

Chemistry of *m*-Carbon-substituted Aromatic Amino Acids

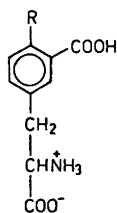
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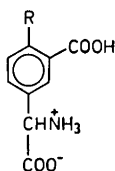
Syntheses are reported for 3-(3-carboxy-4-hydroxyphenyl)-DL-alanine, DL-3'-carboxyphenylglycine, DL-3'-carboxy-4'-hydroxyphenylglycine, 3-(3-hydroxymethylphenyl)-DL-alanine, and 3-(3-aminomethylphenyl)-DL-alanine, all naturally occurring amino acids, and also for 3-(4-amino-3-carboxyphenyl)-DL-alanine, hitherto undescribed but a potential metabolite of chorismic acid. 3-(3-Cyanophenyl)-DL-alanine, DL-3'-carbamidophenylglycine, 3-(3-chloromethylphenyl)-DL-alanine, and 3-(3-carboxy-4-nitrophenyl)-DL-alanine have been isolated as by-products or intermediates. A synthesis is also reported for [2-¹⁴C]3-(3-carboxy-4-hydroxyphenyl)-DL-alanine, and contents of crystal water, p*K*-values, UV-spectra, and *R_F*-values in PC have been determined for the amino acids.

m-Carbon-substituted aromatic acids with the formulas 1*a*, 1*b*, 2*a*, 2*b*, 3*a*, 3*b*, and 4 are occurring in various higher plants.¹⁻⁴ We have studied their distribution and biosynthesis (Refs. 1, 2, and 5 and references cited there). The studies have been used as basis for a coherent representation of all derivations of amino acids from chorismic acid (2-(5-carboxy-1,2-dihydro-2-hydroxyphenoxy)acrylic acid).⁶ These studies have depended on the availability of the amino acids produced by chemical syntheses and in special cases labelled with radioactive isotopes and on knowledge of their chemical and analytical properties. The present paper reports new syntheses for a number of these amino acids and also presents details of their analytical and chemical properties. Furthermore, since the representation of possibilities for transformation of chorismic acid implies 3-(4-amino-3-carboxyphenyl)alanine (5) as a potential chorismic acid metabolite we also report the synthesis and properties of this hitherto undescribed compound.

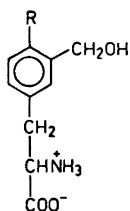
Traditional methods have previously been described for the synthesis of 3-(3-carboxyphenyl)-DL-alanine (1*a*),⁷ 3-(3-carboxy-4-hydroxyphenyl)-L- and -DL-alanine (1*b*)^{8,9} DL-3'-carboxyphenylglycine (2*a*)¹⁰ D-2*a*,¹¹ and DL-3'-carboxy-4'-hydroxyphenylglycine (2*b*).^{12,13} We have now repeated the synthesis of 1*a* and obtained the hitherto undescribed 3-(3-cyanophenyl)-DL-alanine (6) as a byproduct. Furthermore, we have developed a new synthesis of DL-1*b* and ¹⁴C-labelled DL-1*b* by the azlactone method, a new synthesis of DL-2*a* via *m*-cyanobenzaldehyde and 5-(*m*-cyanophenyl)hydantoin, and a new synthesis of DL-2*b* from salicylic acid and benzoylaminoglycolic acid (*cf.* Ref.



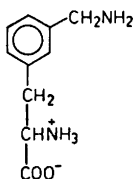
1*a*: R=H
1*b*: R=OH



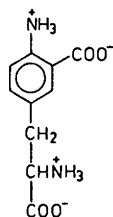
2*a*: R=H
2*b*: R=OH



3*a*: R=H
3*b*: R=OH



4



5

14). During the synthesis of *2a* a small amount of the hitherto undescribed DL-3-carbamido-phenylglycine (*7*) was isolated.

3-(3-Hydroxymethylphenyl)alanine (*3a*) has been isolated from two different sources^{3,15} but synthetic material has not been available. Synthesis of 3-(3-aminomethylphenyl)-DL-alanine (*4*) by catalytic reduction of DL-*6* mentioned above has been reported although without experimental details.⁴ By use of the diethyl acetamidomalonate ester route we have now obtained DL-*3a*, DL-*4* as the dihydrochloride, and 3-(4-amino-3-carboxyphenyl)-DL-alanine (*5*). In the first of these syntheses the hydrochloride of 3-(3-chloromethylphenyl)-DL-alanine (*8*) was isolated as a by-product and characterised, whereas in the last of the syntheses the intermediate 3-(3-carboxy-4-nitrophenyl)-DL-alanine (*9*) was characterised.

For all analytical work with the amino acids a knowledge of molecular weights and contents of crystal water as a function of drying conditions is essential. However, a number of references contain no or conflicting information on these matters. We have therefore collected, verified, and supplemented the available information as described under Experimental. Special attention is called to the behaviour of the dihydrate of DL-*2b* which by drying over P₂O₅ at 50 °C loses the water of crystallization. The anhydrous sample by reequilibration in the atmosphere forms a hemihydrate.¹³ X-Ray powder diagrams, have now shown that this procedure involves a definite change in crystal structure. Also the monohydrate of DL-*1a* after prolonged drying over P₂O₅ at 120 °C does not regain all the water when exposed to the atmosphere, but here X-ray powder diagrams show no differences.

In the experimental section is also given UV-data for all the amino acids in their various ionization forms. The p*K*-values of the amino acids have been determined by microtitrations. The observed values, reported in Experimental, are in good agreement with the protolytic properties of the amino acids as exhibited in ion-exchange and paper chromatography and in paper electrophoresis. R_F-values from paper chromatography are reported under Experimental.

The syntheses reported here and those previously described in the literature have made the

racemates of *1a*, *1b*, *2a*, *2b*, *3a*, 3-(4-hydroxy-3-hydroxymethylphenyl) alanine (*3b*),¹⁶ *4*, and *5* and L-*3b*^{17,18} available. Also convenient methods are available for the resolution of *1b*,¹⁹ *2a*,¹¹ and *2b*¹³ and for the production of some of the amino acids labelled with ¹⁴C. On the other hand methods for the direct synthesis of the optically active amino acids and preferentially methods which could be adopted for microscale synthesis of labelled materials would be desirable. Presently experiments are undertaken here with this aim by two approaches. First it is attempted to use the enzyme β-tyrosinase, which catalyses the formation of tyrosine from phenol and serine and of 3-(3,4-dihydroxyphenyl)alanine from resorcinol and serine,²⁰ for the production of *1b* from salicylic acid and serine and of *5* from anthranilic acid and serine. Secondly the recently described production of *N*-acetyl-3-(3-formyl-4-methoxyphenyl)-L-alanine ethyl ester, from *N*-acetyl-*O*-methyl-L-tyrosine ethyl ester, dichloromethyl methyl ether, and TiCl₄,¹⁸ is used as a basis for the synthesis of L-*1b*, and a similar reaction route is envisaged from 3-(4-aminophenyl)-L-alanine to L-*5*.

EXPERIMENTAL

UV-spectra were measured on a Zeiss DMR-21 instrument at 25 °C. ¹H NMR spectra were measured on a JEOL C-60 HL instrument. Melting points are uncorrected. Optical rotations were determined on a Perkin-Elmer Model 141 polarimeter. Microanalyses were performed by Mr. G. Cornali and his staff. H₂O determinations were made by drying weighed samples under the conditions indicated for each compound. When not otherwise stated the water was regained when the sample was exposed to the atmosphere. p*K*-Values are uncorrected and were determined by microtitrations of solutions in 1 N HCl with 1 N NaOH or of solutions in 0.1 or 1 N NaOH with 0.1 N HCl.

DL-*1a* was prepared as described in the literature⁷ with an overall yield from *m*-cyanobenzylbromide and acetamidomalonic ester (0.33 mol) of 58 %. The final isolation of the amino acid was performed by adsorption to a strongly basic ion-exchange resin (Dowex 1 × 8, 200–400 mesh) in the acetate form with subsequent elution with 1 M AcOH. Anal. (air-dried C₁₀H₁₁NO₄·H₂O: C, H, N, H₂O (P₂O₅, 120 °C, 100 h; only 41 % of the water was regained when the sample was exposed to the atmosphere). Ref. 7: No crystal water. UV [0.1 M HCl (ε)]: 278 (1000), 233 (9000), 209 (6000) nm

(cf. Ref. 21). [phosphate pH 7, 0.05 M]: 272 (560), 226 (7500), 202 (22 000) nm. [0.1 M NaOH]: 274 (960), 217 (11 000) nm. Lit. 275 nm (H₂O).⁷ p*K*-values: 2.4; 4.0; 9.4 (cf. Ref. 7).

3-(3-Cyanophenyl)-DL-alanine, (6), was obtained from the effluent from the ion-exchange resin used in the purification of DL-1a by evaporation and recrystallization from water. Yield 2.53 g (4 %). Found after drying over CaCl₂: C 57.13; H 6.01; N 13.32; H₂O 8.57 (P₂O₅, 50 °C, 11 h). Calc. for C₁₀H₁₀H₂O₂·H₂O: C 57.69; H 5.81; N 13.45; H₂O 8.65. The IR showed a sharp peak at 2220 cm⁻¹ (CN). UV [0.1 M HCl (ε)]: 282 (1050), 274 (1090), 227 (13 000), 202 (18 000) nm. [phosphate pH 7, 0.05 M]: 282 (990), 274 (1050), 227 (12 000), 197 (45 000) nm. [0.1 M NaOH]: 284 (1100), 276 (1200), 228 (11 000) nm. p*K*-values: 2.6; 8.7.

1-1a was isolated from seeds of *Lunaria annua* L.²² Found after air drying: C 49.94; H 6.01; N 5.80; H₂O 12.16 (CaCl₂, 20 °C, 48 h). Calc. for C₁₀H₁₁NO₄·1½H₂O: C 50.85; H 5.97; N 5.93; H₂O 11.44. Ref. 7: No crystal water. Ref. 23: One mol of crystal water.

DL-1b. A mixture of 4-(4-acetyloxy-3-carbomethoxybenzylidene)-2-methyl-2-oxazolin-5-one²⁴ (19 g), HI (19 ml, *d* 1.7), red P (17 g) and acetic anhydride (50 ml) was refluxed for 4 h. After cooling, filtration, and washing with glacial acetic acid the solution was taken to dryness. 4 M HCl (20 ml) was added twice to the residue with subsequent evaporation. The residue was then mixed with ethanol (30 ml) and 4 M HCl (125 ml) and refluxed for 10 h. After removal of the ethanol the solution was decolourised with charcoal and after cooling the crystals of the hydrochloride of 1b were collected. Yield 9.3 g (57 %). The hydrochloride was dissolved in water (100 ml), and pH adjusted to 3 with NaOH. After 24 h. at 0 °C the crystals were collected. Yield 6.8 g (46 %). Anal. (dried over CaCl₂) C₁₀H₁₁NO₅·½H₂O: C, H, N, H₂O (P₂O₅, 50 °C, 60 h). Ref. 9: No crystal water. p*K*-values 2.4; 3.5; 9.5; 12.4 (cf. Ref. 8).

DL-2a. 5-(*m*-Cyanophenyl)hydantoin²⁵ (50 g) was refluxed with 100 g of Ba(OH)₂·8H₂O in 1700 ml of water for 10 days. 700 ml of 6 % (NH₄)₂CO₃ was added and after 15 min of boiling the mixture was filtered. The filtrate was taken to dryness, dissolved in 100 ml of water and applied to a strongly basic ion-exchange resin in the acetate form. After flushing with water the column was eluted with 1 M AcOH. Evaporation and recrystallization from water gave 14.5 g (27 %) of DL-2a·H₂O (identified by IR and PC). Anal. (air-dried) C₈H₇NO₄·H₂O: C, H, N, H₂O (P₂O₅, 100 °C, 16 h). Ref. 10: No crystal water. UV [0.1 M HCl (ε)]: 275 (820), 229 (10 000), 200 (29 000) nm. [phosphate pH 7, 0.05 M]: 272 (490), 200 (26 000) nm. [0.1 M NaOH]: 273 (670), 217 (10 000) nm. Lit. 275 nm (H₂O).²⁶ 275, 228, 205 nm (0.05 M HCl).¹³ p*K*-values: 2.4; 3.8; 9.1.

DL-3'-Carbamidophenylglycine, (7), was obtained from the effluat of the ion-exchange resin used in the purification of DL-2a by evaporation and recrystallization from water. Yield 0.15 g. Found (after drying over P₂O₅): C 54.67; H 5.27; N 14.10. Calc. for C₉H₁₀N₂O₃: C 55.67; H 5.19; N 14.43. IR showed the absence of a nitrile group. UV [0.1 M HCl (ε)]: 271 (560), 200 (30 000) nm. [phosphate pH 7, 0.05 M]: 271 (590), 197 (36 000) nm. [0.1 M NaOH]: 275 (750), 217 (12 000) nm.

N-Benzoyl-DL-3'-carboxy-4'-hydroxyphenylglycine, (10). 23.6 g of benzoylaminoglycolic acid²⁷ was dissolved in 230 ml 20 % H₂SO₄ in glacial acetic acid and mixed with a solution of 16.7 g of salicylic acid in 330 ml of the same solvent. After 18 h at room temperature the reaction mixture was diluted with 5 volumes of water, cooled and filtrated to give 12.3 g (32 %) of material, m.p. 232–235 °C. Two recrystallizations from water-ethanol, m.p. 250–251.5 °C. Anal. (dried over CaCl₂) C₁₆H₁₃NO₆: C, H, N.

DL-2b. 8.4 g of 10 was refluxed with 500 ml of 20 % HCl for 48 h. The solution was extracted with diethyl ether to remove benzoic acid and evaporated. The residue was suspended in 100 ml of water and pH adjusted to 2.3 with ammonia. After cooling and filtration 5.8 g (88 %) of the dihydrate of DL-2b was obtained, identified by IR and PC. p*K*-values: 3.1; 9.2; 12 (p*K*₁ undetermined).

Diethyl (3-cyanobenzyl)acetamidomalonate, (11). A solution of Na (1.92 g), diethyl acetamidomalonate (17.4 g) and *m*-cyanobenzyl bromide (15.7 g) in 130 ml of absolute ethanol was refluxed for 8 h with stirring. After addition of water (1000 ml) and cooling, the crystalline material was collected by filtration. Yield 18 g (68 %), m.p. 132–135 °C. Recrystallization from ethanol-water, m.p. 142–143 °C. Anal. C₁₇H₂₀N₂O₆: C, H, N.

Diethyl (3-aminomethylbenzyl)acetamidomalonate.HCl, (12). 8 g of 11 was hydrogenated at atmospheric pressure and room temperature for 8 h in 96 % ethanol (500 ml) and conc. HCl (6 ml) with Pd/C as catalyst. After filtration the solution was taken to dryness. Water (50 ml) was added to the residue, and unreacted material removed by filtration. The filtrate was again concentrated to dryness, and after addition of acetone (500 ml) and cooling the crystals were collected. Yield 7 g (78 %), m.p. 198–200 °C. Recrystallization from ethanol-acetone, m.p. 200–201 °C. Anal. C₁₇H₂₄N₂O₆·HCl: C, H, N.

Diethyl (3-hydroxymethylbenzyl)acetamidomalonate, (13). A solution of 12 (7.45 g), NaNO₂ (1.75 g) and water (10 ml) was heated for 2 h on a water bath, cooled and extracted with ethyl acetate. The extract was washed with 1 M HCl, water, 5 % NaHCO₃, and water, dried over MgSO₄, and taken to dryness. The solid residue was recrystallized from benzene-light-petroleum (b.p. 100–120 °C). Yield 5.2 g

(77 %), m.p. 78–79 °C. Recrystallization from cyclohexane, m.p. 83–85 °C. Anal. $C_{17}H_{23}NO_6$: C, H, N. For deamination conditions, cf. Ref. 28.

DL-3a. A mixture of 13 (2.7 g), Dowex 50W X 8 (50–100 mesh, H^+) (50 g) and water (50 ml) was refluxed for 18 h with stirring. The resin was transferred to a column and washed with water to neutral reaction. 3a was then eluted with 100 ml 1 M NH_3 , aq. The eluate was evaporated and the residue recrystallized from water. Colourless crystals of 3a were obtained. Yield 1.03 g (66 %), m.p. 215–217 °C (decomp.) Anal. (dried over $CaCl_2$ at 50 °C) $C_{10}H_{13}NO_3$: C, H, N. UV [0.1 M HCl (ϵ): 260 (280), 201 (9500) nm. [H_2O]: 260 (280), 193 (41 000) nm. [0.1 M NaOH]: 271 (225), 261 (350), 215 (7800). Lit. 262 nm (without indication of solvent).³ pK-values: 2.5; 9.2.

3-(3-Chloromethylphenyl)-DL-alanine.HCl, (DL-8). 12 (3.7 g) in water (50 ml) and conc. H_2SO_4 (0.4 ml) was mixed at 0 °C with a solution of $NaNO_2$ (4 g) in water (40 ml). Dilute H_2SO_4 was added dropwise until N_2 evolution started. After 2 h at room temperature (pH 4) the reaction mixture was extracted with ethyl acetate. The extract was washed with water, 10 % $NaHSO_3$, and water, dried over $MgSO_4$, and taken to dryness. The residue was refluxed with 20 % HCl (100 ml) for 2 h. After cooling the crystals of 8 were collected. PC of the mother liquor demonstrated the presence of 3a. Recrystallization of the hydrochloride from ethanol produced an analytical sample. Yield 1.2 g (48 %), m.p. 208–214 °C (decomp). Anal. $C_{10}H_{12}ClNO_2.HCl$: C, H, N, Cl. UV [0.1 M HCl (ϵ): 262 (250), 202 (14 000) nm. [phosphate pH 7, 0.05 M]: 262 (270), 202 (13 000) nm. [0.1 M NaOH]: 263 (310), 215 (7800) nm. pK-values: 2.5; 9.3.

DL-4.2HCl.H₂O. 12 (2 g) in 8 M HCl (30 ml) was refluxed in a N_2 atmosphere for 3 h. The solution was then taken to dryness, and the residue was evaporated twice with water. The residue was dissolved in water and 4 absorbed on a column of Dowex 50W X 8 (50–100 mesh) in the ammonium form. After washing with water the column was eluted with 1 M NH_3 , aq. The eluate was taken to dryness, and after evaporation twice from water, 2 M HCl was added, and the solution again evaporated to dryness to give a crystalline residue of DL-4.2HCl.H₂O. Yield 1.36 g (89 %). Recrystallization from 96 % ethanol, m.p. 153–155 °C (decomp). Anal. $C_{10}H_{14}N_2O_2.2HCl.H_2O$: C, H, N, Cl. UV [0.1 M HCl (ϵ): 270 (160), 260 (250), 208 (9600) nm. [phosphate pH 7, 0.05 M]: 270 (180), 260 (260), 208 (9800) nm. [0.1 M NaOH]: 271 (170), 262 (260), 215 (8000) nm. pK-values: 2.3; 8.7; 9.9.

3-Carboxy-4-nitrobenzylbromide, (14). Br_2 (8 ml) was added dropwise over a period of 4 h to a boiling solution of 5-methyl-2-nitrobenzoic acid (18.1 g) in $CHBr_3$ (100 ml). The reaction mixture was refluxed for additional 6 h and the $CHBr_3$ removed by distillation. The re-

maining oil was dissolved in CCl_4 (300 ml) and the solution decolourised with charcoal. After cooling the crystals were collected. Yield 17.4 g of impure material. The crystals were extracted with boiling cyclohexane to remove unreacted starting material and afterwards recrystallized several times from CCl_4 to give an analytical sample, m.p. 104–106 °C. Anal. $C_8H_8BrNO_4$: C, H, N. 1H NMR (60 MHz, $DMSO-d_6$): δ 4.52 (2 H, s, CH_2), 7.2–7.8 (3 H, m, aromatic protons).

Diethyl (3-carboxy-4-nitrobenzyl)acetamidomalonate, (15). To a solution of Na (2.3 g) in absolute ethanol (300 ml) was added diethyl acetamidomalonate (11 g). After stirring for 30 min at 60 °C, 14 (13 g) was added. The reaction mixture was refluxed for 12 h, evaporated to dryness, and the residue dissolved in water. pH was adjusted to 1 with HCl, and the solution extracted with $CHCl_3$. The extract was washed with water and extracted with a saturated $NaHCO_3$ -solution. The extract was acidified with HCl, and after cooling the crystals were collected. Two recrystallizations from ethanol produced an analytical sample. Yield 7.65 g (39 %), m.p. 209–211 °C. Anal. $C_{17}H_{26}N_2O_8$: C, H, N.

3-(3-Carboxy-4-nitrophenyl)-DL-alanine, (9). 15 (3.96 g) in 10 M HCl (25 ml), water (10 ml) and ethanol (20 ml) was refluxed with stirring under N_2 for 8 h. The ethanol was then removed, 10 M HCl (25 ml) was added, and reflux continued for 10 h. After filtration and cooling overnight the crystals of the hydrochloride were collected. Yield 2.3 g (79 %), m.p. 180–215 °C. The crude hydrochloride was dried at 60 °C and boiled with water (50 ml) for 2 h. The hot solution was filtered and ethanol (50 ml) was added. The crystalline precipitate of 9 was isolated. Yield 1.9 g (75 %). Recrystallization from water, m.p. 260 °C (decomp). Anal. $C_{10}H_{10}N_2O_6$: C, H, N. UV [0.1 M HCl (ϵ): 270 (7000), 201 (18 000) nm. [phosphate pH 7, 0.05 M]: 275 (7000), 202 (13 000) nm. [0.1 M NaOH]: 282 (8000), 214 (11 000) nm. pK-values: 1.8; 2.5; 8.7.

DL-5. 9 (314 mg) was hydrogenated at atmospheric pressure in 0.5 M AcOH (50 ml) with PtO_2 (40 mg) for 2 h at 50 °C. After filtration the solution was taken to dryness. The residue was dissolved in water and 5 absorbed to a column of Dowex 1 \times 8 (200–400 mesh) in the acetate form. After washing with water, 5 was eluted with 0.5 M AcOH. The eluate was taken to dryness, and recrystallization was performed twice from water-ethanol. Yield 210 mg (76 %), m.p. 275–277 °C (decomp). Anal. $C_{10}H_{13}N_2O_4$: C, H, N. UV [1 M HCl (ϵ): 278 (1100), 227 (8500), 203 (13 000) nm. [phosphate pH 7, 0.05 M]: 314 (2700), 245 (15 000), 211 (27 000) nm. [0.1 M NaOH]: 314 (2800), 243 (sh, 10 000), 214 (27 000) nm. pK-Values: 1.5; 2.1; 4.2; 9.1. pK-Values for *p*-aminophenylalanine: 2.2; 4.3; 9.1.

[2-¹⁴C]DL-*Ib*. ¹⁴C-4-(4-Acetoxy-3-carbomethoxybenzylidene)-2-methyl-2-oxazolin-5-one was prepared as described previously²⁹ from [2-¹⁴C]glycine (250 μCi) via [2-¹⁴C]N-acetylglycine. The azlactone (20 mg) was refluxed with HI (0.035 ml, *d* 1.7), red P (25 mg) and acetic anhydride (0.15 ml) for 4 h. After cooling, filtration, and washing with glacial acetic acid (2 ml), the solution was taken to dryness. 4 M HCl (2 ml) was added to the residue and the solution taken to dryness again. After repetition of this step, 4 M HCl (2 ml) was added, and the solution was refluxed for 10 h, filtered hot, and taken to dryness. Water was added twice with subsequent evaporation, the residue was dissolved in water and applied to a column of Dowex 1×8, 200–400 mesh, in the acetate form. The column was washed with water and 0.5 M AcOH and eluted with 1 M formic acid. The fractions containing *Ib* (found by the fluorescence in UV) were taken to dryness, and water was added twice with subsequent evaporation to give a crystalline residue of *Ib*. Yield 12.8 mg, 7 μCi/mg. 36 % radiochemical yield. The purity was ascertained by PC followed by autoradiography.

Paper chromatography. The following *R_F*-values were determined for the new amino acids by descending chromatography on Whatman Paper No. 1 at 22 °C in (1) butanol-acetic acid-water (12:3:5), and (2) phenol-water-conc. ammonia (120:30:1) (w/v/v):

Amino acid	(1)	(2)
3-(3-Hydroxymethylphenyl)-alanine (3a)	0.48	0.76
3-(3-Aminomethylphenyl)alanine (4)	0.22	0.87
3-(4-Amino-3-carboxyphenyl)-alanine (5)	0.29	0.31
3-(3-Cyanophenyl)alanine (6)	0.53	0.83
3'-Carbamidophenylglycine (7)	0.24	0.64
3-(3-Chloromethylphenyl)alanine (8)	0.73	0.95
3-(3-Carboxy-4-nitrophenyl)-alanine (9)	0.24	0.29

We are indebted to Dr. Karen Kaas for determination of the X-ray powder diagrams.

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