Synthesis of DL-α(m-Carboxyphenyl)-glycine

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Recently a new addition to the ever growing list of naturally occurring amino acids was reported. An acidic aromatic amino acid, α(m-carboxyphenyl)-glycine was isolated from the bulb of Iris tingitana var. Wedgewood (1). It was also found to be present in another variety, White Perfection. In both these varieties, the amino acid was not detected in the protein fractions. This paper describes the synthesis of dl-α(m-carboxyphenyl)-glycine and its N-acetyl and N-chloroacetyl derivatives and indicates their behavior toward p-amino acid oxidase, l-amino acid oxidase, hog kidney acylase, and carboxypeptidase A.

**EXPERIMENTAL PROCEDURE**

The synthesis was accomplished conveniently by using as starting material m-toluic acid which was oxidized with chromic acid in the presence of acetic anhydride and glacial acetic acid (2). The resulting diacetate of isophthalaldehydic acid was hydrolyzed with sulfuric acid to the corresponding aldehydic acid. The methyl ester was then prepared and was converted to the aminonitrile by the Strecker synthesis with a mixture of NaCN and NH₃ (3). Hydrolysis of the resulting aminonitrile yielded the desired amino acid which was purified by passage through Dowex 50 in the hydrogen form. Final purification was obtained by ion exchange chromatography on Dowex 1 in the acetate form with acetic acid as eluting agent (4). The amino acid crystallizes readily from water-ethanol. It forms crystalline N-acetyl and N-chloroacetyl derivatives.

The free amino acid was not acted upon by D- or L-amino acid oxidase, whereas a synthetic sample of the next higher homologue, α(m-carboxyphenyl)-alanine, was oxidized by L-amino acid oxidase at approximately one-fourth the rate observed with L-leucine as standard (5). The N-acetyl derivative of α(m-carboxyphenyl)-glycine did not serve as a substrate for hog kidney acylase. Likewise, carboxypeptidase A was inactive toward the N-chloroacetyl derivative. It was found that the α-alanine homologue was resolved by leucine aminopeptidase with L-leucine as standard (5). The N-acetyl derivative of α(m-carboxyphenyl)-glycine was inactive toward the N-chloroacetyl derivative. It was found that the α-alanine homologue was resolved by leucine aminopeptidase with L-leucine as standard (5). This work is being extended toward the resolution of the glycine compound.

The synthetic α(m-carboxyphenyl)-glycine gave identical infrared spectra, and cochromatographed in several solvent systems with a racemized sample of the isolated natural isomer (1). Titration studies of αL-(m-carboxyphenyl)-glycine showed that pKₐ' for the aromatic carboxyl is 3.9, pKₐ for the amino group is 9.1, and pKₐ' for the α-COOH is less than 2, probably around 1.5. The isoelectric point is therefore about 2.7.

**Diacetate of Isophthalaldehydic Acid**—This compound was prepared by a procedure similar to that used for the preparation of the diacetate of p-nitrobenzaldehyde (2) with 235 ml of glacial acetic acid, 283 ml (3 moles) of acetic anhydride, 24.5 g (0.18 mole) of m-toluic acid, 43 ml of concentrated H₂SO₄, and 50 g (0.5 mole) of chromium trioxide. The oxidation was accomplished at 5°C in an ice-salt bath. At the completion of the reaction, the mixture was poured into 2 liters of chilled ice and water, resulting in a volume of 2.5 to 3 liters. This mixture was extracted with three 300-ml portions of benzene. The extracts were dried with anhydrous Na₂SO₄ and evaporated under reduced pressure. The residue was dissolved in 100 ml of benzene, 300 ml of petroleum ether were added, and the mixture was cooled in the icebox. The crystals were washed with petroleum ether and air-dried: weight, 23 g (50% of theory). After recrystallization three times from ethyl acetate-petroleum ether and dried in a vacuum over P₂O₅, the product melted at 129-130°C.

C₈H₁₀O₄

Calculated: C, 57.15; H, 4.50
Found: C, 57.44; H, 5.01

**Isophthalaldehydic Acid**—A mixture of 23 g (0.091 mole) of the diacetate of isophthalaldehydic acid, 50 ml of ethyl alcohol, 50 ml of water, and 5 ml of concentrated H₂SO₄ was refluxed for 30 minutes and cooled. The crystals were repeatedly washed with cold H₂O and dried in a vacuum over P₂O₅: weight, 11.8 g (87.4% of theory). After recrystallization twice from hot water and decolorization with activated charcoal, the compound melted at 174-175°C (6).

**Isophthalaldehydic Acid Methyl Esters**—A solution of 7.5 g (0.05 mole) of isophthalaldehydic acid in 100 ml of ether was treated with diazomethane prepared from 14.7 g (0.1 mole) of N-methyl-N-nitroso-N′-nitroguanidine. The crude methyl ester weighed 8.2 g (100% of theory). After decolorization with charcoal and threefold recrystallization from petroleum ether, it was dried in a vacuum over P₂O₅: melting point, 52-53°C (6).

**Dl-α-(m-Carboxyphenyl)-glycine**—This compound was prepared by a procedure based on that used for the preparation of α-phenylglycine (3). To 1.96 g (0.04 mole) of NaCN and 2.35 g (0.044 mole) of NH₃ in 8 ml of H₂O there was added a solution of 6.6 g (0.04 mole) of the methyl ester of isophthalaldehydic acid in 10 ml of hot methyl alcohol. The mixture was refluxed for 1 hour and held at room temperature overnight with mechanical shaking. Then 40 ml of concentrated HCl were cautiously added (with good ventilation in a hood) and the mixture was evaporated in a vacuum on a water bath. The residue was refluxed with...
100 ml of 6 N HCl for 2 hours. The hydrolysate was filtered and the filtrate evaporated to dryness in a vacuum. The residue was dissolved in water; the solution was neutralized with 20% NaOH to pH 6.0, filtered, and passed through a column (32 x 27 cm) of Dowex 50-X4, 50 to 100 mesh, in the hydrogen form. The resin was washed with deionized water. The amino acid was eluted from the column with 2 N acetate and dried with anhydrous Na2SO4 and evaporated in a vacuum to dryness. The residue, weighing 0.32 g (71.9%), was decolorized with charcoal, recrystallized from water, and dried over P2O5 at 105°C.

The product, m.p. 237° with decomposition, gave a negative reaction with ninhydrin. The amino acid was recrystallized three times from 50% ethyl alcohol and dried over P2O5 in a vacuum. It melted at 210–211° with decomposition.

**C6H11NO3**

**Found:** C 55.20, H 4.91, N 7.13

**Properties**—DL-α-(m-Carboxyphenyl)-glycine reacted with ninhydrin with the liberation of 1 mole of CO2 per mole. It is insoluble in chloroform, ether, and benzene, very slightly soluble in ethyl alcohol, and soluble in water. It reacts with ninhydrin on paper at room temperature to give within the first 10 minutes a yellow color which gradually changes to a typical blue color. After chromatography, the final ninhydrin color on paper depends on the solvent used. For instance, with phenol it became rose-brown, with pyridine, reddish violet; with acetic acid and formic acids, chocolate brown; with lutidine, greenish brown; with lutidine-acetic acid, grayish blue; and with neutral solvent the bright yellow color became reddish violet on standing.

**N-Acetyl-DL-α-(m-carboxyphenyl)-glycine—DL-α-(m-Carboxyphenyl)-glycine** (0.41 g, 0.002 mole) was treated with 0.5 g of acetylchloride and 0.83 g of Na2CO3 in 20 ml of H2O under usual Schotten-Baumann conditions. The reaction mixture was acidified to pH 1 and the acetyl derivative was extracted with several small portions of ethyl acetate. The combined extracts were dried with anhydrous Na2SO4 and evaporated in a vacuum to dryness. The residue, weighing 0.32 g (71%), was decolorized with charcoal, recrystallized from water, and dried over P2O5. The product, m.p. 237° with decomposition, gave a negative reaction with ninhydrin.

**C11H18NO4Cl**

**Found:** C 48.68, H 3.93, N 5.16

**Enzymatic Studies**—DL-α-(m-Carboxyphenyl)-glycine was inert toward d-amino acid oxidase (Nutritional Biochemicals Corporation), and l-amino acid oxidase, dried snake venom (Bothrops atrox) (7, 8). N-Acetyl-DL-α-(m-carboxyphenyl)-glycine was unaffected by hog kidney acylase (9), and N-chloroacetyl-DL-α-(m-carboxyphenyl)-glycine resisted the action of carboxypeptidase A (10).

**SUMMARY**

DL-α-(m-Carboxyphenyl)-glycine and its N-acetyl and N-chloroacetyl derivatives have been synthesized. They were not affected by d- and l-amino acid oxidases, hog kidney acylase, and carboxypeptidase A.

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Morris Filadello Irreverre, Herman Kny, Sam Asen, John F. Thompson and Clayton J. Morris

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