DISSYMMETRICAL ION-EXCHANGE SORBENTS BASED ON α-AMINO ACIDS AND THEIR DERIVATIVES

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In our previous communications [1, 2] we described convenient ways of synthesizing sorbents containing α-amino acid residues or their derivatives as the ionogenic groups in a polystyrene framework. Such ion exchangers can also be produced on the basis of optically active amino acids. The dissymmetrical ion-exchange sorbents formed in this case, in our opinion, are very promising for solving a problem of importance in the scientific and practical respects, associated with the creation of convenient methods for the separation of racemic compounds into optical antipodes. The known methods of separation of racemates are extremely laborious and rather ineffective. In most cases they permit isolation in pure form only of part of one of the isomers of the racemate. On the contrary, in the case of chromatographic separation of the racemate, the theoretical separation of the racemate, the theoretical possibility of quantitative isolation of both isomers of the racemate in optically pure form is created even in the case when the dissymmetrical sorbent used does not possess 100% optical purity [3].

Actually, if acid centers (A) of one of the two possible steric configurations (A and B) predominate in the sorbent used, then under the condition of sufficient selectivity of the sorption of optical isomers (a, b) of the racemate, the capacity of such a sorbent with respect to one of the isomers (a) will be greater than with respect to the other (b). The distribution ratio h (ratio of the concentration n of the substance to be sorbed in the mobile phase to the concentration N of the same substance in the phase of the sorbent) will also differ for the two isomers.

It is not difficult to show that the ratio h_b/h_a at low concentrations of the racemate is expressed in terms of the degree α of optical purity of the sorbent as follows:

$$\alpha = \frac{A - B}{A + B} < 1, \quad 1 < \frac{h_b}{h_a} \leqslant \frac{A}{B} = \frac{1 + \alpha}{1 - \alpha}$$

According to the Wilson law, in the case of equilibrium eluent chromatography and a linear isotherm of sorption (h = const), the rate v of displacement of the chromatographic zone is determined by the rate of flow u of the mobile phase and the distribution ratio h

$$v = u \frac{h}{1 + h}, \quad h_a < h_b, \; v_a < v_b$$

From this it follows that the rate of displacement of the zones of the optical isomers at equal values of h will differ, and in the case of a sufficient length of the chromatographic column, there should be a quantitative separation of the isomers.

It should be emphasized that for any other method of separation of the racemate, insufficient optical purity of the separating reagent inevitably reduces either the optical purity or the yield of the antipode to be isolated. A large number of different sorbents have now been tested for the chromatographic separation of racemates [4]. Special attention among them is merited by dissymmetrical (optically active) ion exchangers [4-8].

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TABLE 1. Dissymmetrical Ion Exchangers

No. of ion exchanger	p-DVB, %	Dissymmetrical component	Conditions of synthesis	Analytical capacity, meq/g
1	2	L-Leucine amide	2.5 moles of amide per mole of iodomethyl groups, 40°, 5 h dioxane + water (10:1)	2.90
2	2	L-Leucine	Hydrolysis of amide groups of ion exchanger by 1.20% HCl, 3 h, 110°	2.90
3	0.8	L-Valineamide	2.2 moles of amide and 1 mole NaI per mole of chloromethyl groups, 40°, 10 h, dioxane + ethanol (6:1)	3.04
4	0.8	L-Proline	2.25 moles of amino acid and 1 mole NaI per mole of chloromethyl groups, 60°, 15 h, dioxane + methanol (6:1)	1.96

To carry out any stereospecific process, including stereoselective sorption, it is necessary for the reacting component to interact at least at three points. Therefore, the closest surroundings of the dissymmetrical centers of the sorbent should contain ionogenic and polar groups, capable of forming ionic and hydrogen bonds with the molecules to be sorbed, as well as other substituents, providing the required steric orientation of the molecules to be sorbed. In our opinion, these requirements are satisfied by ion exchangers with dissymmetrical α-amino acid ionogenic groups. They contain two functional groups of opposite polarity and a voluminous hydrocarbon radical directly at the asymmetrical carbon atom. The synthesis of such ion exchangers has been accomplished by interaction of halomethylated copolymers of styrene with divinyl benzene (DVB) with α-amino acids [1], their esters and amides [2]. The conditions of synthesis of some of the ion-exchange resins studied and their exchange capacity are cited in Table 1.

The ion-exchange properties and ability of analogous ion-exchange resins based on racemic amino acids to swell were described earlier [1, 2].

The main requirement set for the synthesis of dissymmetrical ion exchangers should be the absence of racemization of the optically active components during synthesis. On account of the insolubility of the ion exchangers formed, their optical activity unfortunately cannot be measured directly. It is known that the reactions of benzylation of α-amino acids [9] and acid hydrolysis of their amides [10] proceed without appreciable racemization. Sodium iodide used as the catalyst of the reaction of chloromethylated copolymers, does not induce racemization of α-amino acids and their amides. This is evidenced by the complete conservation of optical activity of solutions of L-proline, L-leucineamide, and L-valineamide in a mixture of dioxane with ethanol containing NaI, in the case of prolonged exposure at 60°.

Model benzyl and p-isopropylbenzyl derivatives of L-proline, L-valineamide, and L-leucineamide were produced under the same conditions in which dissymmetrical ion exchangers were synthesized (see Table 1). Their properties are given in Table 2. Since these derivatives have not been described in the literature, the degree of their optical purity cannot be calculated. However, the absence of appreciable racemization in their synthesis and, consequently, in the synthesis of the corresponding ion exchangers, is indicated by the fact that the interaction of L-leucineamide, L-valineamide, and L-proline with benzyl chloride and p-isopropylbenzyl chloride in an aqueous methanol solution of alkali leads to products with the same value of the specific rotation.

The dissymmetrical sorbents synthesized were used for the chromatographic separation of optical isomers of certain compounds. The anion-exchange resin with L-leucineamide groups, just like the amphoteric ion exchanger with L-leucine, possesses a lower affinity for (+) L-amygdalic acid than for its (–) D-antipode [11, 12]. The same order of emergence of the isomers of amygdalic acid is also observed in chromatography on an anion exchanger with L-valineamide groups and on an amphoteric anion exchanger with L-proline. In the first case, frontal chromatography of a solution of D,L-amygdalic acid was conducted in 90% ethanol, since this ion-exchange resin swells better in alcohols than in water. As can be seen from Fig. 1, after saturation of the anion-exchange resin with amygdalic acid, the (+) L-isomer appears first in the eluate. Chromatography permits the isolation of substantial amounts of the acid, 10-15% enriched with the (+) L-isomer. However, a product with a high degree of optical purity is contained in only a small part of the eluate.

The ion-exchange resin based on L-proline swells well in water and in alcohols. The ion exchanger firmly retains amygdalic acid; therefore its elution was performed with a dilute solution (0.15 N) of ammonia As is shown by a measurement of the optical activity of the eluate, (+) L-amygdalic acid is eluted first. The degree of separation of the isomers is low, so that the optical purity of the amygdalic acid eluted does not exceed 11%.

TABLE 2. Properties of Derivatives of α-Amino Acids

Name	Empirical formula	Found, %			Calculated, %			Mp, °C	$[\alpha]_D^{20}$, C = 1	
		C	H	N	C	H	N		ethanol	THF + AA*
N-Benzyl-L-leucine-amide	$C_{13}H_{20}N_2O$	70.35	8.98	12.40	70.87	9.15	12.72	125.5-126	-32.0	+19.7
N-(p-Isopropylbenzyl)-L-leucineamide	$C_{16}H_{26}N_2O$	73.11	9.70	10.92	73.23	9.99	10.68	120-121	-18.9	+15.6
N-(p-Isopropylbenzyl)-L-valineamide	$C_{15}H_{24}N_2O$	72.84	9.70	11.03	72.53	9.74	11.28	111-112	-32.3	+27.0
N-Benzyl-L-proline	$C_{12}H_{15}NO_2$	69.57	7.32	6.73	70.22	7.37	6.82	184-186	-28.3	-38.3 (1 N HCl)

* Mixture of tetrahydrofuran with acetic acid 2:1.

TABLE 3. Frontal Chromatography of a 1.5% Solution of Acetyltryptophan in 50% Ethanol on a Column with an Ion-Exchange Resin Based on L-Proline

Fraction No.	$\alpha_{436} \cdot 10^3$, observable angle (l = 0.2 dm), deg	Concentration, %	$[\alpha]_{436}^{20}$, deg	Fraction No.	$\alpha_{436} \cdot 10^3$, observable angle (l = 0.2 dm), deg	Concentration, %	$[\alpha]_{436}^{20}$, deg
59	—2	0	—	67	—4,0	1,48	—1,25
60	—21	1,06	—9,9	68	—3,7	1,41	—1,1
61	—15	1,13	—6,6	69	—3,0	1,48	—1,0
62	—12	1,30	—4,6	70	—3,0	1,41	—1,0
63	—6	1,38	—2,2	71	—1,7	1,48	—0,6
64	—4,5	1,42	—1,6	72	—1,5	1,48	—0,5
65	—4,5	1,36	—1,65	73	0	1,48	0
66	—4,5	1,42	—1,6				

Note: Column diameter 9 mm, length 500 mm, velocity 6 ml/h; 2 ml fractions.

An ion-exchange resin based on L-proline is also capable of separating racemates of amino acids. A column with the ion-exchange resin, on which 0.1045 g of D,L-proline was sorbed, was washed with water at a rate of 5.15 ml/h. Both isomers of proline are eluted within a single concentration peak. The first half of it contains an excess of L-proline, the second an excess of the D-isomer, more strongly retained by the sorbent (Fig. 2). The initial and final portions of the product to be eluted possess greater optical purity than the intermediate portion.

The curve of the optical density of the eluate is symmetrical. Variation of the temperature of the process in the range 20-55° has practically no effect on the effectiveness of the separation of the antipodes of proline, evaluated as the difference of the maximum observable values of the positive and negative rotation of the eluate. Elution of proline with 50% ethanol, with all other conditions equal, approximately doubles the effectiveness of the separation of the isomers. If the chromatography is conducted in 90% ethanol, the second half of the effluent curve, corresponding to the proline fraction enriched in the D-isomer, is greatly stretched out.

D,L-Tryptophan is well sorbed from aqueous solution by an ion exchanger based on L-proline. However, no appreciable separation of the isomers of tryptophan was observed in the case of frontal chromatography. On the contrary, when a solution of acetyltryptophan in 50% ethanol is passed through a column with the same sorbent, the levorotatory D-isomer emerges first, as can be seen from Table 3.

Partial separation of the optical isomers in eluent chromatography was also observed for D,L-alanine in 50% ethanol and D,L-acetylalanine in water on an ion exchanger with L-proline. It is interesting to note that in both cases (–) D-alanine and (+) D-acetylalanine appear first in the eluate.

The results of the chromatography show that amphoteric compounds, for example, alanine, are retained more weakly by an ion exchanger based on L-proline than acid compounds (acetylalanine, amygdalic acid). The racemic base of the amide of D,L-valine also possesses increased affinity for the sorbent; however, no separation of its isomers during eluent chromatography was observed. Our investigation confirms the correctness of the hypotheses advanced and demonstrates the theoretical possibility of using

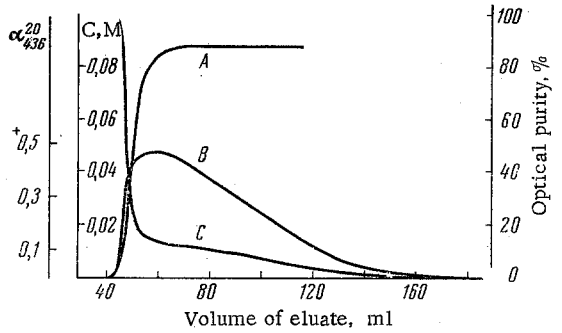

Fig. 1

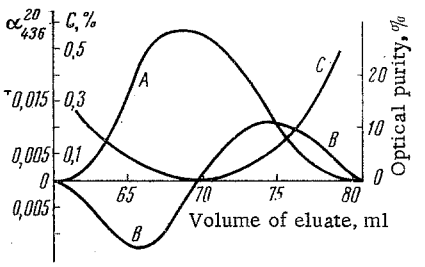

Fig. 2

Fig. 1. Effluent curve of amygdalic acid (A), observable rotation of the eluate (B), and degree of its optical purity (C) in frontal chromatography of a 0.088 N solution of amygdalic acid in 90% ethanol on a column (diameter 9, length 665 mm) with an ion-exchange resin based on L-valineamide; velocity 3 ml/h.

Fig. 2. Effluent curve of proline (A), observable rotation of the eluate (B), and degree of its optical purity (C) in eluent chromatography of 0.1045 g of D,L-proline on a column (diameter 9, length 1475 mm) with an ion-exchange resin based on L-proline; velocity 5.15 ml/h.

the dissymmetrical ion-exchange sorbents obtained for the separation of racemates of α-hydroxyacids, α-amino acids, and their derivatives. A study of the mechanism of stereoselective sorption and the influence of various factors on the process of chromatography permits a development of preparative methods of separation of racemates.

EXPERIMENTAL METHOD

L-Leucineamide was produced analogously to the racemic amide [2] by esterification of L-leucine, followed by ammonolysis of the ethyl ester of L-leucine, mp 104°, $[\alpha]_D^{20}$ +8.65 (C 1, ethanol), +13.83 (C 3, 1 N HCl). According to the data of [13]: $[\alpha]_D^{26}$ +13.8 (C 3, 1 N HCl).

L-Valineamide had $[\alpha]_D^{22.5}$ + 14.7 (C 2, ethanol) and was produced analogously to the racemic amide [2] through the anhydride of N-carboxy-L-valine. The purer product with $[\alpha]_D^{20}$ +17.8 (C 1, ethanol) used for further synthesis was produced by ammonolysis of the ethyl ester of L-valine [2]: mp 92°. Only the hydrochloride of L-valineamide has been described in the literature [14]. L-Proline had $[\alpha]_D^{20}$ −80.9 (C 1, water), which corresponds to an optically pure product.

Dissymmetrical ion exchangers were synthesized according to the method of [1, 2]. The initial copolymer of sturene with 0.8% p-divinylbenzene was produced by emulsion polymerization in the presence of 50% (of the volume of the styrene) toluene. Diameter of the granules 0.03-0.05 mm. Chlorine content after chloromethylation 21.5%. Replacement of chlorine by iodine gives a product containing 41.7% iodine. The conditions of amination of the copolymers and the exchange capacity of the ion exchangers formed are given in Table 1.

N-Benzyl-L-leucineamide, N-(p-Isopropylbenzyl)-L-leucineamide, N-(p-Isopropylbenzyl)-L-valineamide, and N-Benzyl-L-proline. Synthesized under the conditions used for the synthesis of the corresponding dissymmetrical ion exchangers (see Table 1), as well as by the usual alkylation of L-leucineamide, L-valineamide, and L-proline in an aqueous methanol solution of alkali at room temperature [15]. At the end of the process, the reaction mixture was evaporated, and the amides extracted with benzene. The amides are readily soluble in most solvents, with the exception of water, diethyl and petroleum ethers. In the case of L-proline the reaction mixture was neutralized before evaporation, and the extraction was conducted with chloroform.

N-Benzyl-L-proline is readily soluble in water and in alcohols, less soluble in chloroform and dioxane. None of the N-substituted products give a color reaction with ninhydrin. The final purification of the products was performed by chromatography on columns with Al_2O_3 (in such a method of purification, the original ratio of the optical isomers in the product is unchanged). For the properties of the derivatives obtained, see Table 2.

Chromatography of Racemates. The optical activity of the eluate was determined continuously (in the case of chromatography of proline) or in individual fractions on the micropolarimeter produced by Jouan (France), with an accuracy of ±0.001°. The concentration of amygdalic acid in the case of frontal chromatography was determined by titration of the fractions of the eluate with 0.1 N KOH, using the RKS universal indicator. The proline concentration was calculated from the readings of a Konduktolaizer flow-through conductometer produced by LKB Company (Sweden). The amygdalic acid concentration in eluent chromatography, as well as the concentration of acetyltryptophan, were calculated from the readings of a Uvikord flow-through photometer produced by LKB Company (Sweden), using calibration curves.

CONCLUSIONS

1. Dissymmetrical sorbents, containing L-leucineamide, L-leucine, L-valineamide, and L-proline as ionogenic groups were synthesized on the basis of halomethylated copolymers of styrene with divinylbenzene.

2. Model syntheses of benzyl derivatives of L-leucineamide, L-valineamide, and L-proline demonstrated the absence of racemization in the process of synthesis of dissymmetrical ion exchangers.

3. Racemates of amygdalic acid, proline, alanine, acetylalanine, and acetyltryptophan were partially separated by chromatography in dissymmetrical ion-exchange resins.

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