

RESOLUTION OF DL-PENTAMETHYLPHENYLALANINE

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The synthesis of N-acetyl-DL-pentamethylphenylalanine and its resolution with brucine are described. Assignment of the configuration of both antipodes was performed with O.R.D.-measurements and checked by enzymic hydrolysis.

Carrión et al. (1) have synthesized a derivative of phenylalanine in which the phenylring had been permethylated. This amino acid resembles tryptophan in its electron-donor properties. In order to find a possible role of tryptophan as an electron-donor in biologically active peptides, one might consider replacement of the latter by this amino acid as a "functional analogue". A prerequisite to this application is that the analogue be resolved into its antipodes, the configurations of which are known.

MATERIALS AND METHODS

Because the synthesis which is given by Carrión et al. (1) was conducted via an acetamidomalonate, we decided to prepare N-acetyl-pentamethylphenylalanine by partial hydrolysis and decarboxylation of this intermediate and to investigate its resolution by enzymic or chemical methods. The turnover of enzymic hydrolysis with carboxypeptidase A (2) proved impractically slow (0.5 $\mu\text{mol/mg/h}$) for a usable resolution. The enzymic hydrolysis could be used, however, for corroboration of the eventually assigned configuration. For chemical resolution several bases, (-)ephedrine, (+)dexamphetamine, quinine and brucine were attempted. It turned out that only the brucine salts of the acetylated antipodes differed sufficiently in solubility to obtain good resolution.

A favorable feature of the procedure was the independent crystallizability of both diastereomeric salts from different solvent systems once they had been roughly separated. So ethyl acetate/propanol-2 dissolves preferably the salt containing the dextrorotating acid, and methanol the other. Methanol also is an excellent solvent for the mixture of both salts. Thus, the fundamental problem in chemical resolutions concerning the stereochemical purity of the "second, better soluble" fraction did not arise in this case.

Decomposition of both diastereomeric salts was carried out with an excess of hydrochloric acid in propanol-2. When a primary alcohol was used, partial esterification occurred rapidly. The antipodes were isolated in nearly theoretical yield, and their absolute specific rotations were equal within the limits of experimental error.

Hydrolysis of the acetylated antipodes was performed by heating their solutions in acetic acid with concentrated aqueous hydrochloric acid under reflux. In both cases the product crystallized on cooling in the form of its monohydrochloride monohydrate. (On drying in a high vacuum at 80°C over P_2O_5 , efflorescence was observed and the hydrochloride was left. In moist air the hydrate reconstituted itself.) Treatment of its solution in acetic acid with ammonium acetate gave the neutral amino acid.

As the deacetylation procedure was rather drastic, the risk of partial racemization at this

step is not merely imaginary. Extensive racemization could be ruled out by reacetylation of a sample: the rotation for the product appeared to be unaltered.

The resolution was performed twice, with different batches of brucine. The results confirmed each other.

Assignment of the configuration was performed by rotatory dispersion measurements (3). Positive Cotton effects were recorded for dextrorotating N-acetyl-pentamethylphenylalanine as well as for N-acetyl-L-phenylalanine and L-phenylalanine. The (+)-enantiomer was also susceptible to enzymic attack: digestion with carboxypeptidase A, although very slow, resulted in precipitation of the free amino acid after one day of incubation. The optical rotatory dispersion curve of the precipitated amino acid revealed also a positive Cotton effect. The levorotating enantiomer was not digestible with carboxypeptidase A.

From these observations we attribute L-configuration to (+)-acetyl-pentamethylphenylalanine and D-configuration to its enantiomer. As deacetylation did not alter the sign of the rotation, dextrorotatory pentamethylphenylalanine has to be looked upon as belonging to the class of amino acids having "natural" asymmetry.

EXPERIMENTAL PROCEDURES

Melting points were determined with a Heitzsch Microscope (Leitz) and are uncorrected. Specific rotations and O.R.D. curves were measured with a Perkin Elmer 141 and a Jasco O.R.D./U.V.-5 instrument. N.M.R. Spectra were recorded on a Varian HA 100 apparatus and were in accordance with theory.

For thin layer chromatography on silica, pre-coated plates (Kieselgel F₂₅₄, Merck) (TS) were used with the solvent systems A = benzene-acetone (1:1), B = n. butanol-acetic acid-water (10:1:3), C = ethyl acetate-pyridine-acetic acid-water (62:21:6:11) and D = acetic acid-0.1 N hydrochloric acid (4:1). The chromatograms were developed according to Reindel & Hoppe (4) with chlorine and o. tolidine as spray reagents (5).

Pentamethylbenzylchloride

Bis(chloromethyl)mesitylene was synthesized from mesitylene by chloromethylation, according to Shacklett & Smith (6). The compound was recrystallized from acetic acid and was obtained in

78% yield. M.p. 103–105°C. It contained some 6% of tris(chloromethyl)mesitylene.

The product was hydrogenated in acetic acid with palladium on charcoal as a catalyst. On concentration of the filtered solution, some hexamethylbenzene crystallized. Pentamethylbenzene, contaminated with 2 to 3% hexamethylbenzene (N.M.R.), was obtained in 94% yield.

Without further purification, the compound was chloromethylated according to Carrión (1). The product was recrystallized from 90% acetic acid yielding 85% of pure pentamethylbenzylchloride with m.p. 82–84°C.

Analysis:

C₁₂H₁₇Cl Calcd.: %C 73.27 %H 8.71 %Cl 18.02 (196.72) Found: %C 73.55 %H 8.92 %Cl 18.15

Diethyl-(2-pentamethylbenzyl-2-acetamido)malonate

This intermediate was synthesized according to Carrión (1). The product was purified either by recrystallization from 70% acetic acid, giving large rectangular plates, or by treatment with hot methanol-water. The yield was 70%, m.p. 133–135°C, TS:Rf = 0.60, system A.

Analysis:

C₂₁H₃₁NO₅ Calcd.: %C 66.82 %H 8.28 %N 3.71 (377.48) Found: %C 66.68 %H 8.28 %N 3.66

If the reaction mixture contains some water the alkylation of the malonate is not impaired, but the product isolated is N-acetyl-DL-pentamethylphenylalanine ethyl ester, melting at 142–143°C rather than the malonate. This was confirmed by, azeotropic esterification of N-acetyl-DL-pentamethylphenylalanine with ethanol. N.M.R. spectroscopy revealed identity of both compounds. TS:Rf = 0.51, system A.

N-Acetyl-DL-pentamethylphenylalanine

By alkaline hydrolysis and decarboxylation of the foregoing compound, the racemic pentamethylphenylalanine derivative was obtained. Therefore, diethyl-(2-pentamethylbenzyl-2-acetamido)malonate was dissolved in a mixture of dioxane and water (2:1). A solution of 4 N sodium hydroxide (about 20% excess) was added, and the solution refluxed for about 18 h. Dioxane was removed under reduced pressure and the resulting solution extracted with ethyl acetate to remove some 5% of unreacted starting material which was recycled. The aqueous layer was poured into an excess of 6 N hydrochloric acid with stirring, then cooled and filtered. After recrystallization from 90%

acetic acid, about 96% of chromatographically pure product was obtained, m.p. 247–248°C, TS: Rf=0.67, system B; =0.81, system C.

Analysis:

$C_{16}H_{23}NO_3$ Calcd.: %C 69.29 %H 8.36 %N 5.05 (277.36) Found: %C 68.88 %H 8.49 %N 4.99

Resolution of N-acetyl-DL-pentamethylphenylalanine

Because of the limited solubility in the solvent system used for the resolution, 65.0 g = 234.3 mmol of N-acetyl-DL-pentamethylphenylalanine and 92.3 g = 234.3 mmol of brucine were dissolved in 650 ml of warm methanol. The solvent was removed by evaporation, and evaporation was repeated after the addition of some propanol-2 to eliminate residual methanol. The residue containing the diastereomeric brucine salts was dissolved in 300 ml of warm propanol-2, the same volume of ethyl acetate was added and the solution stored in a refrigerator for 7–8 d. After filtration and washing of the precipitate (I) with ethyl acetate, the combined filtrates were evaporated to dryness, the residue taken up in ethyl acetate and the solvent evaporated again. To remove the last traces of propanol-2 this procedure was repeated. The residue was dissolved in some 300 ml of warm ethyl acetate, the solution cooled and kept at about 0°C for 1 week. A small amount of precipitate (II) was filtered and the filtrate evaporated to dryness. Upon dissolution of the residue in 300 ml of warm methanol immediate crystallization occurred upon cooling, giving fraction III.

Fractions I and III were recrystallized from ethyl acetate/propanol-2 (1:1) and methanol, respectively.

| Fraction | Yield | M.p. | $[\alpha]_D^{24}$ (c=1, MeOH) |
|----------|--------------|-----------|----------------------------------|
| I | 69.2 g=43.9% | 216–218°C | -16.0°±0.5° |
| II | 2.5 g= 1.6% | 130–240°C | -2.4°±0.5° |
| III | 57.8 g=36.7% | 129–134°C | -6.2°±0.5° |

N-Acetyl-L-pentamethylphenylalanine

In 200 ml of propanol-2 were dissolved 54.84 g of the brucine salt III (80.3 mmol). Fifty ml 4 N hydrochloric acid (200 mmol) were added with stirring followed by ca. 1300 ml of water. After cooling, the precipitate was filtered, washed with dilute hydrochloric acid and dried.

Yield: 21.05 g = 94.5% of a chromatographically pure product (TS: Rf=0.80, system C). M.p.: 246–248°C. (The crystals melted at about 222°C, but then suddenly gave long thin needles, which melted again at 246–248°C).

$[\alpha]_D^{21} = 15.8 \pm 0.5^\circ$ (c=1, MeOH); $[\alpha]_{578} = 16.7^\circ$, $[\alpha]_{546} = 19.3^\circ$, $[\alpha]_{436} = 38.0^\circ$, $[\alpha]_{365} = 74.2^\circ$.

Analysis:

$C_{16}H_{23}NO_3$ Calcd.: %C 69.29 %H 8.36 %N 5.05 (277.36) Found: %C 69.24 %H 8.47 %N 5.00

N-Acetyl-D-pentamethylphenylalanine was obtained from its brucine salt (I) in a similar manner in 88% yield. M.p.: 242–245°C. (The crystals melted at about 218°C, giving long thin needles which melted again at 242–245°C).

$[\alpha]_D^{21} = -16.0^\circ \pm 0.5^\circ$ (c=0.98, MeOH); $[\alpha]_{578} = -16.5^\circ$, $[\alpha]_{546} = -19.4^\circ$, $[\alpha]_{436} = -38.1^\circ$, $[\alpha]_{365} = -74.4^\circ$.

Analysis: Found: %C 69.29 %H 8.54 %N 5.01

L-Pentamethylphenylalanine monohydrochloride monohydrate

The N-acetyl compound (20.03 g = 72.2 mmol) was dissolved in 200 ml of concentrated aqueous hydrochloric acid and 200 ml of glacial acetic acid, and the solution refluxed for 5 h. When heating was discontinued crystallization started at once, and 20.37 g = 97.5% of H-L-Phe(Me)₅-OH.HCl.H₂O could be isolated. TS: Rf=0.50, system D. M.p.: 256.5–258°C.

$[\alpha]_D^{21} = 55.2^\circ \pm 0.5^\circ$ (c=0.59, HOAc + 0.1 N HCl (4:1)); $[\alpha]_{578} = 57.2^\circ$, $[\alpha]_{546} = 65.8^\circ$, $[\alpha]_{436} = 120.9^\circ$, $[\alpha]_{365} = 211.8^\circ$.

Analysis: $C_{14}H_{24}NO_3Cl$ (289.80)

Calcd.: %C 58.02 %H 8.35 %N 4.83 %Cl 12.23 Found: %C 57.83 %H 8.34 %N 4.73 %Cl 12.36

The same procedure was used to get the D-isomer, m.p. 254.5–256°C. $[\alpha]_D^{21} = -54.9 \pm 0.5^\circ$ (c=0.64, HOAc + 0.1 N HCl (4:1)); $[\alpha]_{578} = -57.0^\circ$, $[\alpha]_{546} = -65.7^\circ$, $[\alpha]_{436} = -120.3^\circ$, $[\alpha]_{365} = -211.3^\circ$.

Analysis: Found:

%C 57.88 %H 8.34 %N 4.74 %Cl 12.42

L-Pentamethylphenylalanine

The neutral amino acid was obtained from its hydrochloride upon dissolution in hot acetic acid (100%), addition of an excess of ammonium acetate, and dilution with water. Consistent analytical data were obtained after thorough drying (80°C in high vacuum for about 6 h). M.p.: 242–

245°C, $[\alpha]_D^{21} = 65.7 \pm 0.5^\circ$ (c=0.46, HOAc + 0.1 N HCl (4:1)); $[\alpha]_{578} = 68.8^\circ$, $[\alpha]_{546} = 78.5^\circ$, $[\alpha]_{436} = 144.7^\circ$, $[\alpha]_{365} = 254.4^\circ$.

Analysis:

$C_{14}H_{21}NO_2$ Calcd.: %C 71.46 %H 9.00 %N 5.95 (235.33) Found: %C 71.51 %H 9.06 %N 5.90

The same procedure was used to get after drying the D-isomer, m.p. 243–246°C. $[\alpha]_D^{21} = -65.4 \pm 0.5^\circ$ (c=0.47, HOAc + 0.1 N HCl (4:1)); $[\alpha]_{578} = -68.3^\circ$, $[\alpha]_{546} = -78.1^\circ$, $[\alpha]_{436} = -144.0^\circ$, $[\alpha]_{365} = -254.3^\circ$.

Analysis: Found: %C 71.17 %H 8.96 %N 5.82

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