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SYNTHESIS OF D,L-β-CARBOXYASPARTIC ACID FROM HYDANTOIN-5-MALONIC ACID DIETHYL ESTER

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Abstract—Hydantoin-5-malonic acid diethyl ester was synthesized by reduction of parabanic acid (oxalyl urea) to 5-hydroxy-hydantoin, conversion to 5-chlorohydantoin and condensation with malonic ester. Alkaline hydrolysis gave D,L-β-carboxyaspartic acid.

The occurrence of aminomalonic acid in certain peptides of marine origin[1] and the increasingly important role of γ-carboxyglutamic acid in calcium binding proteins[2,3] has prompted us to seek a synthesis of the analogous carboxylated amino acid, β-carboxyaspartic acid. While the natural occurrence of β-carboxyaspartic acid has not as yet been reported, it appears to be an interesting compound whose synthesis and characterization we have undertaken. Patchornik[4] while at this laboratory, showed the formation of putative β-carboxyaspartic acid in trace levels in the alkaline hydrolysate of a synthetic poly-α-aspartic acid, which had been treated at low temperature in tetrahydrofuran with n-butyl-lithium and gaseous carbon dioxide. This acidic product isolated by ion exchange chromatography was shown to decarboxylate to aspartic acid under the conditions of 6N HCl heating at 100°.

In an unambiguous synthesis of D,L-β-carboxyaspartic acid, we have utilized as the intermediate, 5-chlorohydantoin (III), which has both the desired blocked amino and carboxyl groups, which are alkaline labile, and the necessary alkylating function attached to the α C atom. Alkylation of III with malonic ester afforded hydantoin-5-malonic acid diethyl ester (IV) which although obtained in relatively low yield, required only alkaline hydrolysis to generate D,L-β-carboxyaspartic acid. The reaction scheme is represented in Fig. 1.

EXPERIMENTAL

Thionyl chloride (Fisher Chemical Co.) was redistilled at atmospheric pressure. Parabanic acid was purchased from Sigma Chemical Co.; other chemicals were of reagent quality.

M.ps were determined on a Fisher–Johns apparatus and are uncorrected. Mass spectra low resolution were recorded on a Hitachi RMU-6E Mass Spectrometer. High resolution mass spectrum performed by Shrader Analytical Labs, Detroit, Michigan. Amino acid analysis was carried out with a Beckman 121M amino acid analyzer. The cation exchange column (2.8 × 330 mm) was operated at 51°, and eluted with 0.2 M citrate buffer (0.16 M Na^+) at pH 2.57 (until 4 min after injection) followed by a similar buffer at pH 3.10 as previously described.[6] Details of the analytical determination of β-carboxyaspartic acid are contained in another paper.[7] The ninhydrin color yield for β-carboxyaspartic acid is 78% that of aspartic acid.[7]

5-Hydroxyhydantion (II). 5-Hydroxyhydantoin was prepared by the procedure of Abblard and Meynaud.[5] However, it was found that recrystallization of the crude 5-hydroxyhydantoin from AcOH–n-PrOH, afforded a product of greater purity. The 5-hydroxyhydantoin obtained by this modified procedure has a m.p. of 157–158° (lit m.p. 140–142°[5]). (Found: C, 31.00; H, 3.50; N, 24.16. Calc. for $C_3H_4O_3N_2$: C, 31.03; H, 3.47; N, 24.12%). MS, M^+, *m/e* (relative intensity) 116 (20%), 99 (90%), 98 (55%), 88 (95%), 73 (100%).

5-Chlorohydantoin (III). In the procedure of Abblard and Meynaud,[5] difficulty was experienced in handling the 5-chlorohydantoin and in eliminating the excess thionyl chloride from the product. Instead, the following modification was employed:

Protected from moisture with a drying tube, 0.81 g (7 mmoles)

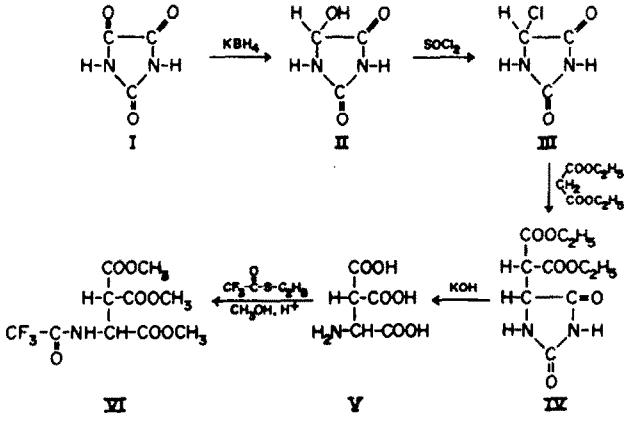

Fig. 1. Scheme for synthesis of β-carboxyaspartic acid (V). Derivation by trifluoroacetylation and esterification in acidic methanol produced (VI) for mass spectrometric proof of structure.

of II and 1.5 ml redistilled $SOCl_2$ (21 mmoles) were refluxed in 30 ml $CHCl_3$ for 4 hr. After cooling, the solvent and excess $SOCl_2$ were removed in a rotary evaporator by repeated addition of benzene and evaporation (3 times). The residue analyzed by mass spectrometry showed molecular ions at *m/e* 136 (7%) and 134 (20%), also ions at *m/e* 108 (3%), 106 (9%), 99 (20%), 98 (100%), 93 (10%) and 91 (30%).

Hydantoin-5-malonic acid diethyl ester (IV). Without purification, the residue of 5-chlorohydantoin in 20 ml DMF was warmed to 50°, excluding moisture with a drying tube; sodio malonic acid diethyl ester (7.7 mmoles in 20 ml DMF was added dropwise over 0.5 hr. The mixture was then left at 60–70° for 2 hr. The yield of IV ranged from 6 to 10% of theoretical based on quantitative amino acid analysis of β-carboxyaspartic acid in aliquots of the mixture after alkaline hydrolysis. After cooling, 200 ml water was added and the soln extracted with 3 × 5 ml portions EtOAc. The EtOAc layer was washed twice with water and dried over Na_2SO_4. After removal of solvent an oil remained which partially crystallized on standing in a refrigerator for 2 days. The oil was recrystallized from EtOH–cyclohexane to give 0.09 g (5%) crystaline IV, (m.p. 117°). (Found: C, 46.43; H, 5.46; N, 10.83. Calc. for $C_{10}H_{14}O_6N_2$: C, 46.51; H, 5.46: N, 10.85%). MS, M^+, *m/e* (relative intensity) 258 (10%), 213 (15%), 212 (9%), 185 (76%), 160 (5%), 139 (100%), 115 (27%).

Alkaline hydrolysis of hydantoin-5-malonic acid diethyl ester (IV) *to* D,L-*β-carboxyaspartic acid* (V). 2.8 mg (10 μmoles) of IV were hydrolyzed[6] using 1 ml 2M KOH at 100° for 24 hr. After addition of 0.3 ml sat $KHCO_3$ aq the soln was neutralized on ice to pH 9 with conc HCl. The stock soln of V was stored at −20°. Amino acid analysis of 10 nmoles of V (Fig. 2a) shows a prominent β-carboxyaspartic acid peak at 16.6 min, with a trace of aspartic acid at 43.0 min. Figure 2(b) indicates that the $t_{1/2}$ for decarboxylation of β-carboxyaspartic acid is 1.7 min at 100° in 1 M HCl,[7] and quantitative decarboxylation to aspartic acid is achieved within 30 min (Fig. 2c). At the 51° of the ion-exchange column, the $t_{1/2}$ for decarboxylation in 1 M HCl is 6 hr.[7] When run on the amino acid analyzer before alkaline hydrolysis, IV contained no ninhydrin-positive substances.

As a standard for hydantoin hydrolysis, hydantoin-5-acetic acid ethyl ester m.p. 91–93°, MS, M^+, *m/e* (relative intensity), 186 (21%), 141 (33%), 140 (34%), 113 (25%), 112 (100%), 99 (44%) was prepared from hydantoin-5-acetic acid (Aldrich) by esterification with ethanolic HCl. Before alkaline hydrolysis, this compound contained no ninhydrin positive substances on the amino acid analyzer. After alkaline hydrolysis under conditions identical to those used for IV above, quantitative conversion to aspartic acid was determined on the amino acid analyzer.

Characterization of D,L-*β-carboxyaspartic acid as the* N-*trifluoracetyl, trimethyl ester derivative.* After adjusting the alkaline hydrolysate of 2.8 mg of IV to pH 9, 0.03 ml (200 μmoles) ethyl trifluorothioacetate was added and the soln stirred at room temp for 1 hr. Water and volatile substances were removed by rotary evaporation several times with MeOH to dryness. The dried residue was cooled in an icebath while 2 ml of an icecold sat soln of HCl in MeOH was added. The mixture was allowed to come to room temp and stirred overnight while protected from moisture. The solvent and HCl were removed in a stream of N_2 5 ml MeOAc and filtered. The MeOAc soln was reduced in volume to 1 ml and used directly for mass spectroscopy.

Low Resolution MS, M^+, *m/e* (relative intensity), 315 (3%), 296 (2%), 284 (3%), 283 (4%), 256 (27%), 212 (8%), 202 (8%), 171 (100%). High resolution MS.

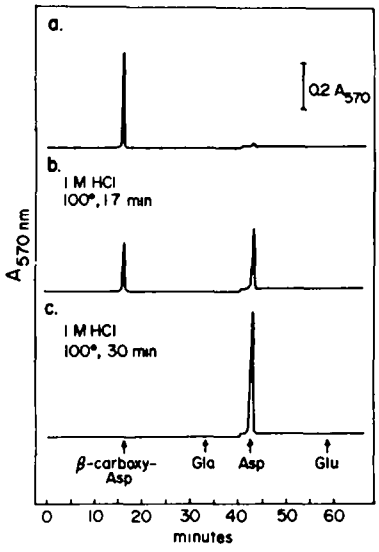

Fig. 2. Ninhydrin profile of D,L-β-carboxyaspartic acid after ion-exchange chromatography on an amino acid analyzer. (a) Authentic β-carboxyaspartic acid (10 nmoles) elutes 16.6 min after injection; the column front is 13.0 min, based on elution of cysteic acid, and the buffer change effect occurs 2 min before the aspartic acid peak at 43.0 min. Elution positions of glutamic acid (Glu, 59.0 min) and γ-carboxyglutamic acid (Gla, 33.0 min) are indicated for reference. (b) Profile of 10 nmoles β-carboxyaspartic acid after 1.7 min heating in 1 M HCl at 100°, showing 50% decarboxylation; the ninhydrin color factor for aspartic acid is 1.28 times treater than for β-carboxyaspartic acid.[7] (c) Profile of 10 nmoles β-carboxyaspartic acid after quantitative decarboxylation to aspartic acid by 30 min at 100° in 1 M HCl.

By contrast, the low resolution mass spectrum of trifluoroacetyl-l-aspartic acid dimethyl ester (British Drug House, Ltd.) showed a molecular ion at *m/e* 257, and the absence of any measurable mass at *m/e* 256:

M^+, *m/e* (relative intensity) 257 (2%), 226 (5%), 225 (4%), 198 (100%), 166 (42%), 144 (5%).

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Ion	ORIGIN	COMPOSITION	OBS. MASS	CALC. MASS
315	M^+	$C_{10}H_{12}O_7NF_3$	NOT SEEN	
256	M^+-59	$C_8H_9O_5NF_3$	256.0425	256.0420
212	M^+-(59+44)	$C_7H_9O_3NF_3$	212.0524	212.0515
202	M^+-113	$C_8H_{10}O_6$	202.0490	202.0503
171	M^+-(113+31)	$C_7H_7O_5$	171.0298	171.0292