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Short Communications

Studies on Histidyl Peptides

II. The Synthesis of L-Histidyl-L-Leucine and its Optical Homogeneity

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The potentialities of the new procedure [1] for the incorporation of an histidine residue into synthetic peptides have been demonstrated during the synthesis of several histidine and histidyl peptides [2]. The latter were obtained through condensation of carbobenzoxy-*im*.benzyl-L-histidine (I) with amino acid esters for which purpose the carbodiimide method [3] for the peptide bond formation was employed. Owing to the slight solubility of (I) in various organic solvents and especially in cold, its condensation was accomplished in dimethylformamide solution although at a higher temperature than that customarily used. This alteration naturally raises speculations regarding the optical homogeneity of peptides thus produced. It has also been reported recently from two different laboratories [4,5] that, as a condensing agent, carbodiimide yields partly racemized peptides in certain cases. Among the factors which influence racemization, it has been found that temperature and solvents predominate.

Consequently, it was considered desirable to reveal whether racemization takes place while (I) is being coupled with amino acid esters under the experimental conditions previously described [2], and if possible, to eliminate such a risk by carrying out the reaction at a lower temperature.

When (I) was coupled with L-leucine benzyl ester in the same way as previously reported [2], the product, carbobenzoxy-*im*. benzyl-L-histidyl-L-leucine benzyl ester (IIa) showed an $[\alpha]^{22}D$ value of $-13.4°$ (*c* 3.6 in glacial acetic acid).

Alternatively, the same product was synthesized by mixing (I) with the equivalent amount of triethylamine in dimethylformamide or methylene chloride, thus causing the carbobenzoxy derivative to dissolve, followed by addition of the equivalent amount of L-leucine benzyl ester *p*-toluenesulfonate [6] and carbodiimide. The above modification not only facilitates the condensation of (I) at room temperature but more-over, produces better yields. Carbobenzoxy-*im*.benzyl-L-histidyl-L-leucine benzyl ester (IIb) was thus obtained at a rate of 65 % as against 50 % yield produced by the previously described procedure [2].

It is signifficant that compounds (IIa) and (IIb) showed identical optical rotations and melting points.

This evidence, however, does by no means exclude the possibility that compounds (IIa) and (IIb) have suffered racemization to the same extent during their syntheses. Carbobenzoxy-*im*.benzyl-L-histidyl-L-leucine benzyl ester, prepared by either of the two above mentioned processes, was therefore converted into free peptide L-histidyl-L-leucine, and the optical value of the latter was compared to that reported by Holley and Sondheimer [7]. These authors synthesized L-histidyl-L-leucine by he azide procedure which, up to the present, has been believed to cause no racemization.

Carbobenzoxy-*im*.benzyl-L-histidyl-L-leucine benzyl ester was hydrogenated in the presence of palladium black or reduced by sodium in liquid amonnia [8], to produce L-histidyl-L-leucine.

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The obtained peptide, L-histidyl-L-leucine, showed and $[\alpha]^{22}D$ value of 45.2° as a 1 % solution in 0.10 N sodium hydroxide, practically identical with the reported $[\alpha]^{22}D$ value of 43.5° as a 1 % solution in 0.10 N sodium hydroxide. This finding emphasizes the capability of the new procedure [1] in producing optically active histidyl peptides.

EXPERIMENTAL

Carbobenzoxy-im.*benzyl*-L-*histidyl*-L-*leucine benzyl ester. A. Modified procedure.* To a suspension of 1.89 (0.005 mole) of carbobenzoxy-*im*.benzyl-L-histidine in 30 ml of dimethylformamide, 0.5 g (0.005 mole) of triethylamine was added. This caused the carbobenzoxy derivative to dissolve almost completely. A solution of 1.89 g (0.005 mole) of L-leucine benzyl ester *p*-toluenesulfonate in 10 ml dimethylformamide was then added, followed by the addition of 1.1 g of carbodiimide. The mixture was stirred overnight at room temperature, and di*cyclo*hexylurea was subsequently removed by filtration. The solution was treated in the manner described below and the peptide derivative thus obtained at 65 % yield, melted at 112—114° and showed an optical rotation of $[\alpha]^{22}D$ —13.8° (*c*, 3.5 in glacial acetic acid). (Found: C 70.2; H 6.80; N 9.80. Calc. for $C_{34}H_{38}O_5N_4$: C 70.08; H 6.57; N 9.61.) B. Carbobenzoxy-*im*.benzyl-L-histidine (0.005 mole) was treated with L-leucine benzyl ester (0.005 mole) and 1.1 g of carbodiimide as previously described [2]. Di*cyclo*hexylurea was filtered and the solution diluted with 500 ml of water. The precipitating product was recrystallized by dissolving in ethylacetate followed by addition of petroleum ether. Yield 1.4 g (50 %), needles, m. p. 112—114°, $[\alpha]^{22}D$ —13.4° (*c*, 3.6 in glacial acetic acid).

Carbobenzoxy - im.*benzyl* - L - *histidyl* - im.*benzyl* - L - *histidine benzyl ester.* Carbobenzoxy-*im*.benzyl-L-histidine (1.89 g) was dissolved in 30 ml of methylene chloride by addition of 1.01 g of triethylamine. To this solution 3.4 g of *im*.benzyl-L-histidine benzyl ester di-*p*-toluenesulfonate dissolved in 5 ml of methylene chloride and 1.1 g of di*cyclo*hexylcarbodiimide were then added. After 12 h the di*cyclo*hexylurea was filtered and the solution washed with water. It was then dried over Na_2SO_4 and the solvent evaporated *in vacuo*. The residue was crystallized by addition of ether. It was recrystallized from ethylacetate-petroleum ether. Yield 2.2 g (65 %), small needles, m. p. 137°, (reported [2] 137°), $[\alpha]^{22}D$ —23.3° (*c*, 4.5 in glacial acetic acid), reported $[\alpha]^{22}D$ —22.3° (*c*, 5.3 in glacial acetic acid).

L-*Histidyl*-L-*leucine. By catalytic hydrogenation.* A solution of 1.48 g of carbobenzoxy-*im*.benzyl-L-histidyl-L-leucine benzyl ester in 25 ml of dimethylformamide-water (1:1) was hydrogenated in the presence of 200 mg of palladium black. Half an h later a thick mass was formed and then an equal portion of 50 % ethanol followed by 500 mg of palladium on charcoal were added. Since no more CO_2 was evolved, the hydrogenation continued in a closed apparatus for 24 h. At that time all the precipitate was redissolved. The catalyst was filtered, washed with 50 % ethanol and the combined filtrates evaporated *in vacuo*. The product was recrystallized in accordance with the directions of Holley and Sondheimer [7]. Yield 300 mg (70 %), m. p. 220—222° (reported [7] 214—217°), $[\alpha]^{22}D$ —45.2° (*c*, 1 in 0.10 N NaOH), reported [7] $[\alpha]^{22}D$ —43.5° (*c*, 1 in 0.10 N NaOH).

im.*Benzyl* - L - *histidine benzyl ester di-p-toluenesulfonate.* This compound was prepared in the same way as described for the corresponding dibenzenesulfonate derivative [2]. The product was obtained in 80 % yield when recrystallized by dissolving in *iso*propyl alcohol and precipitating by addition of ether, m. p. 176—177°. (Found: N 6.05. Calc. for $C_{34}H_{39}O_8N_3S_2$: N 6.16.)

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