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The Synthesis and Asymmetric Adsorption of an Optically-active Basic Polymer Starting from L-Proline

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N-Prolyl-*p*-aminostyrene was synthesized by the reaction of *N*-carboxy-L-proline anhydride with *p*-aminostyrene phosphate. This optically-active basic monomer was then copolymerized wtih *N*,*N*′-bis(*p*-vinylphenyl)-adipamide to give a basic polymer. Similar basic polymers were also obtained, starting from other α-amino acids, through this procedure. Of these polymers, the polymer involving L-proline was used for the chromatographic resolution of the phthalamidic acid derivatives of DL-phenylalanine, DL-valine, and DL-phenylglycine. The polymer resolved partially DL-*N*-(*o*-carboxybenzoyl)phenylalanine.

There have been many reports[1–5] concerning the use of synthetic optically-active basic polymers as adsorbents for the chromatographic resolution of racemates.

Most such polymers have been synthesized by the method in which an optically-active group is introduced into an optically-inactive "reactive polymer"[6] (*e.g.*, chloromethylated polystyrene[2–5] and polymethacroyl chloride[1]).

On the other hand, we[7] previously reported several optically-active basic polymers synthesized by a different method, which involves either the hydrazinolysis or hydrogenolysis of the neutral copolymers composed of *N*-(*N*-phthaloyl or *N*-tosyl-α-amino acyl)-*p*-aminostyrene and *N*,*N*′-diacroylhexamethylenediamine. However, this synthetic method has two limitations.

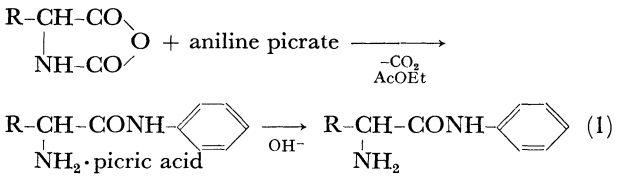
The first limitation is that this method cannot be applied to imino acids or to the α-amino acids with *s*-alkyl, acetoxyl, and/or indole groups. The second one is the difficulty of removing completely the *N*-blocking group from the polymer; the optimum yield of the dephthalization of the polymer upon treatment with hydrazine hydrate was below 60%.

This article will describe the procedure of synthesizing directly the optically-active basic monomers by

1) N. Grubhofer and L. Schleith, *Z. Phys. Chem.*, **296**, 262 (1954).
2) S. Tsuboyama and M. Yanagita, *Sci. Pap. Inst. Phys. Chem. Res.*, **53**, 245 (1959).
3) H. Suda and R. Oda, *Kanazawa Daigaku Kiyo*, **2**, 215 (1960).
4) J. A. Lott and W. Rieman, *J. Org. Chem.*, **31**, 561 (1966).
5) G. Maneck and W. Lamer, *Naturwissenschaften*, **52**, 539 (1965); **54**, 140 (1967).
6) G. W. Roberts and D. H. Haigh, *J. Org. Chem.*, **27**, 3375 (1962).
7) N. Nakamura, T. Yamashita, and T. Uemura, *Nippon Kagaku Zasshi*, **88**, 1238 (1967).

the use of several *N*-carboxy-α-amino acid anhydrides and *p*-aminostyrene phosphate.

Wessley and John[8] reported a synthetic procedure of α-amino acid anilides in which aniline picrate was allowed to react with some *N*-carboxy-α-amino acid anhydrides (Eq. (1)).



(1)

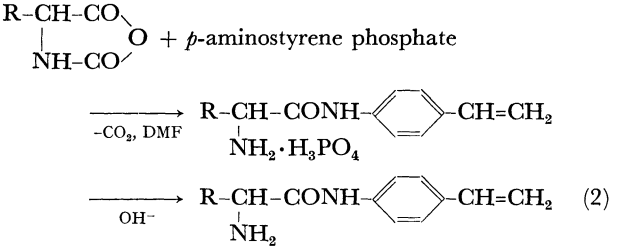
In this reaction, they nicely utilized the difference between the pK_a's of aniline and the resulting anilide, the former being an aromatic amine, and the latter, aliphatic. The picric acid formed a salt with the anilide, whose pK_a is higher than that of the aniline. The salt formation also prevented any further reaction between the still-remaining *N*-carboxy-α-amino acid anhydride and the resulting anilide.

If *N*-carboxy-α-amino acid anhydride is allowed to react with a similar salt of *p*-aminostyrene in lieu of aniline, *N*-(α-amino acyl)-*p*-aminostyrene should be obtained as an optically-active basic monomer.

The synthesis of the monomer was attempted with several salts of *p*-aminostyrene with acids of different pK_a's.

When the salt of a weak acid, such as acetic acid or formic acid, was used, the amine formed was not stabilized as a salt, but it attacked successively the unreacted *N*-carboxy-α-amino acid anhydrides to give only a polyamide. *p*-Aminostyrene hydrochloride polymerized itself, to poly-*p*-aminostyrene, while the salt of *p*-toluenesulfonic acid gave a small amount of the monomer, accompanied mostly by complex by-products.

When the phosphate or picrate was allowed to react with *N*-carboxy-α-amino acid anhydride, the synthesis of the monomer was successful, especially in the former case (Eq. (2)).



(2)

Table 1 shows the physical data on the monomers synthesized by the above procedure.

Table 1. Monomers derived from α-amino acids

Monomers started from	Mp (°C)	$[\alpha]_D$	Yield (%)
Cystine	171—172	−10(THF)	50
Proline	100—102	−62(AcOEt)	50
Tryptophan	115	−68(AcOEt)	84
O-acetyl-Tyrosine	125—150	−77(AcOEt)	45
Methionine	Oil	−55($CHCl_3$)	76

8) F. Wessley and M. John, *Monatsch. Chem.*, **48**, 1 (1927).

In order to obtain an insoluble basic polymer suitable as a chromatographic sorbent, the monomers were copolymerized with some crosslinking agents [divinyl benzene, *N*,*N*′-diacroylhexamethylenediamine,[7] *N*,*N*′-bis(*p*-vinylphenyl)adipamide].

N,*N*′-bis(*p*-vinylphenyl)adipamide was found to be especially useful among these crosslinking agents.

The homogeneous copolymerization of monomer with *N*,*N*′-bis(*p*-vinylphenyl)adipamide was performed, by the use of azo-bis-isobutyronitrile as an initiator, in tetrahydrofuran under refluxing conditions.

Of these polymers, the polymer from L-proline was subjected to hydrolysis in order to examine whether or not racemization occurred during the synthesis. The crude proline obtained after hydrolysis was purified as the picrate. No apparent racemization was detectable from the observed value of the optical rotation of the proline freed from the picrate with aniline.[9]

The chromatographic resolutions of phthalamidic acid derivatives of DL-phenylalanine, DL-valine, and DL-phenylglycine by means of this proline polymer were attempted. As eluent solvents, 10^{-3}, 10^{-4}, and 10^{-5}N aqueous ammonia were used successively.

When DL-*N*-(*o*-carboxybenzoyl)phenylalanine was eluted chromatographically with 10^{-5}N aqueous ammonia, the observed value of the specific rotation for the first fraction was $[\alpha]_D = -18°$ (optical purity, 33%).

The results of the resolution of DL-*N*-(*o*-carboxybenzoyl)phenylalanine eluted with 10^{-4}N aqueous ammonia are given in detail in Table 2.

Table 2. Result of resolution of ammonium salt of DL-*N*-(*o*-carboxybenzoyl)phenylalanine with proline polymer

Fraction No.	Weight (mg)	*c*	α_D	$[\alpha]_D$(°)	α_{546}	$[\alpha]_{546}$(°)
1	13.5	0.45	−0.070	−15	−0.090	−20
2	51.0	1.70	−0.086	− 5	−0.123	− 7
3	21.0	0.70	0	0	0	0
4—14	29.0	0.96	+0.040	+ 4	+0.046	+ 5
15—34	21.0	0.70	+0.042	+ 6	+0.045	+ 6
35—74	3.0	0.10	+0.010	+10	+0.012	+12
last fraction	45.0	1.50	+0.105	+ 7	+0.120	+ 8

In the case of the elution of DL-*N*-(*o*-carboxybenzoyl)-valine with 10^{-4}N aqueous ammonia, the fraction at the earliest stage of the elution showed only an unreliable, low specific rotation.

As was indicated in the previous paper[10] concerning acidic polymers, the substrate involving a benzyl group was more efficiently resolved than that with an isopropyl group in the corresponding moiety of the molecule.

The parallel result in the present study seems quite interesting.

DL-*N*-(*o*-carboxybenzoyl)phenylglycine was less stable than the two above-mentioned substrates under the basic condition; it was, however, found that this sub-

9) G. J. Cox and H. King, *J. Biol. Chem.*, **84**, 533 (1929).

10) T. Yamashita and N. Nakamura, This Bulletin, **43**, 1809 (1970).

strate unfortunately suffered from hydrolysis during the elution despite the expected resolution.

In the optical resolution of the racemates of amino acids, amino acid derivatives, and mandelic acid by means of synthetic optically-active polymers[5,6,10,11] derived from the starting materials of the L-configuration, it has been reported that these polymers are apt to adsorb the substrates of the L-configuration more strongly than those of the D-configuration.

In the present work, the *N*-(*o*-carboxybenzoyl)-phenylalanine of the L-configuration was also more adsorptive, on the L-polymer, than that of the D-configuration.

However, further experimentation and consideration are necessary to explain this observation fully.

Experimental

The Synthesis of p-*Aminostyrene Phosphate.* When 98% phosphoric acid (23 g) in ethanol (35 m*l*) was added to an ether solution of *p*-aminostyrene[12] obtained by the reduction of *p*-nitrostyrene[13] (34 g) with an aluminum amalgam, a white solid salt separated. The solid was collected on a filter, washed with ethanol and ether, and dried in a vacuum desiccator. Yield, 72%. mp 138—140°C.

The salt in a sealed tube was stable for a long period in a refrigerator.

The Syntheses of N-*Carboxy-α-amino Acid Anhydrides.* All of the *N*-carboxy-α-amino acid anhydrides except that of L-cystine[14] were prepared by the phosgenation procedure.[15-18]

The Synthesis of the L-*Proline Monomer.* *p*-Aminostyrene phosphate (0.1 mol) was dissolved, in small portions, in a solution of *N*-carboxy-L-proline anhydride (0.1 mol) in anhydrous dimethylformamide; the evolution of CO_2 gas thus occurred. The solution was then allowed to stand for 2—3 days at room temperature. The end point of the reaction was confirmed by the absence of the spot of *p*-aminostyrene on a silica-gel thin-layer chromatogram [benzene-ethyl formate-methanol (5:4:1) and 95% ethanol-28% aqueous ammonia (4:1) as solvents.]

The dimethylformamide was distilled off from the solution under reduced pressure below 40°C. The residue was decomposed with aqueous sodium bicarbonate, and then extracted with chloroform or ethyl acetate. The extract was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure.

The *N*-prolyl-*p*-aminostyrene was recrystallized from ethyl acetate-petroleum ether to give needles; mp 100—102°C, $[\alpha]_D^{20}$ −62° (*c* 0.5, AcOEt). IR (cm^{-1}): 3200—3400 (NH amide and amine), 1670 (C=O), 990, 910 (–CH=CH_2), 835 (*p*-substitution). Found: C, 72.60; H, 7.31; N, 12.80%. Calcd for $C_{13}H_{16}N_2O$: C, 72.19; H, 7.46; N, 12.95%.

The monomers starting from some other α-amino acids were prepared by procedures similar to that desribed above.

The Synthesis of N,N′-*Bis(*p-*vinyl phenyl)adipamide.* Adipic acid dichloride[19] (0.05 mol) was added, under external cooling with ice water, to an ether solution of *p*-aminostyrene (0.1 mol) and pyridine (0.1 mol) to separate a white, solid material. After standing overnight at room temperature, the solid was collected on a filter, washed with water and ethanol, and then dried in a vaccum desiccator. The *N,N′*-bis(*p*-vinylphenyl)adipamide was recrystallized from ethanol or tetrahydrofuran. Yield, 75%. mp 240°C dec.. IR (cm^{-1}): 3350 (NH), 1655 (C=O), 990, 905 (–CH=CH_2), 840 (*p*-substitution). Found: C, 75.64; H, 7.26; N, 7.65%. Calcd for $C_{22}H_{24}N_2O_2$: C, 75.83; H, 6.94; N, 8.04%.

Copolymerization. A mixture of *N*-prolyl-*p*-aminostyrene (0.01 mol) and *N,N′*-bis(*p*-vinylphenyl)adipamide (0.01 mol) in anhydrous tetrahydrofuran (100—120 m*l*) was stirred, after the addition of a small amount of azo-bis-isobutyronitrile, under reflux for 30—40 hr. The copolymer precipitated as a white powder; it was filtered and then washed thoroughly with dimethylformamide, methanol, and tetrahydrofuran successively. Yield, 60%.

The copolymers containing α-amino acids other than L-cystine were also obtained by procedures similar to that described above. The cystine monomer was polymerized in anhydrous benzene without the addition of any cross-linking agent. The exchange capacities of these polymers, determined by using 0.1 N hydrochloric acid, were as follows: proline, 1.5 *meq*/g; tryptophan, 1.0 *meq*/g; methionine, 0.72 *meq*/g; cystine, 3.64 *meq*/g.

Isolation of Proline from the Hydrolysates of the Polymer Starting from L-*Proline.* The proline copolymer was hydrolyzed and proline was isolated according to the ordinary procedure[9,20] for the isolation of L-proline from protein hydrolysates. The polymer was boiled in 5 N hydrochloric acid for 10 hr under reflux and filtered. The filtrate was decolorized with charcoal and evaporated *in vacuo* to dryness. Picric acid was added to the residue dissolved in ethanol, and the resulting picrate was recrystallized from water. mp 150°C. Free proline was isolated from the picrate after decomposition with aniline by the usual method.[9] mp 219—221°C dec. (from ethanol-ether). $[\alpha]_D^{20}$ −84.5° (*c* 1, in water). Lit,[20] $[\alpha]_D^{20}$ −86° in water.

The Syntheses of Substrates. The substrates were obtained by the ordinary method,[21] in which *N*-phthaloyl-α-amino acids were treated with aqueous alkali.

DL-*N*-(*o*-carboxybenzoyl)phenylalanine, mp 180—182°C. $[\alpha]_D$ +67°, $[\alpha]_{546}$ +80° (*c* 1, 0.5N aqueous ammonia) in L-form. The ammonium salt of the L-configuration, mp 178—182°C. $[\alpha]_D$ +56° (*c* 0.51, 10^{-4}N aqueous ammonia), +54° (*c* 0.52, 10^{-5} N aqueous ammonia).

DL-*N*-(*o*-carboxybenzoyl)valine, mp 164—166°C. $[\alpha]_D$ +12°, $[\alpha]_{546}$ +12° (*c* 1, 0.1 N aqueous ammonia) in L-form.

DL-*N*-(*o*-carboxybenzoyl)phenylglycine,[21] mp 185—188°C. $[\alpha]_D$ +101 (*c* 2, acetone) in L-form.

The ammonium salt of DL-*N*-(*o*-carboxybenzoyl)phenylalanine was prepared by the addition of tetrahydrofuran or acetone to a solution of the acid in aqueous ammonia. The ammonium salts of the other acids failed to crystallize.

Column Chromatography. A proline copolymer (45 g) suspended in a sufficient volume of water was poured into a column (2 cm in diameter; 150 cm in length; the final length

11) H. Suda, Y. Hosono, Y. Hosokawa, and T. Seto, *Kogyo Kagaku Zasshi*, **73**, 1250 (1970).
12) J. H. Boner and H. Alul, *J. Amer. Chem. Soc.*, **81**, 2136 (1959).
13) M. M. Koton, Yu. V. Mittin, and F. S. Florinski, *Zh. Obshch. Khim*, **25**, 1469 (1955).
14) J. L. Bailey, *Nature*, **164**, 889 (1949); *J. Chem. Soc.*, **1950**, 3461.
15) A. C. Farthing, *J. Chem. Soc.*, **1950**, 3213.
16) E. Datchornik, M. Sela, and E. Katchalski, *J. Amer. Chem. Soc.*, **76**, 299 (1954).
17) J. Kurz, G. D. Fasman, A. Berger, and E. Katchalski, *ibid*, **80**, 393 (1958).
18) J. Honzyl and J. Rudinger, *Collect. Czech. Chem. Commun.*, **20**, 1190 (1955).
19) R. Meyer, *Ann. Chem.*, **347**, 49 (1906).
20) M. Bergman, *J. Biol. Chem.*, **110**, 471 (1935).
21) A. Mc Kenzie, *J. Chem. Soc.*, **1928**, 646.

of the packed polymer was *ca.* 80 cm). The ammonium salt of DL-*N*-(*o*-carboxybenzoyl)phenylalanine (200 mg), dissolved in *ca.* 10 m*l* of 10^{-4} N aqueous ammonia, was adsorbed on the upper zone of the sorbent; then was eluted with the same solvent at room temperature. The flow rate of the solvent was *ca.* 6 m*l*/hr under atmospheric pressure; 12.50 m*l* portions of the eluent were collected automatically. Each of the eluents showed only a single spot on a silica-gel thin-layer chromatogram, the R_f value being in agreement with that of authentic *N*-(*o*-carboxy-benzoyl)phenylalanine. Acetic acid-*n*-butanol-water (1:1:1), 95% ethanol-28% aqueous ammonia(4:1), and benzene-ethyl formate-methanol (5:4:1) were used as the solvents. The concentration of the substrate in each fraction was estimated by UV absorption by using a Hitachi EPS-2-photometer. When the observed concentration was too low to determine the optical rotation, 10—30 consecutive fractions were combined and concentrated under reduced pressure. The last fraction was eluted with 0.1 N aqueous ammonia. The optical rotation of the evaporated residue was determined in 10^{-4}N aqueous ammonia by means of a Rex-automatic polarimeter. The chromatographic resolution of the other two racemates were also carried out by a procedure similar to that described above.