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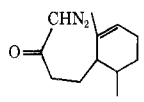

i


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(13) Melting points and boiling points are uncorrected. Infrared spectra were taken on a Perkin-Elmer Model 457 infrared spectrophotometer. Ultraviolet spectra were taken on a Beckman DBGT recording spectrophotometer using 1-cm matched quartz cells. NMR spectra were determined at 60 MHz with a Varian T-60 spectrometer. Signals are reported in parts per million (δ) downfield from internal tetramethylsilane. Mass spectra were obtained using a Hitachi Perkin-Elmer RNU-7 or a Varian M-66 spectrometer. Microanalyses were obtained by Atlantic Microlab, Inc., Atlanta, Ga. Gas–liquid chromatography was carried out using a Perkin-Elmer 881 or an Aerograph A-90-P3 gas chromatograph. The following columns were used: A (6 ft × 0.125 in., 20% Carbowax K-20M on Chromosorb W); B (10 ft × 0.25 in., 20% Carbowax K-20M on Chromosorb W).


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# Amino Acids and Peptides. 44. Synthesis of DL-γ-Carboxyglutamic Acid, a New Amino Acid


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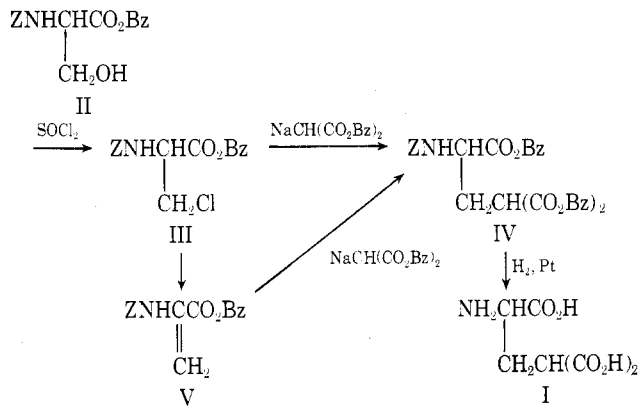
Vitamin K dependent blood clotting proteins, such as prothrombin and factor X, have been found to contain a novel amino acid, L-γ-carboxyglutamic acid (I),[1-3] which is implicated in the formation of calcium binding sites.[4,5] The initial formulation relied exclusively on spectral data, so a synthesis seemed desirable, both to confirm the structure and to obtain a larger quantity for other biochemical activities.

Benzyl $N^{\alpha}$-benzyloxycarbonyl-L-serinate (II)[6] was refluxed with thionyl chloride in benzene to give benzyl $N^{\alpha}$-benzyloxycarbonyl-3-chloro-L-alaninate (III). Addition of chloride III to a solution of monosodium dibenzyl malonate in tetrahydrofuran then afforded tribenzyl 3-benzyloxycarbonylamino-DL-1,1,3-propanetricarboxylate (IV). Ester IV was optically inactive, which suggested that its mode of formation must have involved a racemization step. To clarify this situation, a solution of chloride III on stirring with sodium hydride formed benzyl $N^{\alpha}$-benzyloxycarbonyl-2-methyleneglycinate (V). The readdition of monosodium dibenzyl malonate to the unsaturated ester V went smoothly and furnished the condensation product IV. Thus, the actual conversion of III to IV goes by way of a β-elimination sequence, which was first reported to occur on alkaline treatment of (di-*O*-phenylphospho)serine derivatives.[7] Hydrogenation of compound IV in methanol afforded DL acid I and a comparison with a natural specimen showed common chromatographic behavior.[8] A tentative $pK_a$ value indicates that it is the most acidic natural amino acid.




While this work was in progress (or at an end), five other approaches to this amino acid appeared in the literature. The first procedure began with DL-serine and proceeded via the same intermediates described here, but the report did not provide any experimental details.[9] The second route involved the preparation of methyl $N^{\alpha}$-benzyloxycarbonyl-3-iodo-L-alaninate or methyl $N^{\alpha}$-benzyloxycarbonyl-*O*-tosyl-L-serinate, followed by condensation with di-*tert*-butyl malonate. The resulting ester was subjected to sequential deprotection involving hydrogen, base, and acid treatment. Methyl $N^{\alpha}$-benzyloxycarbonyl-2-methyleneglycinate was identified, but only as a by-product of the alkylation step. A low rotation was tabulated for the condensation ester, but this observation must be erroneous, as the final amino acid had no rotation.[10] One of the intermediates in this synthesis has now been crystallized[11] and used in a resolution.[12] The third path started with the tosylate mentioned above and several DL derivatives of blocked I were generated, but there was no report of the free amino acid.[13] The fourth preparation used a condensation between ethyl $N^{\alpha}$-acetyl-2-methyleneglycinate and diethyl malonate to yield triethyl DL-acetamidopropane-1,1,3-tricarboxylate. Alkaline hydrolysis, desalting, and treatment with ammonium hydroxide formed the monoammonium salt of the DL amino acid I.[14] The last scheme required the synthesis of benzyl-di-*tert*-butyl $N^{\alpha}$-butyloxycarbonyl-DL-γ-carboxyglutamate, which after a two-step removal of the protecting groups was changed into the trifluoroacetate salt of I.[15]

In summary, the synthesis described here is the simplest and most direct for the preparation of free DL-γ-carboxyglutamic acid.

## Experimental Section[16]

**Benzyl $N^{\alpha}$-Benzyloxycarbonyl-3-chloro-L-alaninate (III).** A mixture of magnesium oxide (0.160 g, 4 mmol) and benzyl $N^{\alpha}$-benzyloxycarbonyl-L-serinate (0.624 g, 2 mmol) in benzene (20 ml) was treated with thionyl chloride (0.440 g, 4 mmol) and refluxed for 3 h. The solvent was removed in vacuo, the residue was extracted with hot benzene (3 × 10 ml), and the pooled organic phase was washed with water and dried ($Na_2SO_4$). Evaporation of the benzene gave the desired product, which was crystallized from ethanol–water: mp 90–92 °C (0.232 g, 33%); $R_f$ 0.26; $\nu_{max}$ 3410 (NH), 3045, 3030 (aromatic CH), 2960 (aliphatic CH), 1740, 1715 (CO), 1640 (C=C), and 697 $cm^{-1}$; $[\alpha]^{21.0}D$ −20.0° (*c* 1.0); $\delta_{Me_4Si}$ 7.45 (aromatic), 5.75 (NH), 5.25 ($CH_2C_6H_5$), 5.20 ($OCH_2C_6H_5$), 4.80 (CH), and 3.90 ($CH_2Cl$), $J$ = 2 Hz. Anal. Calcd for $C_{18}H_{18}ClNO_4$ (347.79): C, 62.16; H, 5.22; Cl, 10.20; N, 4.03. Found: C, 62.17; H, 5.22; Cl, 10.68; N, 3.94.

**Tribenzyl 3-Benzyloxycarbonylamino-DL-1,1,3-propanetricarboxylate (IV).** A suspension of sodium hydride (50% in mineral oil, 0.149 g, 31 mmol) in tetrahydrofuran (15 ml) under a nitrogen atmosphere was stirred with dibenzyl malonate (0.966 ml, 34 mmol) for 5 min, after which the now cloudy solution was diluted with more tetrahydrofuran (30 ml) and agitated for 2 h. A solution of the

aforementioned chloroalanine (0.982 g, 29 mmol) in tetrahydrofuran (30 ml) was added and the mixture was stirred for 2 days. Water (5 ml) was injected, the solvent was removed in vacuo, and the residue was redissolved in hot benzene (3 × 10 ml). The pooled organic phase was washed with water, dried ($Na_2SO_4$), and evaporated to dryness. On TLC analysis, the crude product was shown to consist of five components: a trace of oil at the solvent front, benzyl $N^{\alpha}$-benzyloxycarbonyl-L-alaninate ($R_f$ 0.90), the desired ester ($R_f$ 0.58), unreacted dibenzyl malonate ($R_f$ 0.40), and monosodium dibenzyl malonate ($R_f$ 0.04). Chromatography over silica gel using benzene gave a pure fraction (0.326 g), as well as another cut contaminated with some dibenzyl malonate (0.438 g): $\nu_{max}$ 3540 (NH), 3065, 3040 (aromatic CH), 2960 (aliphatic CH), 1800, 1740 (CO), and 697 $cm^{-1}$; $\delta Me_4Si$ 7.35 (aromatic), 5.90 (NH), 5.2 ($CH_2C_6H_5$ and $OCH_2C_6H_5$), and 4.80 (CH); $[\alpha]^{20.0}D$ 0.0° (c 1); mass spectrum m/e 595.2148 ($C_{35}H_{33}NO_8$, parent ion), 460 (loss of $C_6H_5CH_2OCO$), 396 (loss of $C_6H_5CH_2OH$), 108 ($C_6H_5CH_2OH$), and 91 ($C_6H_7$).

**Benzyl $N^{\alpha}$-Benzyloxycarbonyl-2-methyleneglycinate (V).** A solution of benzyl $N^{\alpha}$-benzyloxycarbonyl-3-chloro-L-alaninate (0.180 g, 5.1 mmol) in tetrahydrofuran (3 ml) was added to a suspension of sodium hydride (50% in mineral oil, 0.031 g, 6.0 mmol) in tetrahydrofuran (5 ml) and stirred for 3 h. The solvent was removed in vacuo and the residue was redissolved in benzene (10 ml). The organic phase was washed with water, dried ($Na_2SO_4$), and evaporated to leave an oily residue. Chromatography of a benzene solution over a silica gel column gave the pure product as an oil (0.082 g, 52%): $R_f$ 0.38; $\nu_{max}$ 3420 (NH), 3045, 3030 (aromatic CH), 2960 (aliphatic CH), 1740, 1720 (CO), 1635 (C=C), and 697 $cm^{-1}$; $\delta_{Me_4Si}$ 7.3 (aromatic), 6.20 (=CH), 5.75 (=CH), J = 1 Hz, 5.09 ($CH_2C_6H_5$), and 5.02 ($OCH_2C_6H_5$); $\nu_{max}$ 245 nm (ε 6600); mass spectrum m/e 311.1278 ($C_{18}H_{17}NO_4$, parent ion), 220 (loss of $C_6H_5CH_2$), 176 (loss of $C_6H_5CH_2OCO$), 107 ($C_6H_5CH_2O$), and 91 ($C_6H_7$).

**DL-γ-Carboxyglutamic Acid (I). A. From Hydrogenolysis of Tribenzyl 3-Benzyloxycarbonylamino-DL-1,1,3-propanetricarboxylate.** A solution of the aforementioned tribenzyl ester (0.260 g, 0.9 mmol) was dissolved in methanol (50 ml), 10% palladium on charcoal catalyst (0.060 g) was added, and hydrogen was bubbled through the suspension for 2.5 h at room temperature. At this time the reaction was judged complete both by monitoring the rate of precipitation of $BaCO_3$ and the disappearance of starting ester by TLC analysis. The filtered solution was evaporated to leave a clear residue, which on lyophilization afforded a white powder (0.060 g, 72%): mp 90–92 °C, followed by evolution of a gas at 114 °C; ninhydrin positive; $[\alpha]^{22.5}D$ 0.0° (c 1); no observable chiroptical property with 2-methoxy-2,4-diphenyl-3(2H)-furanone.[17] Chromatography of an aqueous solution of the synthetic amino acid at pH 3.25 on a Dow X-50 column gave the same retention time as that of authentic natural γ-carboxyglutamic acid. The latter compound was obtained by the hydrolysis of prothrombin. This value was different from that observed for glutamic acid or alanine. Hydrolysis of a synthetic sample with 6 N HCl for 4 h at 110 °C formed glutamic acid, identical in all aspects with DL-glutamic acid. The thiohydantoin derivative possessed the same $R_f$ values as observed for natural γ-carboxyglutamic acid thiohydantoin.

**B. From the Reaction of Benzyl $N^{\alpha}$-Benzyloxycarbonyl-2-methyleneglycinate with Dibenzyl Malonate.** A suspension of sodium hydride (50% in mineral oil, 0.014 g) in tetrahydrofuran (5 ml) under a nitrogen atmosphere was stirred with dibenzyl malonate (0.072 g) for 5 min, then the methylene ester V (0.062 g) was added in tetrahydrofuran (3 ml). After 2 h at room temperature, the solution was refluxed for an additional 2 h. The reaction was treated as previously described to yield DL-γ-carboxyglutamic acid (0.096 g), identical with the previous sample.

**Acknowledgment.** We thank both L. H. Ericsson and D. L. Enfield, Department of Biochemistry, University of Washington, for calling the problem to our attention and for the amino acid analysis, plus other technical help. The National Science Foundation supported this work through an Undergraduate Research Participation Program (EPP 75-04525).

**Registry No.**—I, 56271-99-9; II, 21209-51-8; III, 55822-82-7; IV, 60064-83-7; V, 59524-07-1; dibenzyl malonate, 15014-25-2.

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# Preparation and Grignard Reactions of 2-Benzoyl-4,4-dimethyl-2-oxazoline

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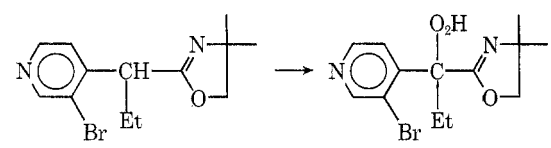
The autoxidation of organic compounds containing acidic carbon–hydrogen bonds is a well-known reaction.[1] For example, Gersmann and Bickel report that high yields of α-hydroperoxy esters are formed when oxygen is bubbled through cold solutions of esters in the presence of a base.[2] However, with methyl phenylacetate, the expected hydroperoxy ester was reported as a minor product, apparently undergoing further reaction to yield as the major product the α-keto ester, methyl phenylglyoxylate, along with some methyl mandelate. Gersmann and Bickel demonstrated that for ketones and nitriles the α positions are also susceptible to autoxidation and suggested that the reaction should be general for other compounds containing similarly activated acidic carbon–hydrogen bonds.

The extensive work of Meyers and co-workers with 2-alkyl-2-oxazolines has demonstrated the utility of these compounds for the protection and synthesis of carboxylic acid derivatives.[3] Since the protons adjacent to the ring in the 2-alkyl substituent are activated by the oxazoline, it seemed likely that these compounds, like those studied by Gersmann and Bickel, might be susceptible to autoxidation. Indeed, oxidation of 1 has been reported to give 2 in high yield.[4] It was hoped that autoxidation of appropriate 2-alkyl-2-oxazolines might provide a useful route to the previously unreported 2-acyl-2-oxazolines which would have potential utility as synthetic reagents.

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