules through the polymer network is considerably hindered; in such materials, the formation of a 2:1 complex is not possible because of the macro-net rigidity. As seen in Table I, approximately one L-proline unit is associated with one copper(II) ion in

TABLE I

PROPERTIES OF THE RESINS

Resin	*DVB content (%)*	*CH_2Cl content (%)*	*L-Proline content (%)*	*L-Proline capacity (mequiv./g)* *	*Copper capacity (mequiv./g)*		*Swelling (g/100 g of resin)*		
					Calculated	*Found*	*Water*	*1M NH_3*	*Methanol*
Bio-Rad	1	57.7	22.3	1.51	0.74	0.81	60	70	88
	2	52.7	17.4	1.24	0.61	0.67	55	60	70
	3	53.7	17.0	1.21	0.60	0.56	60	70	80
	4	53.5	12.7	0.94	0.47	0.70	55	62	66
	4	60.5	16.1	1.14	0.56	0.81			
	8	41.8	5.4	0.44	0.22	0.40	38	48	54
Fluka	2	56.0	17.7	1.25	0.62	0.63	50	60	65
	2	52.8	17.5	1.25	0.61	0.60			
	2	49.2	16.5	1.19	0.59	0.60			

* Determined by titration, with acid, of a weighed amount of the sodium salt of the resin.

the resin with 8 moles % DVB. The peculiar behaviour of this resin was confirmed by swelling capacity measurements.

Chromatographic elution

Pure enantiomers of proline were injected independently into the chromatography system. The two elution volumes were widely different, the D-isomer being retained to a much greater extent than the L-form on the column-packing material. By measuring the optical rotations of the residues obtained after evaporation of the eluent, we checked that no racemization had occurred during any stage of the procedure.

The results reported in Table II were obtained exclusively with Bio-Rad resins, as the columns could not be packed with the Fluka resins (which contained a large amount of very fine beads). In this Table, the parameter X is the ratio of the number of active copper(II) sites of the stationary phase to the number of moles of DL-proline to be resolved. As shown in Fig. 1, four types of chromatograms (I–IV) were registered according to the resolution of the peaks. The second column had no significant influence on the chromatogram, probably because its contribution to the dead volume was negligible in view of the high selectivity of the separation. The result of each run is expressed by three parameters (given in Table II) for every combination of fractions, *viz.*, elution volume, percentage of proline eluted, and enantiomeric composition.

DISCUSSION

Effect of degree of cross-linking

Runs 1, 2, 3, 4 and 5 were carried out on resins with variable DVB contents under similar conditions. Elutions with 1 M aqueous ammonia were made at a flow-rate of 0.3 ml/min., and the value of X was *ca.* 10. From the change of the type of chromatogram and the results reported in Table II, it can be concluded that the lower the degree of cross-linking, the higher is the optical resolution achieved. It should be emphasized, however, that with resins containing 4 moles % and 8 moles % of

TABLE II

OPTICAL RESOLUTIONS OF DL-PROLINE

Resolution		*Elution*		*Proline racemate*		*Separation*			
Run No.	*DVB in resin (%)*	*Eluent*	*Flow-rate (ml/min)*	*Weight (mg)*	*X**	*Type (see Fig. 1)*	*Elution volume (ml)*	*Eluted proline (%)*	*Enantiomeric composition L–D (%)*
1	1	1*M* NH_3	0.27	154	10.4	IV	50 to 100	50	100–0
							110 to 320	48	0–100
2	2	1*M* NH_3	0.33	148	10.7	III	50 to 100	44	95–5
							100 to 110	4	71–29
							110 to 120	5	27–73
							120 to 310	46	7–93
3	3	1*M* NH_3	0.30	123	9.7	III	30 to 70	32	90–10
							70 to 90	19	70–30
							90 to 100	5	20–80
							100 to 250	44	15–85
4	4	1*M* NH_3	0.28	159	10.4	II	20 to 70	46	85–15
							70 to 80	10	56–44
							80 to 90	10	44–56
							90 to 280	34	10–90
5	8	1*M* NH_3	0.24	101	10.4	I	6 to 28	67	54–46
							28 to 33	11	48–52
							33 to 70	21	45–55
6	1	0.5*M* NH_3	0.33	155	10.4	IV	60 to 160	50	100–0
							180 to 650	50	0–100
7	2	0.5*M* NH_3	0.34	149	10.7	IV	50 to 180	50	95–5
							180 to 230	3	50–50
							230 to 400	32	5–95
8	3	0.5*M* NH_3	0.32	124	9.7	IV	50 to 150	50	95–5
							150 to 390	42	5–95
9	4	0.5*M* NH_3	0.25	159	10.4	II	30 to 100	40	80–20
							100 to 130	15	50–50
							130 to 350	44	25–75
10	8	0.5*M* NH_3	0.28	102	10.4	I	6 to 22	48	55–45
							22 to 28	22	50–50
							28 to 80	30	45–55
11**	2	H_2O	0.30	28	56.5	IV	0 to 1100	0	—
		0.5*M* NH_3	0.30				1170 to 1460	50	100–0
		1*M* NH_3	0.30				1480 to 1750	50	0–100
12	1	Methanol–					150 to 340	50	100–0
		1*M* NH_3	0.29	160	10.0	IV	350 to 930	50	0–100
13	8	0.12*M* NH_3	0.20	160	6.6	I	11 to 30	64	38–42
							30 to 90	26	49–51
14	3	1*M* NH_3	0.33	162	7.4	III	40 to 90	40	85–15
							90 to 110	13	40–60
							110 to 300	45	20–80
15	3	1*M* NH_3	0.29	55	21.9	III	50 to 100	42	95–5
							100 to 250	58	15–85
16	3	1*M* NH_3	0.26	29	40.9	IV	60 to 110	50	100–0
							120 to 250	50	0–100
17	2	1*M* NH_3	0.21	154	10.3	III	40 to 70	43	95–5
							70 to 80	7	80–20
							80 to 90	12	15–85
							90 to 260	38	5–95

TABLE II

Resolution		*Elution*		*Proline racemate*		*Separation*				
Run No.	*DVB in resin (%)*	*Eluent*	*Flow-rate (ml/min)*	*Weight (mg)*	*X***	*Type (see Fig. 1)*	*Elution volume (ml)*		*Eluted proline (%)*	*Enantiomeric composition L–D (%)*
18	2	1*M* NH_3	0.60	154	10.3	III	40 to	80	38	95–5
							80 to	110	26	45–55
							110 to	330	53	5–95
19	2	1*M* NH_3	0.95	154	10.3	II	30 to	70	32	90–10
							70 to	120	41	40–60
							120 to	290	27	15–85
20	2	1*M* NH_3	1.10	154	10.3	II	30 to	70	33	90–10
							70 to	110	32	45–55
							110 to	300	35	15–85
21	2	1*M* NH_3	1.30	154	10.3	II	30 to	70	33	90–10
							70 to	110	35	45–55
							110 to	300	32	15–85

* See text.
** Elution with three successive eluents.

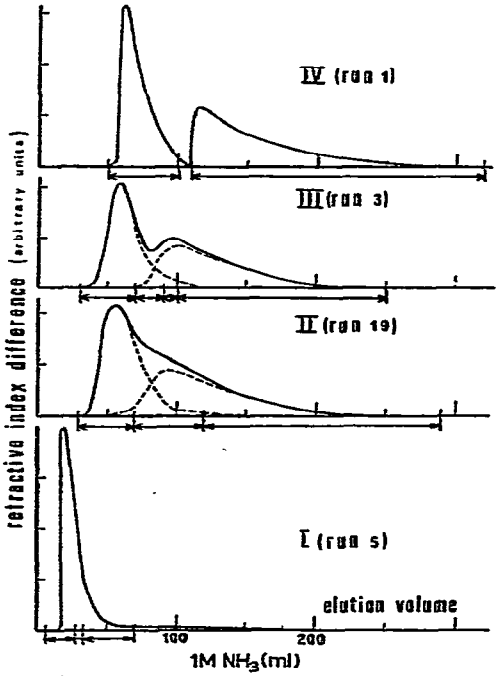

Fig. 1. The four typical chromatographic elutions, illustrated by runs 1, 3, 19 and 5. The eluates were divided into several parts, which are indicated by the arrows. The broken lines represent the distribution of the enantiomers *versus* elution volume. The distribution was calculated by using the optical rotation values of every fraction and by assuming that the first and last fractions contained pure L- and D-proline, respectively.

DVB, the effect of cross-linking cannot be differentiated from the possible influence of the average number of fixed L-proline ligands bound to one copper(II) ion.

Effect of eluent

Runs 6, 7, 8, 9 and 10 were carried out with 0.5 *M* aqueous ammonia as eluent, the other conditions being as in the preceding runs. Results show that the peak width is increased considerably when the ammonia concentration of the eluent is decreased, and the efficiency of the separation increases (see Table II). It follows that the concentrations of the enantiomers in the eluate rapidly become too low for industrial application. The following conclusions can be drawn from the use of other eluents: in 0.1 *M* aqueous ammonia, a large volume of eluent is necessary to recover the L-isomer, whereas elution of the D-form needs an eluent more concentrated in ammonia. The two enantiomers cannot be eluted by water (run 11). In methanol–1 *M* ammonia, optical resolution of DL-proline is achieved (run 12), but elution volumes are large.

The results presented above agree with the view of the chromatographic separation as consisting of competition between ammonia and proline molecules for the active sites of the resin. Peculiar behaviour occurs with the resin containing 8 moles % of DVB (runs 5, 10 and 13), since the usual effect of a change in the ammonia concentration of the eluent is not observed. This is interpreted as additional proof of the lack of interaction between DL-proline and the active sites in the highly cross-linked packing.

Effect of DL-proline load

Runs 14, 3, 15 and 16 were performed in 1 *M* aqueous ammonia at a flow-rate of 0.3 ml/min., on a packing with 3 moles % of DVB. As expected, the efficiency of the resolution increased when the load of the column was decreased. However, the choice of the load must also take into account the isomer concentration in the elution volume.

Effect of flow-rate

Runs 17, 2, 18, 19, 20 and 21 were carried out under similar conditions, except that the flow-rate was increased from 0.2 to 1.3 ml/min. No significant influence was observed on the maximum optical purity of the isomers emerging from the column; however, the separation yield was greatly affected. Each chromatogram in the series was analysed in order to show the two L- and D-enantiomer peaks. Maxima were found, respectively, at elution volumes of 60 and 110 ml; the overlap was such that one-third of the amount of DL-proline injected could not be separated at flow-rates larger than 0.6 ml/min. Higher flow-rates could be used however, if the unseparated racemate were to be recycled.

CONCLUSION

Fairly good conditions have been established for total or partial optical resolution of DL-proline by preparative liquid chromatography, but improvements can still be effected; for example, the use of polystyrene micro-particles may reduce the width and the asymmetry of the peaks and give more rapid separation.

The effects of several parameters on the elution of DL-proline in ligand-ex-

change chromatography have been clarified. Failure to resolve a racemic mixture may be attributable to numerous factors, not necessarily involving only the chemical features of the interaction between a given stationary phase and the racemic mixture; many other factors have also to be studied in order completely to understand the phenomena.

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