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The Absolute Configuration of *m*-Carboxyphenylglycine, an Amino Acid From Higher Plants

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The absolute configuration of levorotatory *m*-carboxyphenylglycine is established by its synthesis from D-phenylglycine through a series of steps not involving the asymmetric centre. These include *m*-nitration, catalytic reduction to the *m*-amino derivative, Sandmeyer reaction to the nitrile, and, finally, conversion of the latter into *m*-carboxy-D-phenylglycine (III) by way of mild alkaline hydrolysis of an iminoester intermediate.

m-Carboxyphenylglycine, with unspecified optical properties, has previously been reported as a constituent of an *Iris* species. It appears likely that the natural specimen is, in fact, the D-enantiomer, yet heavily contaminated with the racemic modification.

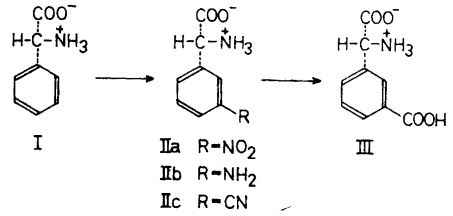
The resolution of *m*-carboxy-DL-phenylglycine into enantiomers with L-arginine as a resolving base is described.

From the non-protein nitrogen fraction of bulbs of *Iris tingitana* var. Wedgewood, Morris *et al.*[1] recently isolated a new aromatic amino acid which they identified as *m*-carboxyphenylglycine by degradation reactions and by comparison of the infra-red spectrum of a racemized specimen with that of authentic *m*-carboxy-DL-phenylglycine, the latter synthesized according to directions given in a subsequent publication.[2] In the original paper [1] no rotation data were presented for the amino acid of natural origin; according to a private communication from Dr. J. F. Thompson, however, the natural sample possessed a specific rotation of $-6°$ in aqueous solution at the D-line.

Because of this unprecedented isolation of a phenylglycine derivative from higher plants we were attracted by the problem of establishing the relationship between the optical rotation and absolute configuration of *m*-carboxyphenylglycine and we sketch the result below.

Since the absolute configuration of optically active phenylglycine is known with certainty, both from chemical correlations (*cf.* Ref.[3]) and kinetic studies,[4] it lay near at hand to attempt a direct chemical correlation between this simple amino acid and its *m*-carboxy derivative. Several attempts to decarboxylate the aromatic ring selectively by heating the amino acid or its copper

complex were fruitless, *m*-carboxybenzylamine being the major degradation product in both cases as estimated from paperchromatographic data. Therefore, a synthesis was planned departing from configurationally known (−)-D-phenylglycine (I) and proceeding through the steps indicated in the annexed scheme:



I IIa R=NO_2 IIb R=NH_2 IIc R=CN III

The individual reactions were first studied in the racemic series. The combined *m*-directing influence of the two side-chain substituents (COOH and NH_3^+) renders *m*-nitro-DL-phenylglycine accessible in good yield by direct nitration of DL-phenylglycine, in accord with older statements in the literature.[5] The described oxidative degradation of the nitro derivative to *m*-nitrobenzoic acid [5] was repeated and confirmed during the present work. Catalytic hydrogenation of racemic *m*-nitrophenylglycine proceeded smoothly to give *m*-amino-DL-phenylglycine, possessing properties agreeing with those previously reported for a sample prepared by reduction with tin and hydrochloric acid.[5] *m*-Cyano-DL-phenylglycine, a new compound, was obtained by a Sandmeyer reaction, performed essentially as described in an analogous synthesis of *m*-carboxy-L-tyrosine,[6] yet in the present case proceeding in a somewhat lower yield. As demonstrated by paper chromatography, prolonged heating at 110° for more than 24 h with 20 % hydrochloric acid was required to convert the cyano-compound into racemic *m*-carboxyphenylglycine, conditions likely to produce extensive racemization in the optically series when the rather facile acid-induced racemization of optically active phenylglycine is considered.[7]

The same sequence of reactions was then repeated in the optically active series, starting with (−)-D-phenylglycine (I). It was soon realized that the nitration reaction was accompanied by partial racemization since fractional crystallization of the crude reaction product resulted in the production of fractions with correct compositions but different solubilities and rotations, those of lowest solubility possessing the lowest rotations. Assuming identical molecular rotations of D-phenylglycine and its *m*-nitro-derivative, an analytical specimen of (−)-*m*-nitro-D-phenylglycine (IIa), produced upon repeated recrystallizations, was estimated to possess an optical purity of about 80 % (*cf.* Table 1). For the subsequent catalytic reduction a product containing about 60 % of the optically active nitro-derivative was employed, resulting in the production of partly racemic reduced material. Upon repeated recrystallizations a preparation was obtained consisting of optically pure or nearly pure (−)-*m*-amino-D-phenylglycine (IIb), as estimated from the molecular rotation data (Table 1). When this material was subjected to the Sandmeyer reaction, (−)-*m*-cyano-D-phenylglycine (IIc) was produced and could be

Table 1. Molecular rotations measured in aqueous 1 N hydrochloric acid (*c* about 1) at the D-line.

Compound	Formula	Molecular rotation
D-Phenylglycine	I	−250°[a]
m-Nitro-D-phenylglycine	IIa	−176°[b]
m-Amino-D-phenylglycine	IIb	−213°
m-Cyano-D-phenylglycine	IIc	−224°
m-Carboxy-D-phenylglycine	III	−248°

[a] in 4 N HCl. [b] Not considered sterically homogeneous.

isolated as the pure or almost pure enantiomer, inferred again from the molecular rotation values (Table 1).

The necessity of conserving the optical activity during conversion of the cyano derivative (IIc) into the carboxylic acid (III) excluded direct hydrolysis with hot aqueous acids or bases. Hence, the desired transformation was accomplished through an iminoester intermediate, followed by alkaline hydrolysis of the latter at room temperature and resulting in the production of the levorotatory amino acid. Consequently, *levorotatory* m-*carboxyphenylglycine possesses the* D-*configuration*, indicated in (III).

From this correlation the conclusion must be drawn that the specimen of *m*-carboxyphenylglycine isolated by Morris *et al.*[1] from *Iris* bulbs, though heavily contaminated with the racemic modification, possessed the D-configuration.* The somewhat surprising conclusion that the *Iris* amino acid belongs to the "unnatural" D-series is strongly supported by the recent isolation in this laboratory of the closely related D-2-(3-carboxy-4-hydroxyphenyl)-glycine from *Reseda luteola* L.,[8] to our best knowledge the first rigorously established occurrence of a D-amino acid in higher plants (*cf.* Ref.[8]).**

In connexion with the present studies, racemic *m*-carboxyphenylglycine was resolved into its enantiomers. Due to the strongly acid character of the present amino acid, attempts to utilize (+)-camphorsulphonic acid, a long-established resolving agent in the case of phenylglycine,[10] were of no avail. Crystalline N-menthoxyacetyl-phenylglycine was readily produced on reaction of *m*-carboxy-DL-phenylglycine with (−)-menthoxyacetyl chloride, a reagent successfully employed for the resolution of phenylglycine,[9] but proved of no use in the present case. However, the application of L-arginine as a resolving base, first utilized in connexion with work described in the subsequent paper,[8] was of great help also in the present case. After repeated recrystallizations of the more soluble arginine salt, levorotatory *m*-carboxyphenylglycine was obtained, possessing almost the same specific rotation as a synthetic specimen produced from D-phenylglycine as described above (Table 1).

* It appears likely that the American workers, inadvertent of the considerable difference in solubility between the enantiomers and the racemic modification, concentrated the less soluble racemate in the most extensively purified fractions.

** *Added in proof.* After the present paper was submitted for publication, a malonyl conjugate of D-tryptophane has been reported as a compound widely distributed in higher plants.[11]

EXPERIMENTAL

Melting points are uncorrected and determined in capillary tubes in an Anschütz-Hershberg apparatus with a standard rate of heating of 2° per min. Rotations are measured in a 1 dm tube.

Syntheses

m-*Nitro*-DL-*phenylglycine*. Racemic phenylglycine (42 g), dissolved in conc. sulphuric acid (210 ml), was nitrated with fuming nitric acid (*d* 1.52) (15.5 ml) under ice-cooling, essentially following the directions of Plöchl and Loë.[5] After recrystallization from water by addition of ethanol, the yield was 24 g (45 %), m.p. 166° (reported:[5] 172°).

In order to confirm the *m*-position of the nitro-group, a small sample of the reaction product was converted into *m*-nitromandelic acid and the latter oxidized with potassium permanganate to give an ether-soluble acid, as previously reported.[5] This was identified as *m*-nitrobenzoic acid by infra-red comparison and mixed melting point determination with an authentic specimen. Paper chromatography demonstrated the absence of observable amounts of other nitro-isomerides.

m-*Amino*-DL-*phenylglycine*. The previously reported reduction of the nitro derivative with tin and hydrochloric acid [5] proceeded quite satisfactorily, but catalytic hydrogenation was found to be a more convenient procedure. A solution of *m*-nitro-DL-phenylglycine (4.0 g) in water (250 ml), containing one equivalent of hydrochloric acid, was treated with hydrogen at room temperature in the presence of Adam's PtO_2 catalyst (300 mg). The theoretical amount of hydrogen was consumed in the course of 3 h. Sodium acetate was then added to the filtrate to bring pH to about 5, and the amino-phenylglycine separated on cooling. An additional crop was obtained by adding ethanol to the filtrate. The total yield was 82 % of a slightly brownish product, m.p. 204—205° (decomp.) (reported [5] 214° (decomp.)).

m-*Cyano*-DL-*phenylglycine*. Racemic *m*-aminophenylglycine (3 g) was dissolved in 2 N hydrochloric acid (45 ml), and the solution was cooled and kept at 0—5° during the addition of a solution of sodium nitrite (1.55 g of a 85 % preparation) in water (10 ml) to the vigorously stirred solution in the course of 20 min. Sodium carbonate was added to bring the pH-value to about 6 and the solution was added, in the course of 20 min, to another solution prepared by dissolving cuprous cyanide (3.2 g), sodium cyanide (5.3 g), and sodium carbonate (5.3 g) in water (40 ml). The stirred mixture was then kept at 60° for 2.5 h. After cooling, it was made strongly acidic, and cuprous cyanide was filtered off. The filtrate was brought to pH 5.5 with ammonia, and the solution (150 ml) was applied to a strongly acid ion exchange resin (Zeo-Karb 225, 3 × 66 cm) on the acid form (hood!). The column was washed with water (2 l), and the racemic *m*-cyanophenylglycine (743 mg) was eluted with 1 N ammonia. After dissolution in water and addition of ethanol, a preparation was obtained, which, after two additional recrystallizations from water, afforded an analytical specimen, m.p. 181° (Found: C 60.40; H 4.64; N 15.59. Calc. for $C_9H_8N_2O_2$, 1/6 H_2O: C 60.33; H 4.69; N 15.64). The infra-red spectrum exhibited a very strong C≡N-band at 2220 cm^{-1}. As estimated by paper chromatography, the cyano-derivative was homogeneous except for a trace of unchanged starting material.

Acid hydrolysis of m-*cyano*-DL-*phenylglycine*. One-milligram samples of the cyano-compound were heated at 110° in 20 % hydrochloric acid (50 µl) for various lengths of time (0.5, 1.5, and 2 h). Paperchromatographic analysis, in butanol:acetic acid:water (12:3:5), indicated that hydrolysis was not complete after 2 h. Another sample, kept at 110° for 109 h, had undergone complete hydrolysis as estimated from paper chromatography.

m-*Nitro*-D-*phenylglycine* (IIa). Optically pure D-phenylglycine (I), produced by resolution of the racemic acid as described by Betti and Mayer,[10] was converted into the *m*-nitro derivative following the procedure utilized in the racemic series, with the sole exception that barium hydroxide rather than lead carbonate was used for removing sulphate ions. The crude reaction product (25.1 g) was recrystallized, first from water, and then from water on addition of ethanol, to give a preparation with the rotation value $[\alpha]_D^{24}$ −68° (*c* 1.0, 1 N HCl). This preparation, probably containing about 25 % of the enantiomer (*cf.* Table 1), was employed in the subsequent step.

Upon repeated fractional crystallization of a small aliquot of the crude reaction product, it was observed that material of decreasing numerical rotation accumulated in the fractions of lowest solubility. Accordingly, the mother liquors were utilized for the isolation of crystalline fractions with relatively high contents of the D-enantiomer. Fractionation was stopped at a point where a preparation, m.p. 162°, with the rotation $[\alpha]_D^{24}$ −89.5° (*c* 0.82, 1 N HCl) separated, containing less than 30 % of the racemic modification (*cf.* Table 1). A sample, containing about 45 % of the latter ($[\alpha]_D$ −72°), m.p. 162°, was analyzed (Found: C 48.85; H 4.32; N 14.22. Calc. for $C_8H_8N_2O_4$: C 48.96; H 4.11; N 14.28).

m-*Amino*-D-*phenylglycine* (II b). The catalytic hydrogenation was carried out essentially as described above for the racemic isomeride. In the present case, however, the nitro-acid ($[\alpha]_D$ −68°) was reduced in aqueous solution (2 g/325 ml) without addition of hydrochloric acid. The reduction product was dissolved in water, and ethanol was added to give a partly racemic preparation, $[\alpha]_D^{22}$ −60° (*c* 1.0, 1 N HCl), containing about 60 % of the racemic modification (Table 1). On further fractionation of this preparation from aqueous ethanol, the less soluble racemate separated. The mother liquor contents were purified by one additional recrystallization from the same solvent mixture to give the virtually homogeneous D-enantiomer, $[\alpha]_D^{24}$ −128° (*c* 1.0, 1 N HCl), which was employed for the subsequent Sandmeyer reaction.

Three additional recrystallizations of a small aliquot produced an analytical specimen with unchanged rotation in 1 N hydrochloric acid. In aqueous solution (*c* 0.84) the following rotations were measured: $[\alpha]_D^{23}$ −90°; $[\alpha]^{23}_{546}$ −106°; $[\alpha]^{23}_{436}$ −189°. The analytical specimen, m.p. 186°, was dried over calcium chloride at room temperature (Found: C 55.91; H 6.17; N 16.26. Calc. for $C_8H_{10}N_2O_2$, 1/3 H_2O: C 55.79; H 6.24; N 16.28). After drying at 100° over phosphorus pentoxide in an oil pump vacuum, the loss of weight was 3.56 %; calculated for 1/3 mole of water: 3.49 %.

m-*Cyano*-D-*phenylglycine* (II c). The Sandmeyer reaction was performed as described for the racemic series above. After ion exchange on a strongly acid resin, a preparation was obtained with the rotation value $[\alpha]_D^{23}$ −127° (*c* 0.93, 1 N HCl), considered to be the pure or almost pure D-isomeride (Table 1), except for its contents of trace amounts of unchanged *m*-aminophenylglycine, disclosed by paper chromatography. This preparation was used for the last synthetic step described below.

An analytical specimen, yet of much lower rotation, $[\alpha]_D^{23}$ −12°, was produced by preparative paper chromatography of 18 mg of the amino acid on 4 sheets of Whatman paper No. 3 MM, with butanol: ethanol:water (12:3:5) as the solvent system. Elution of the appropriate zones gave a solution, which, after ion exchange, was evaporated to give the homogeneous amino acid. This was recrystallized twice from water:ethanol and dried over calcium chloride at room temperature before analysis, m.p. 170° (decomp.) (Found: C 60.37; H 4.77; N 15.17. Calc. for $C_9H_8N_2O_2$, 1/6 H_2O: C 60.33; H 4.69; N 15.64).

m-*Carboxy*-D-*phenylglycine* (III). The cyano amino acid (316 mg) was suspended in anhydrous methanol (55 ml) and brought into solution by saturating the cooled solution with a stream of dry hydrogen chloride. The well-stoppered flask was kept at room temperature for 12 days, when the mixture was taken to dryness *in vacuo*. The residue was taken up in water (5 ml) and neutralized with 1 N NaOH (1.7 ml). 0.2 N NaOH (45 ml) and ethanol (40 ml) were added, and the mixture was set aside for 18 h. The solution was then neutralized with HCl and ethanol was removed. Paper chromatography demonstrated that complete conversion of the cyano compound had taken place. The aqueous solution was applied to a weakly basic ion exchange resin (Dowex 3, 6 × 100 cm) on the chloride form. After thorough washing with water, whereby a ninhydrin-positive contamination was removed, *m*-carboxyphenylglycine was eluted by passing 1 N HCl through the column. The ninhydrin-positive residue (173 mg) was further purified by passage through a small strongly basic ion exchange column (Dowex 1−8) on the acetate form. After washing with water, the amino acid was eluted with 1 N acetic acid. The acid (98 mg) was recrystallized thrice from water by addition of ethanol and dried over calcium chloride before analysis (Found: C 51.96; H 5.11; N 6.65. Calc. for $C_9H_9NO_4$, 5/6 H_2O: C 51.43; H 5.11; N 6.67). The following rotations were determined in aqueous solution (*c* 0.98): $[\alpha]_D^{23}$ −80°; $[\alpha]^{23}_{546}$ −96°; $[\alpha]^{23}_{436}$ −171°. In aqueous hydrochloric acid (1 N) the rotation was: $[\alpha]_D^{23}$ −115° (*c* 1.3). Comparison with the product obtained on resolution (see below) indicates that the synthetic preparation contains at least 95 % of the D-isomeride.

Resolution experiments

(−)-*N-(Menthoxyacetyl)*-m-*carboxyphenylglycine*. *m*-Carboxy-DL-phenylglycine was treated under Schotten-Baumann conditions with (−)-menthoxyacetyl chloride to give a crude product, which was recrystallized from 50 % ethanol several times, yet without any noticeable fractionation into diastereoisomerides. An analytically pure specimen, m.p. 202°, of unknown steric composition, was readily obtained, $[\alpha]_D^{23}$ −53.5° (Found: C 64.65; H 7.60; N 3.57. Calc. for $C_{21}H_{29}NO_6$: C 64.45; H 7.46; N 3.57). Further attempts to utilize this derivative for the resolution were abandoned.

Resolution with L-*arginine*. *m*-Carboxy-DL-phenylglycine (1.32 g) and L-arginine monohydrochloride (1.18 g) were dissolved in hot water (20 ml). Ethanol (4 ml) was slowly added to the 60° hot solution, which was then allowed to cool and set aside in the ice-box overnight. A crop of crystals (1.20 g), $[\alpha]_D^{25}$ + 34° (*c* 0.9, H_2O), was filtered off and a little ethanol was added to the mother liquor. After being kept at 0° overnight, the solution had deposited a second crop of crystals $[\alpha]_D^{23}$ −10.5° (*c* 0.9, H_2O). Both crystalline fractions were subjected to repeated fractional recrystallizations, yet without preparations of sterical homogeneity were obtained.

Therefore, the mother liquor from the second recrystallization was diluted with more ethanol, and the resulting crystalline material (380 mg), $[\alpha]_D^{22}$ −29° (*c* 1.2, H_2O), was subjected to two additional recrystallizations from aqueous methanol resulting in separation of the pure *salt of* L-*arginine and* (−)-m-*carboxyphenylglycine* (34 mg), m.p. 203° (decomp.), $[\alpha]_D^{26}$ −40.4° (*c* 0.8, H_2O) (Found: C 45.44; H 6.56; N 17.52. Calc. for $C_{15}H_{21}N_5O_5$, 2 1/2 H_2O: C 45.45; H 6.61; N 17.67). Attempts to remove the water of crystallization at 100° over phosphorus pentoxide led to gradual decomposition of the salt.

The aromatic amino acid was liberated from the salt by passage of the latter in aqueous solution through a strongly basic ion exchange resin (Dowex 1−8, 0.8 × 5 cm) in the acetate form. Arginine passed through the column, and the strongly acid, aromatic amino acid was then eluted with 1 N acetic acid. After two recrystallizations from hot water, pure (−)-*m*-carboxyphenylglycine was obtained as colourless crystals (28 mg), $[\alpha]_D^{25}$ −89° (*c* 0.6, H_2O). An analytical specimen was dried at room temperature over calcium chloride (Found: C 53.20; H 5.08; N 7.03. Calc. for $C_9H_9NO_4$, 0.5 H_2O: C 52.93; H 4.94; N 6.86). This sample, recrystallized from pure water, possessed a solid phase infra-red spectrum significantly different from that of the synthetic preparation described above. The latter was recrystallized from aqueous ethanol, and the spectroscopic differences are obviously attributable to the different degrees of solvation, apparent also from the analytical compositions. The solid phase infra-red spectra of the hydrochlorides and triethylammonium salts of the two preparations were identical in all details.

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