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166. L(+)- and D(−)-γ-Carboxyglutamic acid. Absolute Configuration, Properties and Synthesis by Resolution of DL-N-Benzyloxycarbonyl-γ-carboxyglutamic acid γ, γ′-di-*t*-butylester

Preliminary communication[1])

dedicated to the memory of our late friend, *Beat Iselin*

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(9. VI. 76)

The optical properties of natural γ-carboxyglutamic acid have not yet been described. It can be assumed that this amino-tricarboxylic acid belongs to the L-series, because it is produced biosynthetically through carboxylation of protein L-glutamic acid residues [1]. We have recently completed a synthesis of DL-γ-carboxyglutamic acid and of crystalline derivatives that could be useful for preparing pep-

[1]) Part of the thesis of *W.M.* We thank the *Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung* for financial aid. A detailed account is in preparation for Helv. chim. acta.

tides [2]. In this report, we wish to present a preliminary account of the resolution of DL-N-benzyloxycarbonyl-γ-carboxyglutamic acid γ, γ'-di-*t*-butylester [Z·Gla (O-*t*Bu)$_2$·OH], the determination of the absolute configuration of the enantiomers, and the optical properties of L(+)- and D(–)-γ-carboxyglutamic acid (L- and D-Gla).

From an equimolar solution of DL-Z·Gla(O*t*Bu)$_2$·OH and quinine in ethyl acetate, the *laevo*-enantiomer of the amino-acid derivative crystallized as the diastereomeric salt: needles, m.p. 131–135°, – 72.4° (c = 1, $CHCl_3$)[2]). From the mother liquor, the *dextro*-enantiomer was obtained partially pure by removal of the quinine by the strongly acidic ion exchanger Amberlyst A15. Crystallization from carbon tetrachloride and pentane gave pure (+)–Z·Gla (O-*t*Bu)$_2$·OH : m.p. 84–86°, + 11.9° (c = 1.2, $CHCl_3$) [(–) – Z·Gla (O-*t*Bu)$_2$·OH : m.p. 86–88°, – 11.3° (c = 1.1, $CHCl_3$)]. Removal of the benzyloxycarbonyl group (Z–) by catalytic hydrogenation gave (+) – H·Gla (O-*t*Bu)$_2$·OH : + 5.5° [(–) – H·Gla (O-*t*Bu)$_2$·OH : – 5.7°], both (c = 1, MeOH). The *t*-butylester groups were removed by dissolution in cold conc. hydrochloric acid and immediate evaporation *in vacuo*. (+)-Gla, HCl : + 34.6° (c = 1.2, 6N HCl) [(–) – Gla, HCl : – 37.5° (c = 1, 6N HCl)].

The absolute configuration was derived from a comparison of the optical rotations of the (electrophoretically pure) glutamic acid hydrochloride obtained by standard hydrolysis[3]) of L(–)– Z·Glu(O*t*Bu)·OH [3] (+ 20.6 ± 0.2°; optical purity assumed to be 100%), (+) – Z·Gla (O-*t*Bu)$_2$·OH (+ 20.4 ± 0.2°; calculated optical purity 99%), and (–) – Z·Gla (O-*t*Bu)$_2$·OH (– 20.5 ± 0.2°; calculated optical purity 99–100%). It follows that (+) – Z·Gla (O-*t*Bu)$_2$·OH, (+) – H·Gla (O-*t*Bu)$_2$·OH, and (+) – Gla, HCl have the L-configuration at the α-carbon atom (this was also shown by asymmetric synthesis of (+) – Gla[4]).

NMR. investigations revealed the (slow) exchangeability of the γ-proton against a deuteron in D_2O, and the restricted rotation of the side chain (inequivalence of the two β-protons).

Gla is the most acidic natural amino-acid: it emerges first from an analytical column (at about 20–25 min), and its I.P. (electrophoresis) lies between 2 and 2.5, comparable to that of nitrilo-triacetic acid.

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[2]) Optical rotations $[\alpha]_D^{20}$. All elemental analyses as expected (± 0.3%).
[3]) The starting materials were weighed, hydrolysed *in vacuo* with 6N HCl at 110° for 3 h, the solvent evaporated over solid KOH and P_2O_5, and the residue dissolved in water to c = 1 for rotation experiments without purification or further handling.
[4]) To be published by *M. Oppliger & R. Schwyzer* in Helv. Chim. Acta.