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SYNTHESIS OF N-(1-CARBOXY-3-PHENYLPROPEN-2-YL)ALANYLPROLINE

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A method has been developed for the synthesis of N-(1-carboxy-3-phenylpropen-2-yl)alanylproline by reductive alkylation of alanylproline by the sodium salt of 2-oxo-4-phenylbutenoic acid, using sodium cyanoborohydride and sodium borohydride as reducing agents. The products were separated chromatographically. Under the conditions of the reaction, sodium borohydride in a neutral medium preferentially reduces the double bond of the Schiff base. Sodium cyanoborohydride reduces, in addition, the double bond of the 2-oxo-4-phenylbutenoic acid, forming enalaprilate.

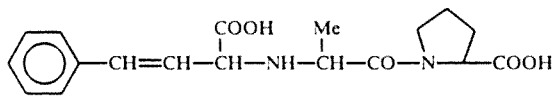
Deviation of the renin–angiotensin–aldosterone system from the norm is the source of a number of pathological states, among which the most widely encountered is arterial hypertension [1]. According to data reported by the American Cardiology Association, more than 25 million persons in the USA suffer from this illness, with some 50% of the cases due to functional breakdown of the renin–angiotensin–aldosterone system [1, 2]. Hence there is an urgent need for research on this system with the goal of developing new, highly effective drugs.

The renin–angiotensin–aldosterone system is characterized by a pressor effect due to the octapeptide angiotensin II formed from angiotensin I under the influence of dipeptidyl peptidase or angiotensin-converting enzyme. The increased blood pressure caused by angiotensin II is due to constriction of the vessels and stimulation of the system of aldosterone and catecholamines by the adrenal glands.

Clinical and pharmacological studies have shown that inhibitors of angiotensin-converting enzyme are highly effective drugs for the treatment of arterial hypertension and cardiac insufficiency [3]. The use of angiotensin-converting enzyme, in contrast to other known vasodilators, diminishes the role of tachycardia and myocardial infarction as side effects in the treatment of hypertension. Captopril, enalapril, and lisinopril are recognized as the best drugs for the treatment of arterial hypertension; however, enalapril is the most popular preparation among the inhibitors of angiotensin-converting enzyme.

The basis for the design of these preparations is the model of active centers of the angiotensin-converting enzyme proposed by Ondetti and Cushman [4, 5]. According to this model, the active center of the enzyme consists of seven structural elements: 1) a positively charged ion; 2) a "pocket" for the hydrophobic group of the C-terminal amino acid; 3) a hydrogen donor; 4) a "pocket" for the hydrophobic group of the penultimate amino acid; 5) a hydrogen acceptor; 6) the zinc ion of the enzyme, which is a complexing agent with the substrate and the enzyme inhibitors; 7) "pockets" for the zinc ion.

With the aim of varying the N-end of the enalaprilate molecule, which interacts with a "pocket" for the zinc ion (active site S_1) of the Cushman and Ondetti model [4, 6], we synthesized the unsaturated analog N-(1-carboxy-3-phenylpropen-2-yl)alanylproline (I):



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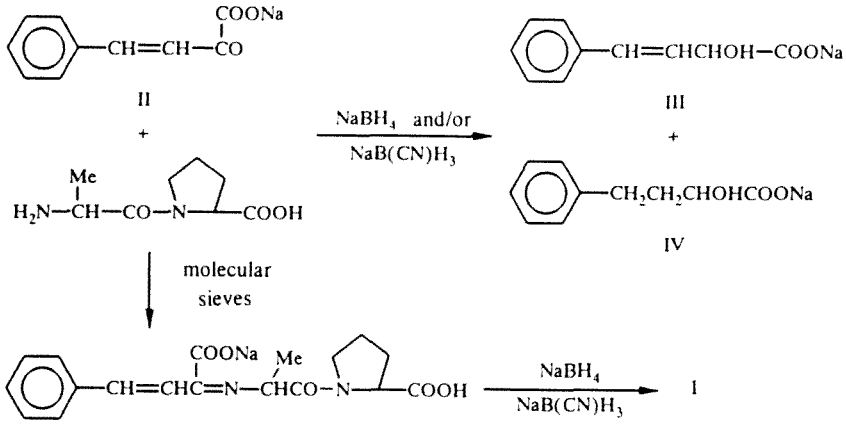
We carried out the reductive alkylation of the dipeptide alanylproline by the sodium salt of benzylidienepyruvic acid in the presence of sodium borohydride and sodium cyanoborohydride as hydrogenating agents (see Scheme 1). Two parallel reactions take place: formation of the sodium salt of the unsaturated analog of enalapril (I) and reduction of the sodium salt of 2-oxo-4-phenylbutenoic acid to the sodium salt of 2-hydroxy-4-phenylbutenoic acid (III). Also, in an alkaline medium, compound III is isomerized to the sodium salt of 2-oxo-4-phenylbutanoic acid [7]. The latter, with the participation of the borohydrides, is reduced to the sodium salt of 2-hydroxy-4-phenylbutanoic acid (IV). Here, the reductive conversion of the sodium salt of 2-oxo-4-phenylbutenoic acid (II) proceeds at a high rate, owing to the large excesses of compound II and the borohydrides in the reaction mixture. E-isomers of the (R,S,S) and (S,S,S) derivatives of I are obtained. Also found in the reaction mixture are the (R,S,S) and (S,S,S) diastereomers of enalaprilate. Their content in the reaction mixture increases when $NaB(CN)H_3$ is used as the reducing agent.

Scheme 1



We also investigated the reductive alkylation of alanylproline by the ethyl ester of 2-oxo-4-phenylbutenoic acid in an absolute ethanol medium in the presence of molecular sieves, using $NaB(CN)H_3$ and $NaBH_4$ as the reducing agents. In addition, the reductive alkylation of the dipeptide by the ethyl ester of 2-oxo-4-phenylbutenoic acid was accomplished in the presence of molecular sieves under conditions of catalytic hydrogenation over 1.6% Pd/C catalyst. However, the corresponding unsaturated analog of enalapril was formed only in a small amount, and we were unable to isolate it from the complex reaction mixture of products. Under these conditions, diastereomers of enalaprilate are formed preferentially [9].

Thus, for the synthesis of the unsaturated analog of carboxymethylalanylproline, the optimal path is condensation of the sodium salt of 2-oxo-4-phenylbutenoic acid with the dipeptide Ala-Pro in the presence of molecular sieves, with $NaBH_4$ as the reducing agent.

EXPERIMENTAL

The hydrochloride of the benzyl ester of L-proline was synthesized by interaction of L-proline with benzyl alcohol in the presence of thionyl chloride. The purity of the compound after recrystallization was 99%.

Alanylproline was synthesized by condensation of BocAla with ProOBzl by the DCC method, followed by acidolysis of the Boc group and hydrogenolysis of the Bzl group. The purity of the product was 96.2%.

The original reactants and the end-product were analyzed by HPLC in a DuPont 850 chromatograph with a UV spectrometer detector, under the following conditions: for the sodium salt of 2-oxo-4-phenylbutenoic acid: sorbent Zorbax ODS, column 4.6 × 150 mm, eluent 15% isopropanol, 0.2% H_3PO_4, 84.8% H_2O, λ = 230 nm; for the dipeptide Ala-Pro: sorbent Silasorb CPH C_{18}, column 4.6 × 150 mm, eluent 0.1 M phosphate buffer solution, pH 2.5, λ = 220 nm; for the N-(1-carboxy-3-phenylpropen-2-yl)alanylproline: sorbent Zorbax ODS, column 4.6 × 250 mm, eluent 5% CH_3CN and 95% 0.2 M $AcONH_4$, λ = 220 nm.

The following reagents were used in this work: Bu^t-hydroxycarbonylalanine and proline (Reanal, Hungary); ethyl ester of pyruvic acid (Fluka AG).

Interaction of Sodium Salt of 2-oxo-4-Phenylbutenoic Acid with Alanylproline Hydrochloride. A round-bottom flask equipped with a magnetic stirrer was charged with 4.45 g (22.5 mmoles) of the sodium salt of 2-oxo-4-phenylbutenoic acid, which was suspended in 4 ml of distilled water; 0.9 g (4.0 mmoles) of alanylproline hydrochloride, suspended in 4 ml of distilled water, was added; and the mixture was stirred. The pH of the reaction mixture was brought to 7 by adding 25% NaOH, and the mixture was diluted with 4 ml of distilled water. Then 0.06 g (0.9 mmole) of $Na(CN)BH_3$ dissolved in 1 ml of distilled water was added to the reaction mixture, which was then stirred for 2 h. Next, 0.1 g (2.6 mmoles) of $NaBH_4$ was added, holding the pH at 7 by means of 4 N HCl. The reaction mixture was stirred for 10 h. Then 0.1 g (2.6 mmoles) of $NaBH_4$ was added, and the pH was again adjusted to 7. Over the course of 2 h, 1.1 g (5.5 mmoles) of the sodium salt of 2-oxo-4-phenylbutenoic acid was added, alternating with $NaBH_4$ while holding the pH at 7. During the course of the reaction, the presence of unreacted dipeptide was monitored by means of TLC. The original compound disappeared in 10 days of holding at room temperature. The mole ratio of reagents, dipeptide:sodium salt of 2-oxo-4-phenylbutenoic acid:sodium cyanoborohydride:sodium borohydride was 1:20.2:0.23:5.8. Purification of the reaction mixture was accomplished in a column packed with the anion exchange resin Dowex $AG^0$50W-X2 (acid form). The column dimensions were 31 × 490 mm. Impurities were removed by eluting the column first with 1 liter of a 1:2 mixture of methanol and water, and then 2 liters of water. The desired product was then eluted with a 2% aqueous solution of pyridine. This operation gave a mixture of the diastereomeric forms of N-(1-carboxy-3-phenylpropen-2-yl)alanylproline, with admixtures of diastereomeric forms of enalaprilate [9]. The final purification was performed by HPLC with the sorbent Zorbax ODS, column 21.2 × 250 mm, eluent 22% CH_3CN and 78% $AcONH_4$. The eluent input rate was 16 ml/min (the detector was a UV spectrometer, λ = 230 nm).

Obtained a mixture of (S,S,S) and (R,S,S) diastereomers, R_f = 0.45 and R_f = 0.52 (system n-butanol:pyridine:acetic acid:water, 15:10:3:6). PMR spectra, ppm: 1.2 (3H, m, ^3J = 11.4 Hz, Ala-CH_3), 1.75-1.90 (5H, m, CβH, CγH, Pro), 1.95-2.25 (H, m, CβH, Pro), 3.8 (1H, m, CαH, Ala), 4.31 (1H, m, CαH, Pro), 6.11 (H, dd, ^3J = 16.3, ^4J = 3.1 Hz, Ph−CH=), 6.48-6.66 (1H, m, =CH−CH), 7.15-7.44. The multiplicity of the PMR signals is doubled in connection with the presence of rotational isomers around the peptide bond of the Ala-Pro.

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