RESOLUTION OF RACEMIC α-AMINO ACIDS*

II. ACIDIC α-AMINO ACIDS

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The successful application of phthaloylamino acids to synthetic peptide procedures was announced first by Kidd and King[1] in 1948 and independently by Sheehan and Frank[2] a year later. The preparation of the phthaloylamino acids, employed in such procedures, was achieved generally through the direct fusion of a mixture of phthalic anhydride and the free amino acid.[3–5] For some amino acids good yields are obtained by these methods, but when the optically active glutamic and aspartic acids are used, considerable racemization occurs.[6] This made Kidd and King[7] and Balenovic[8] look for alternative routes to the optically active phthaloyl derivatives of these amino acids and very tedious alternative syntheses for optically pure phthaloyl glutamic and aspartic acids were devised. Finally, Nefkens[9] showed that *N*-carbethoxyphthalimide has excellent properties for the phthaloylation of amino acids under mild conditions; but yields of phthaloyl-L-glutamic acid were only about 60%.

The need to use optically active glutamic and aspartic acids in the fusion has now been overcome by an efficient method of resolving their racemic phthaloyl derivatives. We have found that the metal complex cation *cis*-dinitrobis(ethylenediamine)cobalt(III) ion[10] can be used for the resolution of racemic phthaloylglutamic and aspartic acids. By procedures analogous to those described previously,[11,12] well-defined diastereoisomeric salts of the type *l*-$[Co(en)_2(NO_2)_2]^+_2$D-[Phthaloyl A]$^=$ (en = ethylenediamine, $A^=$ = glutamate or aspartate) are obtained from aqueous or aqueous alcoholic solutions. The resolved phthaloyl derivatives have outstanding optical purity after a single recrystallization of the diastereoisomer; tritium tracer experiments[13] showed that only 0·1% of the other antipode was present. Removal of the protecting group with hydrazine gave the optically pure amino acids.

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[1] Kidd, D. A. A., and King, F. E. (1948).—*Nature* **162**: 776.

[2] Sheehan, J. C., and Frank, V. S. (1949).—*J. Amer. Chem. Soc.* **71**: 1856.

[3] Reese, L. (1887).—*Liebigs Ann.* **242**: 1.

[4] Fling, M., Minard, F. N., and Fox, S. W. (1947).—*J. Amer. Chem. Soc.* **69**: 2466.

[5] Billman, J. H., and Hartung, W. F. (1948).—*J. Amer. Chem. Soc.* **70**: 1473.

[6] Tipson, R. S. (1956).—*J. Org. Chem.* **21**: 1353.

[7] King, F. E., and Kidd, D. A. A. (1949).—*J. Chem. Soc.* **1949**: 3315.

[8] Balenovic, K., Gaspert, B., and Stimac, N. (1957).—*Croat. Chem. Acta* **29**: 93.

[9] Nefkens, G. H. L., Tesser, G. I., and Nivard, R. J. F. (1960).—*Rec. Trav. Chim. Pays-Bas* **79**: 688.

[10] Dwyer, F. P., and Garvan, F. L. (1960).—*Inorg. Synth.* **6**: 195.

[11] Dwyer, F. P., and Halpern, B. (1962).—*Nature* **196**: 270. (Aust. Pat. Appl. No. 16,783/62 (Apr. 18); No. 20,694/62 (Aug. 6).

[12] Dwyer, F. P., Halpern, B., and Turnbull, K. R. (1963).—*Aust. J. Chem.* **16**: 510.

[13] Garnett, J., Halpern, B., Law, S. W., and Turnbull, K. R., unpublished data.

Experimental

*Resolution of Phthaloyl-*DL*-glutamic Acid.*—A suspension of phthaloyl-DL-glutamic acid (5·54 g) prepared according to the direction of Kidd and King,[7] in water (15 ml), was exactly neutralized with $NaHCO_3$ (3·36 g) and the resulting solution added to a solution of *d-cis*-dinitrobis(ethylenediamine)cobalt(III) acetate (prepared from *d-cis*-dinitrobis(ethylenediamine)-cobalt(III) iodide (9 g) and silver acetate (3·73 g) in water (25 ml)).[10] After filtration from a small insoluble residue, ethanol (130 ml) was added to the filtrate, and the diastereoisomeric salt *d-cis*-dinitrobis(ethylenediamine)cobalt(III)-phthaloyl-L-glutamate [A] was collected, dried, and recrystallized by dissolving in warm water (40 ml) and reprecipitating with ethanol (130 ml) [6·5 g, 80%] α_D^{20} +26°, 1% in H_2O. (Found: C, 30·1; H, 5·5; N, 21·5%. Calc. for $C_{21}H_{41}O_{14}N_{13}Co_2.H_2O$ requires C, 30·2; H, 5·2; N, 21·8%).

The filtrates from the above were combined [B] and set aside for recovery of partly resolved phthaloyl-D-glutamic acid. The diastereoisomer [A] (6·5 g) was added in small quantities to a solution of KI (5 g) in water (20 ml), the suspension was stirred at room temperature for 15 min and the *d-cis*-dinitrobis(ethylenediamine)cobalt(III) iodide (4 g) was collected. The filtrate was acidified with 6N HCl and the phthaloyl-L-glutamic acid extracted several times with ethyl acetate. Evaporation of the ethyl acetate extracts followed by recrystallization from a little water yielded phthaloyl-L-glutamic acid, m.p. 158–159°C, α_D^{20} −45·4°, 1% in ethanol. (Tipson[6] reports −42·6° 1% in alcohol.)

The combined filtrates (B) were evaporated to dryness *in vacuo*, redissolved in water (30 ml) and KI (5 g) added. After removal of the precipitated *d*-iodide the filtrate was acidified with 6N HCl and the partially resolved phthaloyl-D-glutamic acid was extracted into ethyl acetate. Evaporation of the extract yielded 2·7 g. The product was again resolved with *l-cis*-dinitrobis(ethylenediamine)cobalt(III) acetate using the same procedure as described above. After recrystallization from water optically pure phthaloyl-D-glutamic acid (2 g), α_D^{20} +45·4°, 1% in ethanol, was obtained.

*Resolution of Phthaloyl-*DL*-aspartic Acid.*—Phthaloyl-DL aspartic acid (6·1 g) was suspended in water (40 ml) and $NaHCO_3$ (4 g) was added to give a neutral solution, which was added to a solution of *l-cis*-dinitrobis(ethylenediamine)cobalt(III) acetate (ex 9·6 g of *l*-iodide) in water (30 ml). The solution was concentrated to 40 ml *in vacuo* and the diastereoisomer *l-cis*-dinitrobis(ethylene-diamine)cobalt(III)-phthaloyl-D-aspartate was filtered and recrystallized from water; α_D^{20} −18°, 1% in water. (Found: C, 28·8; H, 5·1; N, 22·0%. Calc. for $C_{20}H_{39}O_{14}N_{13}Co_2.H_2O$: C, 29·2; H, 5·0; N, 22·1%).

After decomposition of the diastereoisomer with KI, optically pure phthaloyl-D-aspartic acid, α_D^{20} 66° + 1%, in methanol, was obtained. Balenovic[8] reports α_D^{17} −58°, 0·4% in methanol, for phthaloyl-L-aspartic acid.

Recovery of D*-Glutamic Acid from Phthaloyl-*D*-glutamic Acid.*—The phthaloyl derivative (1 g) was suspended in water (5 ml) and the pH of the mixture adjusted to pH 6·5 by the addition of Na_2CO_3 (anhyd.) (0·4 g). Hydrazine hydrate (1 g; 80%) was then added and the solution left at room temperature for 2 days. The reaction mixture was then acidified with HI to a pH of 3·5, the precipitated phthalhydrazide was filtered off, the filtrate was conc. *in vacuo* and the D-glutamic acid was precipitated by the addition of alcohol. After recrystallization from aqueous ethanol optically pure D-glutamic acid was obtained; (0·4 g) α_D^2 −17·2°, 4% in water. (Greenstein[14] reports α_D +17·7° in water for L-glutamic acid.)

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[14] Greenstein, J. P., and Winitz, M. (1961).—"The Chemistry of the Amino Acids." Vol. 3. p. 1929. (Wiley: New York.)