L-Tyrosyl-L-phenylalanine

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Received February 22, 1960

The dipeptides derived from L-tyrosine and L-phenylalanine have been mentioned in the literature from time to time, mainly in connection with enzymatic hydrolysis studies.[1,2] In particular, L-tyrosyl-L-phenylalanine and various derivatives are referred to, but in only a few cases have any of these compounds been obtained pure and characterized. The synthesis of the pure dipeptide and several of its derivatives by conventional methods is described here.

EXPERIMENTAL

N,O-Biscarbobenzoxy-L-tyrosine. There are considerable differences in the reported properties of this derivative.

Treatment of L-tyrosine in the normal way with excess carbobenzoxy chloride and alkali gave a noncrystalline white powder, m.p. 85–86°; $[\alpha]_D^{22}$ + 3.7° (*c* 10, acetic acid). Pannemann, Marx, and Arens[3] give m.p. 115–121° (indefinite); $[\alpha]_D^{24°}$ +4.5° (*c* 10, acetic acid), while Katchalski and Sela[4] report colorless needles, m.p. 117°; $[\alpha]_D^{20°}$ −5° (*c* 10, acetic acid).

N,O-Biscarbobenzoxy-L-tyrosyl-L-phenylalanine. An ice cold solution of 3.0 g. of *N,O*-biscarbobenzoxy-L-tyrosine and 0.7 g. of triethylamine in 14 ml. of tetrahydrofuran was treated dropwise with 0.9 g. of isobutyl chlorocarbonate. After stirring for 5 min. a solution of 1.1 g. of L-phenylalanine in 6.4 ml of *N* NaOH was added in one lot. The mixture was stirred for 30 min., and acidified. The oil which separated soon solidified and was recrystallized from ethyl acetate–hexane, yield 3.1 g. (78%) m.p. 179–181°. Recrystallization from ethanol raised the m.p. to 182–184°· $[\alpha]_D^{21°}$ +6.1° (*c* 1, acetic acid).

Anal. Calcd. for $C_{34}H_{32}O_8N_2$: C, 68.4; H, 5.4; N, 4.7. Found: C, 68.6; H, 5.6; N, 4.7.

N-Carbobenzoxy-L-tyrosyl-L-phenylalanine. A dried ethereal solution of the azide obtained in the usual way[5] from 6.2 g. of *N*-carbobenzoxy-L-tyrosyl hydrazide was mixed with an ether solution of ethyl L-phenylalanate obtained from 4.3 g. of the hydrochloride. The mixed solution was kept overnight at 0° and the precipitated crude ethyl *N*-carbobenzoxy-L-tyrosyl-L-phenylalanate filtered and washed with ether. The yield of amorphous solid was 5.0 g. (55%).

Treatment of 2.0 g. of this ester with 9.2 ml. of *N* sodium hydroxide and 5 ml. of dioxane, followed by acidification, gave *N*-carbobenzoxy-L-tyrosyl-L-phenylalanine, which was recrystallized from aqueous ethanol. Yield, 1.7 g. (90%), m.p. 175–176°; $[\alpha]_D^{22°}$ +2.1° (*c* 1, *N* sodium hydroxide).

Anal. Calcd. for $C_{26}H_{26}O_6N_2$: C, 67.5; H, 5.6; N, 6.0. Found: C, 67.6· H, 5.8; N, 6.0.

The compound was also obtained in low yield (11%; m.p. 174.5–175.5°) by a mixed anhydride coupling of *N*-carbobenzoxy-L-tyrosine with L-phenylalanine using isobutyl chlorocarbonate.

L-Tyrosyl-L-phenylalanine. A solution of 3.4 g. of *N,O*-biscarbobenzoxy-L-tyrosyl-L-phenylalanine in 30 ml. of 4*M* hydrogen bromide in glacial acetic acid was warmed to 70° for 10 min., cooled and diluted with ether. The oily precipitate was dissolved in water and the solution neutralized to litmus with aqueous potassium carbonate. On addition of ethanol the dipeptide separated slowly as colorless needles of the monohydrate, yield 0.8 g. (42%), m.p. 308–310° dec.; $[\alpha]_D^{21°}$ +17.7° (*c* 0.5, 2*N* hydrochloric acid). The water of hydration was very firmly bound.

Anal. Calcd. for $C_{18}H_{22}O_5N_2$: C, 62.3; H, 6.4; N, 8.1. Found: C, 62.4; H, 6.5; N, 8.3.

The dipeptide was also obtained by treating *N*-carbobenzoxy-L-tyrosyl-L-phenylalanine with hydrogen bromide in acetic acid at room temperature. Recrystallized from water, m.p. 310–312° dec.; $[\alpha]_D^{22.5°}$ +17.2° (*c* 0.267, 2*N* hydrochloric acid).

N-Formyl-L-tyrosyl-L-phenylalanine. The ethyl ester[6] was obtained in very poor yield by the *N,N'*-dicyclohexylcarbodiimide[7] coupling of *N*-formyl-L-tyrosine and ethyl L-phenylalanate. It was repeatedly recrystallized from aqueous ethanol, m.p. 174–175°; $[\alpha]_D^{21°}$ +2.4° (*c* 1, ethanol). Treatment of 50 mg. of the ester with 0.4 ml. of *N* sodium hydroxide, followed by acidification gave 40.6 mg. (88%) of the product, which formed colorless needles from aqueous ethanol, m.p. 247–248° dec.

Anal. Calcd. for $C_{19}H_{20}O_5N_2$: N, 7.9. Found: N, 7.8.

This derivative was also obtained by warming 100 mg. of L-tyrosyl-L-phenylalanine with 0.6 ml. of a 2:1 mixture of 90% formic acid and acetic anhydride. It was recrystallized from aqueous ethanol, m.p. 246–247° (alone and mixed with above product), yield 35 mg. (30%).

Acknowledgment. The author is indebted to Dr. V. du Vigneaud in whose laboratory at Cornell University Medical College, New York, this work was partly carried out, and to Mr. J. Albert for the microanalyses.

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An Unusual Transfer Reaction in the Steroid Series

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Received February 26, 1960

The unusual reactivity of the ketolic system, 3α-acetoxy-17α-hydroxy-17β-methyl-D-homo-5β-androstane-11,17a-dione (I), has been referred to recently.[1] The reaction of this substance with methanesulfonyl chloride in pyridine at 0° affords, in addition to the normal mesylate (II),[1a] an isomeric substance, m.p. 142–144° (25–30%), exhibiting strong hydroxyl absorption in the infrared.

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[1] (a) N. L. Wendler, D. Taub, S. Dobriner, and D. K. Fukushima, *J. Am. Chem. Soc.*, **78**, 5027 (1956) Footnote 14. See also (b) N. L. Wendler and D. Taub, *J. Org. Chem.*, **23**, 953 (1958).