Partial Amino Acid Resolutions on a New Resolving Resin

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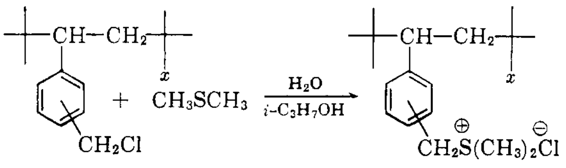
There have been many efforts made to circumvent the classical method of resolution of enantiomers normally achieved by the formation of diastereoisomers, separation of these, and regeneration of the pure enantiomers. Perhaps the most challenging aspect of the problem has been the search for systems wherein the passage of a moving enantiomer-containing phase would result in a moving phase containing only one isomeric species; the other enantiomer would then be recoverable from the stationary phase by elution. The concept of such optically active adsorbents is attributed to Willstatter.[1] Subsequently, varying degrees of success have been achieved in the use of resins for the resolutions of several different types of racemic organic compounds. Monomeric packings such as D-lactose hydrate have been used for the resolution of *p*-phenylenebis(iminocamphor),[2,3] "Troger's base" and other asymmetric compounds containing trivalent nitrogen.[4,5] Recently a patent appeared reporting the preparation and use of a D-lactose column for the separation of D,L-lysine methyl ester dihydrochloride, other derivatives of lysine, and D,L-proline.[6]

The use of *polymeric* materials for resolution has run the gamut from (+)-quartz,[7] and wool for mandelic acid,[8,9] to cellulose (paper) for α-amino acids.[10-12] Modified cellulose (carboxycellulose) has been reported satisfactory for resolutions of D,L-1,2-diaminopropane,[13] and the reaction product of an "Amberlite XE64" resin with thionyl chloride and then with quinine has been reported to give separations to pure (−)-mandelic acid.[14]

Combining the results of the modified resins with the concept that the best resolution should result from asymmetric adsorbents (or sites) of the same pattern as the molecular species to be resolved[15] it was considered practical to synthesize a resin possessing an optically pure substituent far enough away from the resin backbone so as to permit better interaction of this center with the racemic mixture to be resolved.

This paper reports the synthesis of poly [S-(*ar*-vinylbenzyl)-L-cysteine] and its use in the partial resolutions of D,L-methionine. The recent availability of *ar*-vinylbenzyl chloride[16] (60% *para*, 40% *ortho* isomers) prompted us to investigate the reactions of the monomer under known benzylating conditions with cysteine (prepared *in situ*) in liquid ammonia.[17] The benzylation proceeded in 94.5% yield to give the product. Attempts to polymerize this compound alone or in the presence of divinylbenzene failed. The next step was to polymerize the *ar*-vinylbenzyl chloride and attempt the benzylation of the cysteine under conditions which were suitable for the monomeric reaction. Linear poly(*ar*-vinylbenzyl chloride) gave only poor conversions to a poly[S-(*ar*-vinylbenzyl)-L-cysteine], and attempted benzylations with a series of 0.05 to 5% crosslinked poly(*ar*-vinylbenzyl chloride) polymers gave even poorer conversions (10%).

The recent report of the reactivity of sulfonium derivatives of poly(*ar*-vinylbenzyl chloride)[18] in displacement reactions (resulting in benzylation reactions) prompted us to combine this sulfonium intermediation with techniques[19] suitable for its use in aqueous alcoholic systems. Preparation of the linear (I) and lightly cross-linked poly-[dimethyl(*ar*-vinylbenzyl) sulfonium chloride] in aqueous isopropyl alcohol was easily effected[19]; each of the reactive polymers was added to separate preparations of the disodium salt of cysteine freed of ammonia and dissolved in the same concentration of aqueous isopropyl alcohol.



I

Linear or cross-linked

Displacement reactions were run at below 70° and resulted in polymers (II) possessing bound L-cysteine from 80 to 97% (based on nitrogen and sulfur analyses, respectively) for the linear poly-

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[1] R. Willstatter, *Ber.*, **37**, 3758 (1904).

[2] G. Henderson and H. Rule, *Nature*, **141**, 917 (1938).

[3] G. Henderson and H. Rule, *J. Chem. Soc.*, 1568 (1939).

[4] V. Perlog and P. Wieland, *Helv. Chim. Acta*, **27**, 1127 (1944).

[5] N. Leonard and W. Middleton, *J. Am. Chem. Soc.*, **74**, 5114 (1952).

[6] D. L. Garmaise and J. Colucci, U.S. Patent 2,957,886 (October 25, 1960).

[7] R. Tsuchida, M. Kobayashi, and N. Nakamura, *J. Chem. Soc. Japan*, **56**, 1339 (1935); *Chem. Abstr.*, **30**, 926 (1936).

[8] W. Bradley, R. Brindley, and G. Easty, *Discussions Faraday Soc.*, **16**, 152 (1954).

[9] W. Bradley and R. Brindley, *Chem. Ind.* (London), 579 (1954).

[10] N. Nakamura, *J. Chem. Soc. Japan*, **72**, 789 (1951); *Chem. Abstr.*, **47**, 5916 (1953), for lead references.

[11] K. Closs and C. Haug, *Chem. Ind.* (London), 103 (1953).

[12] C. Dalgliesh, *J. Chem. Soc.*, 3940 (1952).

[13] D. L. Garmaise and J. Colucci, U.S. Patent 2,957,917 (October 25, 1960).

[14] N. Grubhofer and L. Schleith, *Naturwissenschaften*, **40**, 508 (1953).

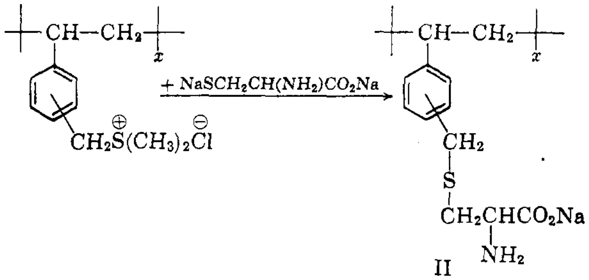
[15] L. Pauling, *Chem. Eng. News*, **27**, 913 (1949).

[16] E. L. McMaster and S. C. Stowe, British Patents 792,859 and 792,860 (April 2, 1958).

[17] R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).

[18] M. Hatch, Abstracts of the 138th Meeting of the American Chemical Society, September, 1960, New York, page 18T. See also British Patent 852,304 (October 26, 1960).

[19] W. G. Lloyd, The Dow Chemical Co., unpublished results.



II

Linear or cross-linked

mer. In the case of the cross-linked polymer (0.05% divinylbenzene) from 66 to 97% (based on nitrogen and sulfur analyses respectively) of the displacement was effected. If we assume that the high sulfur content was due to trapped dimethyl sulfide, then the cysteine had been S-benzylated to at least 80% in the case of the linear polymer and 66% in the case of the cross-linked polymer.

The 0.05% cross-linked poly[S-(*ar*-vinylbenzyl)-L-cysteine] was found to be unaffected (volumewise) on treatment with hydrochloric acid; when the polymeric material was treated with a sodium hydroxide solution, a six to seven hundred per cent volume increase was noted. When the linear polymer was treated with acid a nearly homogeneous "solution" resulted; when the linear material was made alkaline a complete and homogeneous "solution" resulted.

A column containing a cross-linked (1.5% divinylbenzene) poly[S-(*ar*-vinylbenzyl)-L-cysteine] was treated with an aqueous solution of D,L-methionine. Collection of eluent fractions and determination of optical rotation indicated that partial resolution was effected. Although the observed rotations were small, they appeared to be actual. Certain fractions (see Table I) showed as high as 22% separation (44% resolution) of the D,L-methionine, and this could be eluted with water. The columns could be regenerated by washing with acid, alkali, and water.

Experimental[20]

Preparation[17] of S-(*ar*-Vinylbenzyl)-L-cysteine; (Mixed *ortho* and *para* Isomers).—In a 2-l. three-necked, round-bottomed flask fitted with a Dry Ice condenser, air stirrer, and addition port for dry solids and cooled in a Dry Ice–acetone bath was condensed 700 ml. of liquid ammonia. The ammonia was permitted to reflux, and sodium (20 g.; 0.87 g.-atom) and cystine (48 g.; 0.2 mole) were added alternately at a rate controllable by the efficiency of the reflux Dry Ice condenser. When all of the reactants had been added, a slight excess of sodium was added until a permanent blue color persisted; a few crystals of ammonium chloride were added to dispel the blue color. To the solution of the disodium salt of cysteine prepared above was added 60.8 g. (0.4 mole) of *ar*-vinylbenzyl chloride (60% *para*, 40% *ortho*) during 30 min. at a rate to maintain reflux. The reaction mixture was stirred for an additional 30 min., and the ammonia allowed to evaporate during 16 hr. The white residue was chilled in an ice bath and 1-l. of ice water added slowly. The yellow solution which resulted was filtered cold and neutralized with acetic acid to a pH of about 5. The white crystalline product was filtered by suction and washed with a total of 5 l. of water and dried under vacuum at 65–70° for 36 hr. There was obtained 85 g. (94.6%) of product, m.p. 188–190°. (An authentic *para* isomer was also prepared, m.p. 203–205°, in a similar fashion.)

Anal. Calcd. for $C_{12}H_{15}O_2NS$: C, 60.73, H, 6.37; N, 5.90; S, 13.51. Found: C, 60.63; H, 6.18; N, 5.75; S, 13.61.

Attempts to polymerize this vinyl compound in emulsion with peroxide catalyst (cumene hydroperoxide) were unsatisfactory.

Preparation of Cross-linked Poly(*ar*-vinylbenzyl Chloride).—A mixture was prepared from 194.7 g. (1.2 moles) of *ar*-vinylbenzyl chloride (60% *para*, 40% *meta*), 5.3 g. (75% divinylbenzene; 2% of divinylbenzene), 1 g. of benzoyl peroxide, and 1200 ml. of water containing 2% Methocel. The resulting mixture was stirred and heated at a temperature of 75° during 4 hr., at 85° for 4 hr., and at 100° for 1 hr. After cooling the reaction mixture, it was filtered by suction and washed repeatedly with a total of 5 l. of deionized water, to yield a 98% conversion to polymer. Analysis indicated less than 5% hydrolysis of benzylic chlorine.

Preparation of Poly[S-(*ar*-vinylbenzyl)-L-cysteine] from Chloromethylated[21] Polystyrene Beads (0.1% Divinylbenzene).—In a 2-l., three-necked, round-bottomed flask, fitted with a stirrer, Dry Ice condenser, and Dry Ice–acetone bath, was condensed approximately 350 ml. of ammonia. Sodium (10 g.; 0.44 g.-atom) was added alternately with cystine (20.4 g.; 0.1 mole). When all of the cystine was converted to the disodium salt of cysteine, 30 g. (0.2 mole) of dried chloromethylated polystyrene beads (0.1% divinylbenzene) was added during approximately 0.5 hr. The solution was then allowed to reflux for 20 hr., at the end of which time the ammonia was allowed to evaporate. The material in the flask was cautiously treated with 20% acetic acid to pH 5.5, and allowed to stir overnight. The solution was filtered; the solid washed with 10-l. of deionized water and dried under reduced pressure for 6 hr. at 35°.

Anal. Calcd. for $(C_{12}H_{15}O_2NS)_x$: S, 13.15; N, 5.90. Found: S, 1.29; N, 0.55.

According to the elemental analysis received, only about 10% of the displacement reaction proceeded.

Preparation of Poly[dimethyl(*ar*-vinylbenzyl) Sulfonium Chloride].—Poly(*ar*-vinylbenzyl chloride) (200 g.) from the polymerization described before was suspended in 3 ml. of a mixture of equal weights of water and isopropyl alcohol. To this mixture was added 100 ml. of redistilled dimethyl sulfide, and the resulting mixture was shaken in a closed system at 25° for 96 hr. to assure complete conversion of the poly(*ar*-vinylbenzyl chloride) to the dimethylsulfonium form. In this form, the crosslinked poly[dimethyl(*ar*-vinylbenzyl) sulfonium chloride] was a thick, viscous gel. This gel could be stored and was used in certain of the subsequent preparations. Comparable dimethyl sulfonium chloride derivatives were prepared from chloromethylated polystyrene beads (0.05–10% divinylbenzene cross-linking compound) and from the linear polymer.

Preparation of Poly[S-(*ar*-vinylbenzyl)-L-cysteine] from Linear Dimethylsulfonium Resin.—In a 2-l., three-necked, round-bottomed flask, fitted with a stirrer, Dry Ice condenser, and Dry Ice–acetone bath, was condensed approximately 800 ml. of ammonia. Sodium (20 g.; 0.87 g.-atom) was added alternately with cystine (48 g.; 0.2 mole). When all of the cystine was converted to the disodium salt of cysteine, the flask was allowed to warm to room temperature to evaporate the ammonia. The flask was then placed under vacuum (12 hr.) to insure *complete* removal of the ammonia. A mixture of 859 g. (0.4 mole) of a 10% linear

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[20] Analytical data supplied by S. Shrader and L. Swim.

[21] G. D. Jones, U.S. Patent 2,694,702 (November 16, 1954).

sulfonium solution diluted with 700 ml. of 30% isopropyl alcohol was added with cooling, and the temperature of the mixture was raised to 55° (internal temperature) and kept at that temperature for 6 hr. The mixture was cooled to room temperature, allowed to stand for 16 hr., then the polymer was coagulated with glacial acetic acid and filtered to give a white powder. This powder was found to be acid-insoluble, dispersable at pH 6–8, and base soluble; nitrogen analysis indicates 82% of theoretical cysteine present.

Anal. Calcd. for $(C_{12}H_{15}O_2NS)_x$: C, 60.73; H, 6.37; N, 5.90; S, 13.51. Found: C, 61.36; H, 6.74; N, 4.75; S, 13.10.[22]

Preparation of Poly[S-(*ar*-vinylbenzyl)-L-cysteine] from Cross-linked Dimethylsulfonium Resin.—In a 2-l., three-necked, round-bottomed flask, fitted with a stirrer, Dry Ice condenser, and Dry Ice–acetone bath, was condensed approximately 700 ml. of ammonia. Sodium, (15 g.; 0.65 g.-atom) was added alternately with cystine (31.2 g.; 0.13 mole). When all of the cystine was converted to the disodium salt of cysteine, the flask was allowed to warm to room temperature to evaporate the ammonia. The flask was then placed under vacuum for 16 hr. to ensure complete removal of the ammonia, and 428 g. (0.2 mole) of a 10% "microgel" sulfonium suspension (60% *para*, 40% *ortho*), diluted with 750 ml. of 30% isopropyl alcohol, was added and the temperature of the mixture was raised to 50° (internal temperature) and kept at that temperature for 4 hr. After cooling, the polymer was coagulated with glacial acetic acid, filtered, and washed with 5 l. of water. Nitrogen analysis indicates 67% of theoretical cysteine present.

Anal. Calcd. for $(C_{12}H_{15}O_2NS)_x$: S, 13.51; N, 5.90. Found: S, 13.19; N, 3.92.[23]

Several additional preparations of this type were run; the reaction conditions were important. Addition of the sulfonium polymer to the *ammonia-free* disodium cysteineate is exothermic; dilution with aqueous isopropyl alcohol is mandatory. Analysis of each dried polymeric product indicated better than 60% yields of bound cysteine. The cross-linked resins were washed with water and/or dilute hydrochloric acid until the washings gave negative ninhydrin tests. Resins prepared in this fashion were let stand in 0.1 *N* hydrochloric acid for 3 months; the aqueous portion gave only a very slight ninhydrin color. Washed and dried resins of this type were found to be suitable for resolution experiments.

Preparation of Poly[S-(*ar*-vinylbenzyl)-D-cysteine] from Sulfonium Suspension Beads.—In a 2-l., three-necked, round-bottomed flask, fitted with a stirrer, Dry Ice condenser, and Dry Ice–acetone bath, was condensed approximately 700 ml. of ammonia. Sodium (25 g.; 1.09 g.-atoms) was added alternately with cystine (60 g.; 0.25 mole) until all of the cystine was converted to the disodium salt of cysteine; the flask was allowed to warm to room temperature to evaporate the ammonia. The flask was then placed under vacuum to insure complete removal of the ammonia, and 77 g. (0.5 mole) of suspension polymerized *sulfonium beads* in 500 ml. of dioxane was added, and the temperature of the solution was raised to 65° (internal temperature) and kept at that temperature for 4 hr. It was then chilled in an ice bath, and acetic acid was added until a pH 5.5 was reached. The polymer was filtered and washed with deionized water until the eluent gave a negative ninhydrin test.

TABLE I

RESOLUTION OF D,L-METHIONINE USING POLY[S-(*ar*-VINYLBENZYL)-L-CYSTEINE]

Eluent sample	Concentration, g./100 ml.	Rotation		Resolution, %
		α	$[\alpha]^{25}D$	
1	0.000	0.000	0.00	0
2	.036	.000	.00	0
3	.070	.000	.00	0
4	.068	+ .015	+1.1	+14
5	.134	+ .020	+0.7	+ 8
6	.722	.000	0.0	0
7	1.118	+ .030	+1.3	+16
8	1.476	+ .020	+0.7	+ 8
9	1.794	+ .060	+1.7	+22
10	1.786	+ .050	+1.4	+18
11	1.768	+ .050	+1.4	+18
12	1.780	+ .020	+0.6	+ 7
13	1.726	+ .050	+1.5	+19
14	1.812	.000	0.0	0

Attempted Resolution of D,L-Methionine Using Poly[S-(*ar*-vinylbenzyl)-D-cysteine).—A volume of 175 ml. of resin (70% cysteine-containing) was packed in a column of 24 mm. diameter and 340 mm. length. The prepared resin was washed again with 10 l. of deionized water. The resin was treated with a solution of D,L-methionine, 30.0 g. in 1 l. This solution was passed through the resin bed at a rate of 14 ml./hr. Each 14 ml. fraction was separately collected. Two or three consecutive samples were combined in a volumetric flask and diluted to 50 ml. From each sample thus prepared was withdrawn an aliquot; this was taken to dryness and the weight of residue used to determine the concentration of methionine in each sample, expressed in g./100 ml. of solution. Each volumetric sample was used to determine observed rotation, α, in a 2-dm. polarimeter tube. The data from these determinations are in Table I. Calculations of specific rotations from $[\alpha]^{25}D = \frac{\alpha \cdot 100}{c\,l}$ are summarized in the table along with percentages of resolution for each fraction. These percentages are based on the assumption that D-methionine in water has an $[\alpha]^{25}D$ $+8.12°$,[24] and that an $[\alpha]^{25}D$ of a solution containing D,L- and excess D-methionine is directly proportional to the concentration of excess D-methionine.

Elution of the column with deionized water at 14 ml./hr. gave four fractions (nonconsecutive) having observed rotations of -0.02 to -0.04 at concentrations of methionine such that $[\alpha]^{25}D$ varied from -1.39 to $-7.81°$. The enantiomer held on the column was slowly and irregularly released.

The column washed with 0.1 *N* hydrochloric acid and water to a negative ninhydrin reaction could be reused.

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[22] High sulfur analysis results from dimethyl sulfide trapped in the swollen polymer.

[23] See ref. 22. In the cross-linked swollen gel a limited percentage of the benzylic sites, either *ortho* or *meta* to the polymer backbone are sterically unavailable.[19]

[24] W. Windus and C. S. Marvel, *J. Am. Chem. Soc.*, **53**, 3490 (1931).