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SYNTHESIS OF N-[1-BENZYLOXYCARBONYL-2-(R,S)-ETHOXYCARBONYL-4-PYRROLIDINYL]ALANYLPROLINE

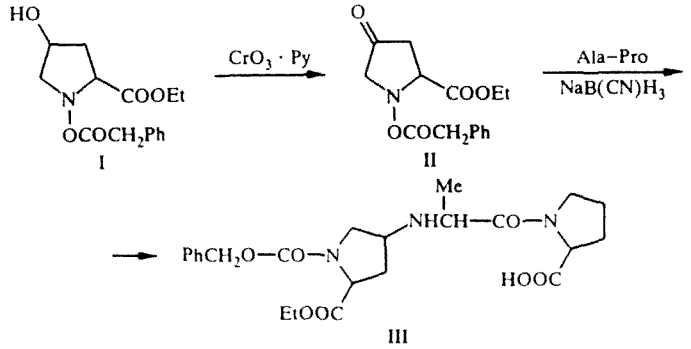
M. Yu. Katkevich, Dz. É. Sile, É. Kh. Korchagova, V. A. Slavinskaya, and É. Lukevits

A method has been developed for the synthesis of the angiotensin-converting enzyme inhibitor N-[1-benzyloxycarbonyl-2-(R,S)-ethoxycarbonyl-4-pyrrolidinyl]-alanylproline by the condensation of N-benzyloxy-carbonyl-4-ketoproline ethyl ester with alanylproline. The intermediate Schiff's base was reduced with $NaB(CN)H_3$. The yield of the corresponding maleate was 5.4%.

Inhibitors of the enzyme converting angiotensin or kininase II are highly effective preparations for the treatment of arterial hypertonia and other diseases of the cardiovascular system [1]. Inhibitors of the enzyme containing no sulfur, e.g., enalapril, lisinopril, and their analogs, have the widest use.

The model for the interaction of enzyme and inhibitor proposed by Cushman and Ondetti [2] is the basis for the synthesis of inhibitors of angiotensin-converting enzyme of a peptide nature. It has also been shown that an important role in the design of active and stable enzyme inhibitors is played by the replacement of the amide bond by other forms of nonhydrolyzing bond. In addition it has proved very fruitful to replace the proline residue in the enalapril molecule by a residue of an unnatural amino acid, and to vary the amino acid sequence, and the N-terminus of the inhibitor.

We considered it expedient to vary the N-terminus of the inhibitor molecule by introducing into it a residue of N-benzyloxycarbonylpyrrolidine. Both the ethoxycarbonyl group and the pyrrolidine ring may therefore participate in complex-formation with the zinc ion of angiotensin-converting enzyme due to the planar disposition of the pyrrolidine ring on the surface of the enzyme. For this purpose we have condensed N-benzyloxycarbonyl-4-ketoproline ethyl ester with alanylproline according to the following scheme.



Oxidation of N-benzyloxycarbonyl-4-hydroxyproline ethyl ester was carried out by the method of [4]. In addition oxidation of compound (I) was also carried out with chromium oxide applied on Al_2O_3. However, in this case complete conversion of the raw material was not achieved and the target product proved to be contaminated with a significant quantity of side products.

Condensation of N-benzyloxycarbonyl-4-ketoproline with the Ala-Pro dipeptide was effected in the presence of calcined molecular sieve in absolute ethanol solution. The reducing agent used was $NaB(CN)H_3$. Product (III) was isolated chromatographically.

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EXPERIMENTAL

The cyclohexylamine (CHA) salt of N-Cbz-hydroxyproline used in the investigations was from Reanal, Hungary. Esterification of N-Cbz-hydroxyproline (after removing cyclohexylamine) was effected with absolute ethanol in the presence of thionyl chloride by the known method of [3]. The purity of compound (I) was 98%.

The ethyl ester of N-Cbz-ketoproline (II) was synthesized by the method of [4]. The content of N-Cbz-hydroxyproline, of N-Cbz-4-ketoproline ethyl ester, and also the purity of the desired product, were determined by HPLC on a Dupont 850 chromatograph. The column used (4.6 × 100 mm) was packed with Silasorb C_{18} reversed-phase sorbent. The eluent for N-Cbz-hydroxyproline and its ethyl ester was a system consisting of 15% CH_3CN and 85% 0.2 M $AcONH_4$, and for N-Cbz-4-ketoproline ethyl ester 40% CH_3CN and 60% 0.1 M pH 2.5 phosphate buffer was used. Detection was at 220 nm. The PMR spectra were taken on a Bruker AM 360 instrument in DMSO solution using TMS as internal standard.

Synthesis of N-[1-Benzyloxycarbonyl-2-(R,S)-ethoxycarbonyl-4-pyrrolidinyl]alanylproline. Powdered calcined molecular sieve 3A (1.6 g) was added to a solution of Ala-Pro·HCl (0.22 g: 1 mmole) and Cbz-Ketopro-OEt (II) (1.17 g: 4 mmole) in absolute ethanol (3.5 ml). After 1 h, $NaB(CN)H_3$ (0.06 g: 1 mmole) in absolute ethanol (1 ml) was added. After 24 h the molecular sieve was filtered off, washed with ethanol, and the solution evaporated. Water (3 ml) was added to the residue, then 10% K_2CO_3 solution to bring the pH to 9, and side products were extracted with ethyl acetate (5 × 3 ml). The aqueous layer was acidified to pH 2 with 1 N HCl. An oil was formed and was triturated for an hour to decompose boron compounds. The pH of the solution was then brought to 4 with K_2HPO_4, NaCl (4 g) was added to the mixture, and the desired product was extracted with ethyl acetate (5 × 5 ml). The AcOEt solution was dried over Na_2SO_4. The latter was filtered off and maleic acid (0.0813 g: 0.7 mmole) was added to the filtrate. The solution was evaporated. The residue was dissolved in acetonitrile (1 ml) and the insoluble solid filtered off. Dry ether was added to the acetonitrile solution and the mixture set aside at 0°C. The oil which separated was rubbed under dry ether to form a white crystalline solid of the maleate salt of substance (III), yield was 0.03 g (5.4%). It had mp 105-108°C and R_f 0.54 in the system AcOEt – BuOH – H_2O – AcOH, 1:1:1:1. An analytical sample was purified by HPLC on Silasorb C_{18}, eluent was CH_3CN – 0.2 M $AcONH_4$ (pH 5.0), 1:3.

PMR spectrum (DMSO-D_6): 7.27-7.42 (5H, Ph), 4.99-5.13 (2H, Ph-CH_2O), 4.30-4.36 (2H, (OCH_2CH_3)), 3.90-4.28 (1H, α-Pro, 1H, α′-Pro, 1H, α-Ala, 1H, γ-Pro), 3.69-3.86 and 3.37-3.50 (2H, δ-Pro, 2H, δ-Pro), 2.15-2.28 (1H, β′-Pro, 1H, β-Pro, 1H, β′-Pro), 1.84-2.08 (2H, γ-Pro, 1H, β-Pro, 1H, β′-Pro), 1.35-1.45 (3H, CH_3Ala), 1.06-1.22 ppm (3H, CH_3). The multiplicity of signals was not determined due to the presence of two enantiomers at Cγ′-Pro and rotational isomers at the two amide bonds of the two proline molecules.

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