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4-Hydroxy-3-phenylprolines were synthesized *via* 1-acetyl-2,2-diethoxycarbonyl-2,3-dihydro-3-phenyl-1*H*-pyrrole. Reversed phase hplc resulted in the isolation of the products which were characterized by  $^1\text{H}$  and  $^{13}\text{C}$  nmr spectroscopy.

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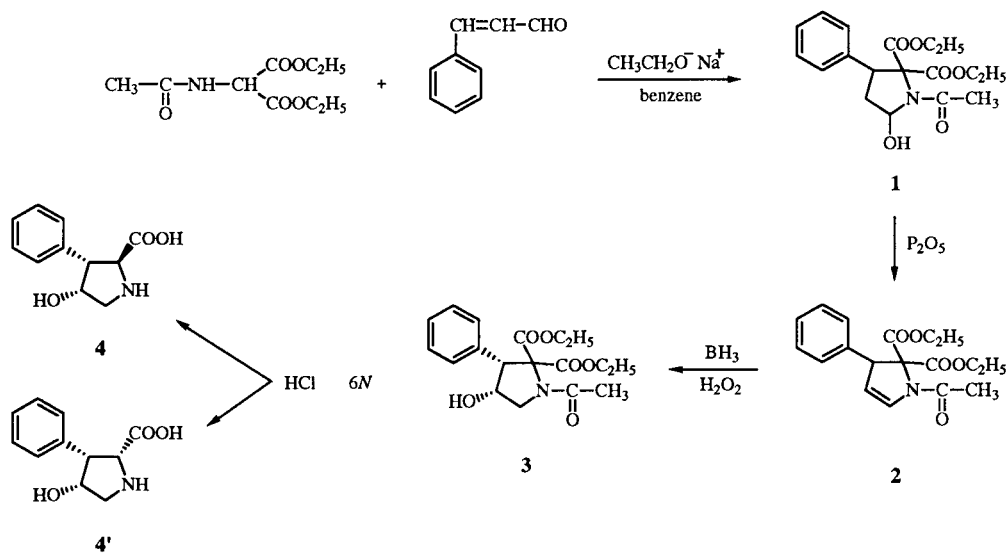
The chemical synthesis of 4-hydroxyproline derivatives is of interest in view of their possible incorporation in modified bioactive peptides which may act as antagonists towards the parent ones [1-2]. As a matter of fact the presence of an -OH group at the 4 position of natural proline could improve the interaction with the enzymatic hydrophilic sites and increase the capability of coordinating inorganic ions such as  $\text{Zn}^{++}$  which take part in numerous enzymatic systems [3-4]. We have recently reported a new method for the preparation of 4-hydroxyproline [5] based on a synthetic strategy previously used to obtain some structural analogues of the antitumor antibiotics anthramycin, tomaymycin and others [6-7]. We wish to describe here the synthesis of a further analogue of pro-

line, namely 4-hydroxy-3-phenylproline. The insertion of a phenyl group on the pyrrole ring of proline causes a remarkable steric burden to the molecule whose incorporation in a modified peptide is expected to reduce its conformational freedom.

The synthetic route utilized for the preparation of 4-hydroxy-3-phenylproline is depicted in Scheme 1 and is similar to that proposed for 4-hydroxyproline with some modifications.

Thus Michael addition of diethyl acetamidomalonate on cinnamaldehyde followed by cyclization afforded, as expected, 1-acetyl-2,2-diethoxycarbonyl-5-hydroxy-3-phenylpyrrolidine **1** in good yield. The  $^1\text{H}$  and  $^{13}\text{C}$  nmr spectra of **1** showed no doubling of any resonance thus

Scheme 1



indicating that the reaction afforded only one of the two pairs of enantiomers (*cis* and *trans*) due to the presence of two chiral carbon atoms. No investigation was attempted to establish the relative stereochemistry of phenyl and hydroxyl groups. In fact, the knowledge of such stereochemistry was judged insignificant since the following reaction had to lead to the disappearance of the chiral center at 5-C. In fact **1**, by treatment with phosphorus pentoxide, underwent a dehydration to give **2** in 79% yield. Successive hydroboration-oxidation of **2** yielded the desired 4-hydroxy derivative **3** as the major product (25% yield). The relative stereochemistry of **3** was established by a nOe difference experiment which showed the spatial proximity of 4-H with 3-H thus pointing to a *cis* relationship between the hydroxyl and phenyl groups. Finally, the complete acidic hydrolysis of **3**, with the creation of a new chiral center at 2-C, afforded the two possible products, **4** and **4'** carrying the carboxyl group on either side of the molecule, which were separated by hplc. The spatial positioning of the carboxyl group in the two products was established by nOe experiments, which showed that the predominant stereoisomer was the one (**4**) characterized by the carboxyl function *trans*-related to the other substituents on the pyrrolidine ring. Thus nOe experiments showed that in **4** the carboxyl group was located on the opposite side of the pyrrolidine ring with respect to the other two groups. In fact, by irradiation at 2-H, a positive nOe effect was observed for the aromatic protons whereas 3-H was completely unaffected. On the other hand, in **4'**, irradiation of 2-H resulted, as expected, in an enhancement of the 3-H signal whereas no detectable effect for the aromatic protons was observed.

## EXPERIMENTAL

Thin layer chromatography on precoated silica gel plates (Whatman K 6 F) was used to control the course of reactions and the purity of products. Detection of the components was made by either uv light or by treatment with iodine vapors. Column chromatography was performed on silica gel Merck (70-230 mesh). Separation of **4** and **4'** (hplc) was accomplished by a Varian 5000 instrument. Melting points were determined with a Kofler hot stage microscope and are uncorrected. The  $^1\text{H}$ ,  $^{13}\text{C}$  and nOe nmr spectra were recorded on Bruker WM 250 and Bruker AMX 500 spectrometers with chemical shift values reported in  $\delta$  (ppm) relative to tetramethylsilane as the internal standard.

1-Acetyl-2,2-diethoxycarbonyl-5-hydroxy-3-phenylpyrrolidine (**1**).

To a stirred solution of 10.9 g (0.05 mole) of diethyl acetamidomalonate in 100 ml of dry benzene a 25% sodium ethoxide solution (5 ml) in dry ethanol was added at room temperature. A solution of 6.25 ml (0.05 mole) of cinnamaldehyde in 10 ml of dry benzene was then added dropwise. After 10

hours, the mixture was filtered. The filtrate was evaporated to dryness *in vacuo*. Diethyl ether was added to the residual oil, obtaining a white solid, which was recrystallized from benzene to give 11.5 g (65%) of **1**, mp 98-100°;  $^1\text{H}$  nmr (deuteriochloroform):  $\delta$  7.2-7.6 (m, 5H, phenyl protons), 5.90 (dd, 1H, 5-H), 4.52 (q, 4H, two ethoxycarbonyl  $\text{CH}_2$ ), 4.17, 3.96 (2 dd, each, 1H, 4- $\text{CH}_2$ ), 2.98 (dd, 1H, 3-H), 2.53 (s, 3H,  $\text{COCH}_3$ ), 1.52, 1.08 (2t, each, 3H, two ethoxycarbonyl  $\text{CH}_3$ );  $^{13}\text{C}$  nmr (deuteriochloroform):  $\delta$  169.9, 169.3 (two ethoxycarbonyl CO), 165.6 (acetamide CO), 135.1 (phenyl quaternary C), 128.7, 128.0, 127.9 (five phenyl CH), 82.5 (5-C), 75.5 (2-C), 62.2, 61.3 (two ethoxycarbonyl  $\text{CH}_2$ ), 50.0 (3-C), 38.7 (4-C), 21.5 (acetyl  $\text{CH}_3$ ), 13.8, 13.3 (two ethoxycarbonyl  $\text{CH}_3$ ).

Anal. Calcd. for  $\text{C}_{18}\text{H}_{23}\text{NO}_6$ : C, 61.88; H, 6.64; N, 4.01. Found: C, 61.74; H, 6.53; N, 4.01.

1-Acetyl-2,2-diethoxycarbonyl-2,3-dihydro-3-phenyl-1*H*-pyrrole (**2**).

A stirred solution of 17.5 g (0.05 mole) of **1** in 150 ml of dry benzene was added with 10 g of phosphorus pentoxide and refluxed for 2.5 hours. After cooling, the mixture was filtered and the filtrate was evaporated to dryness *in vacuo*. After treatment with diethyl ether, a white solid was obtained. This product was recrystallized from benzene to afford 13.1 g (79%) of **2**, mp 91-94°;  $^1\text{H}$  nmr (deuteriochloroform):  $\delta$  7.0-7.4 (m, 5H, phenyl protons), 6.73 (dd, 1H, 5-H), 5.15 (dd, 1H, 4-H), 4.66 (dd, 1H, 3-H), 4.31, 3.47 (2q, each, 2H, two ethoxycarbonyl  $\text{CH}_2$ ), 2.19 (s, 3H,  $\text{COCH}_3$ ), 1.33, 0.76 (2t, each, 3H, two ethoxycarbonyl  $\text{CH}_3$ );  $^{13}\text{C}$  nmr (deuteriochloroform):  $\delta$  166.6, 164.2 (two ethoxycarbonyl CO), 157.2 (acetamide CO), 137.5 (phenyl quaternary C), 130.7 (5-C), 129.2, 128.0, 127.9 (five phenyl CH), 109.5 (4-C), 75.5 (2-C), 62.4, 61.5 (two ethoxycarbonyl  $\text{CH}_2$ ), 57.1 (3-C), 21.9 (acetyl  $\text{CH}_3$ ), 14.4, 13.3 (two ethoxycarbonyl  $\text{CH}_3$ ).

Anal. Calcd. for  $\text{C}_{18}\text{H}_{21}\text{NO}_5$ : C, 65.24; H, 6.39; N, 4.23. Found: C, 65.04; H, 6.27; N, 4.20.

1-Acetyl-2,2-diethoxycarbonyl-4-hydroxy-3-phenylpyrrolidine (**3**).

A stirred solution of 9.9 g (0.03 mole) of **2** in 100 ml of dry tetrahydrofuran was cooled in an ice bath and then added with 6 ml of borane-dimethylsulfide complex (4% of active hydrogen). The mixture was then stirred for 3 hours at room temperature and then added with 30 ml of water/THF (1:1), basified with 3*N* sodium hydroxide solution until pH 8, cooled in an ice bath and added with 15 ml of 30% hydrogen peroxide solution. The mixture was stirred at 0° for 1 hour and then allowed to stand at room temperature for 10 hours. The solution was partitioned on saturated sodium chloride solution: the THF layer was separated, dried on anhydrous sodium sulfate, filtered, concentrated to a small volume and then chromatographed on a silica gel column eluting with diethyl ether/*n*-hexane mixtures with increasing amounts of ether. The product obtained was recrystallized from diethyl ether to give 2.7 g (25%), mp 123-125°;  $^1\text{H}$  nmr (deuteriochloroform):  $\delta$  7.48 (m, 3H) and 7.32 (m, 2H) (phenyl protons), 4.75 (m, 1H, 4-H), 4.36 (q, 4H, two ethoxycarbonyl  $\text{CH}_2$ ), 4.24 (dd, 1H) and 3.92 (dd, 1H) (5- $\text{CH}_2$ ), 3.72 (broad s, 1H, 3-H), 3.53 (d, 1H, OH, deuterium oxide-exchangeable), 2.21 (s, 3H, acetyl  $\text{CH}_3$ ), 1.36, 0.91 (2t, each, 3H, two ethoxycarbonyl  $\text{CH}_3$ );  $^{13}\text{C}$  nmr (deuteriochloroform):  $\delta$  170.0, 168.9 (two ethoxycarbonyl CO), 166.0 (acetamide CO), 134.9 (phenyl qua-

ternary C), 128.9, 128.4, 128.0 (five phenyl CH), 76.0 (2-C), 72.6 (4-C), 62.7, 61.5 (two ethoxycarbonyl CH<sub>2</sub>), 60.1 (3-C), 54.5 (5-C), 22.2 (acetyl CH<sub>3</sub>), 13.9, 13.4 (two ethoxycarbonyl CH<sub>3</sub>).

*Anal.* Calcd. for C<sub>18</sub>H<sub>23</sub>NO<sub>6</sub>: C, 61.88; H, 6.64; N, 4.01. Found: C, 61.68; H, 6.72; N, 3.98.

#### 4-Hydroxy-3-phenylproline (4 and 4').

A solution of 1 g (0.003 mole) of **3** in 10 ml of 6*N* hydrochloric acid was refluxed for 3 hours. After cooling in ice bath, sodium hydrogen carbonate was added to the solution up to pH 6. The solution was evaporated to dryness *in vacuo* and the residue was washed with chloroform. The chloroform insoluble residue (0.15 g, yield 24%) was examined by tlc eluting with BAW mixture (60% butanol, 25% water, 15% acetic acid). This procedure showed the presence of only one spot. However, <sup>1</sup>H and <sup>13</sup>C nmr spectra indicated that the product was a mixture of two diastereoisomers which were separated by hplc (column: Licrospher 100 RP 18.5 mm, eluent water/methanol).

Compound **4** had <sup>1</sup>H nmr (deuterium oxide): δ 7.27 (m, 3H) and 7.21 (m, 2H) (phenyl protons), 4.37 (m, 1H, 4-H), 4.04 (d, 1H, 2-H), 3.49 (dd, 1H, one H of 5-CH<sub>2</sub>), 3.21, 3.18 (2 dd, each, 1H, 3-H and one H of 5-CH<sub>2</sub>); <sup>13</sup>C nmr (deuterium oxide): δ 174.1 (CO), 138.1 (phenyl quaternary C), 131.6, 131.3, 130.1 (five phenyl CH), 77.5 (3-C), 67.3 (2-C), 58.4 (4-C), 52.9 (5-C, assignation by dept).

*Anal.* Calcd. for C<sub>11</sub>H<sub>13</sub>NO<sub>3</sub>: C, 63.75; H, 6.32; N, 6.76. Found: C, 63.61; H, 6.30; N, 6.76.

Compound **4'** had <sup>1</sup>H nmr (deuterium oxide): δ 7.20 (m, 3H) and 7.06 (m, 2H) (phenyl protons), 4.46 (m, 1H, 4-H), 4.40 (d,

1H, 2-H), 3.67 (dd, 1H, one H of 5-CH<sub>2</sub>), 3.55 (dd, 1H, 3-H), 3.11 (dd, 1H, one H of 5-CH<sub>2</sub>); <sup>13</sup>C nmr: δ 175.2 (CO), 139.4 (phenyl quaternary C), 131.7, 131.4, 130.2 (five phenyl CH), 78.0 (3-C), 66.9 (2-C), 56.5 (4-C), 52.2 (5-C).

*Anal.* Calcd. for C<sub>11</sub>H<sub>13</sub>NO<sub>3</sub>: C, 63.75; H, 6.32; N, 6.76. Found: C, 63.52; H, 6.08; N, 6.58.

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