[CONTRIBUTION FROM THE NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

Preparation of the Optical Isomers of Analogous Cyclohexyl- and Phenyl-substituted Amino Acids

BY DANIEL RUDMAN, ALTON MEISTER AND JESSE P. GREENSTEIN

The optical isomers of α-aminocyclohexylacetic, α-amino-β-cyclohexylpropionic and α-aminophenylacetic acids have been prepared by enzymatic resolution of the chloroacetyl derivatives of the corresponding racemic amino acids. The specific optical rotations of these isomers, which were shown to possess an optical purity of more than 99.9%, are given.

The method of enzymatic resolution developed in this Laboratory[1] has been successfully applied to a number of unnatural α-amino acids[2] as well as to those of the naturally occurring group. In the present report, the preparation and optical properties of the isomers of α-amino-β-cyclohexylpropionic acid (cyclohexylalanine), α-aminocyclohexylacetic acid (cyclohexylglycine), and their unsaturated analogs (phenylalanine and phenylglycine) are described. The enzymatic method of preparation and the action of optically-specific oxidase preparations on the isomers obtained establish the relative optical configurations of these amino acid enantiomorphs.

Experimental

Preparation of Racemic Cyclohexylamino Acids.—β-Cyclohexylpropionic acid[3] (2.28 moles) was refluxed with bromine (2.74 moles) and phosphorus trichloride (0.022 mole) for 48 hours. The mixture was distilled under reduced pressure and the α-bromo derivative collected at 145–150° (3 mm.). Ten volumes of 28% ammonium hydroxide were added to the α-bromo acid and the mixture allowed to stand for 3 days at room temperature. The bulky precipitate of α-amino-β-cyclohexylpropionic acid was filtered, washed with acetone, and recrystallized from water and from water–acetone (yield 70%).

Anal. Calcd. for $C_9H_{17}O_2N$: N, 8.2. Found: N, 8.1.

DL-α-Aminocyclohexylacetic acid was prepared in an analogous manner from cyclohexylacetic acid.

Anal. Calcd. for $C_8H_{15}O_2N$: N, 8.9. Found: N, 8.9.

Chloroacetyl Derivatives.—The chloroacetyl derivatives of the amino acids were prepared[4] in the usual way.

Chloroacetyl-DL-α-amino-β-cyclohexylpropionic acid (m.p. 125°). *Anal.* Calcd. for $C_{11}H_{18}O_3NCl$: N, 5.7. Found: N, 5.8.

Chloroacetyl-DL-α-aminocyclohexylacetic acid (m.p. 142°). *Anal.* Calcd. for $C_{10}H_{16}O_3NCl$: N, 6.0. Found: N, 6.1.

Chloroacetyl-DL-α-aminophenylacetic acid (m.p. 127°).[5] *Anal.* Calcd. for $C_{10}H_{10}O_3NCl$: N, 6.2. Found: N, 6.1.

Resolution Procedure.—The acylated derivatives were resolved by the enzymatic procedure previously described.[1] The yields were 60 to 80% for the L-isomers and 40 to 60% for the D-isomers. The nitrogen analyses and optical properties of the isomers are collected in Table I. The resolution of DL-α-amino-β-phenylpropionic acid has been described,[6] and the isomers of this amino acid are included in the table for comparative purposes. The unusually high rotation values of the α-aminophenylacetic acid enantiomorphs are noteworthy.

TABLE I

PROPERTIES OF RESOLVED ISOMERS

Isomers, acid	N, analyses, % Found	Calcd.	$[\alpha]D^a$ (water)	$[\alpha]D^a$ (5 *N* HCl)
L-α-Aminophenylacetic[b]	9.2	9.3	+114.0°[c]	+168.0°[c]
L-α-Aminocyclohexylacetic	8.9	8.9	[d]	+ 35.5°[e]
L-α-Amino-β-phenylpropionic	8.5	8.5	− 34.6°[e]	− 4.5°[e]
L-α-Amino-β-cyclohexylpropionic[f]	8.2	8.2	− 9.0°[e]	+ 15.0°[e]
D-α-Aminophenylacetic[b]	9.2	9.3	−114.0°[c]	−169.0°[c]
D-α-Aminocyclohexylacetic	8.9	8.9	[d]	− 35.0°[e]
D-α-Amino-β-phenylpropionic	8.5	8.5	+ 34.4°[e]	+ 4.5°[e]
D-α-Amino-β-cyclohexylpropionic[f]	8.1	8.2	+ 8.5°[e]	− 15.0°[e]

[a] At 26°. [b] E. Fischer (*Ber.*, **41**, 1290 (1908)) reported $[\alpha]^{20}D$ values of −157.87° (in 0.65 *N*) hydrochloric acid) and −165.43° (in 10% hydrochloric acid) for the D-isomer, and +158.09° (in 0.65 *N* hydrochloric acid) and +112.6° (in water) for the L-isomer. [c] 1.000% solution; 2-dm. tube. [d] These isomers were too insoluble to permit accurate determinations. [e] 2.000% solution; 2-dm. tube. [f] E. Waser and E. Brauchli (*Helv. Chim. Acta*, **7**, 740 (1924)) reported $[\alpha]^{20}D$ values (in 4% hydrochloric acid) of +13.30° and and − 13.32°, respectively, for the L- and D-isomers of this amino acid.

Optical Purity of the Isomers.—The optical purity of the isomers was determined by the enzymatic technique previously described.[7] Hog kidney oxidase was used to detect the presence of D-isomer in the L-amino acids, and rattlesnake and cobra venom oxidases were employed, respectively, to test the D-enantiomorphs of the acetic and propionic acid derivatives.[8] No evidence of contamination was observed under conditions whereby one part in 1000 of the enantiomorph could be detected, indicating an optical purity of greater than 99.9%.

Acknowledgment.—The authors wish to thank Mr. R. J. Koegel for the nitrogen analyses.

BETHESDA, MD. RECEIVED SEPTEMBER 10, 1951

(1) For the literature see S. M. Birnbaum, L. Levintow, R. B. Kingsley and J. P. Greenstein, *J. Biol. Chem.*, **194**, 455 (1952).

(2) C. G. Baker and A. Meister, THIS JOURNAL, **73**, 1336 (1951).

(3) Obtained from Eastman Kodak Company and redistilled before use.

(4) E. Fischer, *Ber.*, **37**, 2486 (1904).

(5) Amino acid obtained from Eastman Kodak Company.

(6) J. B. Gilbert, V. E. Price and J. P. Greenstein, *J. Biol. Chem.*, **180**, 473 (1949).

(7) A. Meister, L. Levintow, R. B. Kingsley and J. P. Greenstein, *ibid.*, **192**, 535 (1951).

(8) The susceptible isomers were rapidly oxidized by the respective enzymes under the conditions employed.