Short Communication

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Direct Resolution of DL-Lysine-3, 5-Dinitrobenzoate

DL-Lysine is resolved into its optical isomers by fractional crystallization or fractional extraction of the diastereoisomeric salts formed with optically active acids such as L-glutamic acid,¹⁾ N- α -phenoxypionic acid,²⁾ D-tartaric acid,^{3,4)} D-camphoric acid,⁵⁾ N-carbamoyl-L-glutamic acid,⁶⁾ L-5-hydantoinpropionic acid,⁶⁾ D-homocysteine acid,⁷⁾ and 1,1-methylene-bis-2-pyrrolidone-5-carboxylic acid.⁸⁾ DL- α -Amino caprolactam, a synthetic precursor of DL-lysine, is resolved by use of optically active 2-pyrrolidone-5-carboxylic acid.⁹⁾ DL- α -Aminocaprolactam hydrobromide is resolved by the preferential crystallization procedure in organic solvents.¹⁰⁾ However, none of literature has ever been appeared which describes the direct resolution of DL-lysine or its derivatives by preferential crystallization due to inoculation.

Here we report the resolution of DL-lysine-3,5-dinitrobenzoate (DL-Lys. B.A.) by the preferential crystallization procedure. Optically active and DL-Lys. B.A. were prepared at first in water as needle crystals by mixing an

equimolar amount of 3,5-dinitrobenzoic acid with optically active and DL-lysine, respectively. Properties of L- and DL-Lys. B.A. are shown in Table I. DL-Lys. B.A. is approximately twice as soluble as L-Lys. B.A. L-Lys. B.A. is insoluble in a saturated solution of DL-Lys. B.A. IR spectrum and powder x-ray diffraction pattern were identical between L- and DL-Lys. B.A.

The resolution was carried out in a batch system. Thus, 67.2 g of DL-Lys. B.A. and 5.4 g of L-Lys. B.A. were dissolved in 200 ml of hot water. When cooled to 46°C, the solution was seeded with 2.2 g of L-Lys. B.A., and further cooled to 30°C in 50 min with stirring. Filtration of the mixture gave L-Lys. B.A. crystals (1st crop). The filtrate was heated after the addition of DL-Lys. B.A., the amount of which was equal to the 1st crop. The resulting solution was seeded at 46°C with 2.2 g of D-Lys. B.A. The mixture was cooled to 30°C in 50 min with stirring, and filtered to give D-Lys. B.A. crystals (2nd crop).

TABLE I. PROPERTIES OF L- AND DL-Lys. B.A.

	Mp, °C	Anal. for:	C ₁₃ H ₁₈ O ₈ N ₄	[α] _D ²⁵	Solubility	
					Temp.	g/100 g·H ₂ O
	(dec.)	Calcd:	$\left\{ \begin{array}{l} \text{C}, 41.49 \\ \text{H}, 5.36 \\ \text{N}, 14.89 \end{array} \right.$		30°	7.48
L	210°	Found:	$\left\{ \begin{array}{l} \text{C}, 41.80 \\ \text{H}, 5.38 \\ \text{N}, 14.96 \end{array} \right.$	+5.2° ($c=4$, H ₂ O)	50°	15.61
					65°	31.58
DL	213°	Found:	$\left\{ \begin{array}{l} \text{C}, 41.47 \\ \text{H}, 5.20 \\ \text{N}, 15.08 \end{array} \right.$		30°	15.17
					50°	36.11
					65°	77.55

1) R. D. Emmick, U. S. Pat., 2556907 (1952).

2) G. H. Suverkropp, Belg. Pat., 655709 (1966).

3) E. Brakebill and J. H. Cornell, Jr., Can Pat., 689164 (1964).

4) J. Cuvillier, Ger. Pat., 1118794 (1962).

5) H. C. White, U. S. Pat., 2859244 (1958).

6) E. C. Britton and H. C. White, U. S. Pat., 2881183 (1959).

7) M. Hara, K. Togo and T. Akashi, *Japanese Pat. publication*, 39-28232 (1964).

8) T. Nagai, T. Yamaura and J. Kawaoka, *ibid.*, 42-3488 (1967).

9) M. Brenner, and H. R. Rickenbacher, *Helv. Chim. Acta*, 41, 181 (1958).

10) M. Shibasaki and K. Ohno, *Japanese Pat. Publication*, 42-3489 (1967).

On repeating alternately the above procedures, additional crops of L- and D-Lys. B.A. (3rd and 4th crops) were obtained. The yield and optical purity of 1st to 4th crops are shown in Table II.

TABLE II. RESULTS OF THE RESOLUTION IN THE BATCH SYSTEM

	Crystals	Yield (g)	Optical purity (%)
1st	L-Lys. B.A.	14.5	91.3
2nd	D-Lys. B.A.	12.5	95.3
3rd	L-Lys. B.A.	8.5	94.7
4th	D-Lys. B.A.	12.5	85.8

The optical purity was determined by specific rotation of L- and D-lysine derived from L- and D-Lys. B.A. An aliquot of aqueous solution of the separated crystals of L- or D-Lys. B.A. was made acidic with hydrochloric acid to liberate 3,5-dinitrobenzoic acid, which was removed by extraction with ether. The acidic solution containing L- or D-lysine was evaporated to dryness. The residue was dissolved in 6N hydrochloric acid, and optical rotation was measured. The content of lysine in the solution used for optical rotation measurement was determined by Kjeldahl-N analysis.

The resolution was also carried out continuously in a fluidized bed system¹¹⁾ consisting of two columns connected in parallel. The column, 8 cm long and 2 cm diameter, was separable in the middle, and fitted with sintered glass discs at both ends. Seed crystals

(1.0 g, 60~115 mesh) of L- and D-Lys. B.A. were added separately to each column. A supersaturated aqueous solutions of DL-Lys. B.A. was circulated through the columns at a constant temperature and flow rate (250 ml per min). At the end of a given time, the selectively grown crystals in each column were separated by filtration, weighed and the optical purity was determined. The results are summarized in Table III.

The temperature of the circulated solution of DL-Lys. B.A. was 35°C and 55°C. It is apparent that the resolution is affected significantly by the temperature of the system. Higher concentration of DL-Lys. B.A. is required at higher temperature to get enough crystal growth. The optical purity of the grown crystals decreases considerably at 35°C, but slightly at 55°C as increasing the growth rate of seed crystals. The result in the case of No. 6 in Table III is useful for practical purpose.

It was also found that DL-lysine-l-chloronaphthalene-4-sulfonate and DL-lysine-anthraquinone- β -sulfonate are resolved by the preferential crystallization procedure. Details will be described elsewhere.

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TABLE III. RESULTS OF THE RESOLUTION IN THE FLUIDIZED BED SYSTEM
Seed crystals, 1.0 g, 60~115 mesh

No.	Concentration of DL-Lys. B.A. (%)	Temperature (°C)	Time (min)	Yield (g)		Optical purity (%)	
				L	D	L	D
1	23.0 $\pm$ 0.2	35	45	2.2	2.4	78.5	72.2
2	23.0 $\pm$ 0.2	35	85	2.8	2.8	71.2	68.3
3	23.0 $\pm$ 0.2	35	160	3.8	3.4	58.1	59.5
4	36.0 $\pm$ 0.2	55	125	2.8	2.8	95.1	95.1
5	36.0 $\pm$ 0.2	55	200	3.2	3.2	92.2	93.1
6	36.0 $\pm$ 0.2	55	300	4.4	4.2	89.7	92.5

11) K. Itoh, T. Akashi and S. Tatzumi, *Japanese Pat. Publication*, 36-17710 (1961).