New Synthetic Method for 4-Hydroxyproline

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A new synthesis of 4-hydroxyproline was carried out starting from diethyl *p*-nitrobenzoylaminomalonate, which was converted by a three steps pathway into diethyl 4-hydroxy-1-(4-nitrobenzoyl)pyrrolidine-2,2-dicarboxylate, which afforded 4-hydroxyproline *via* acid hydrolysis.

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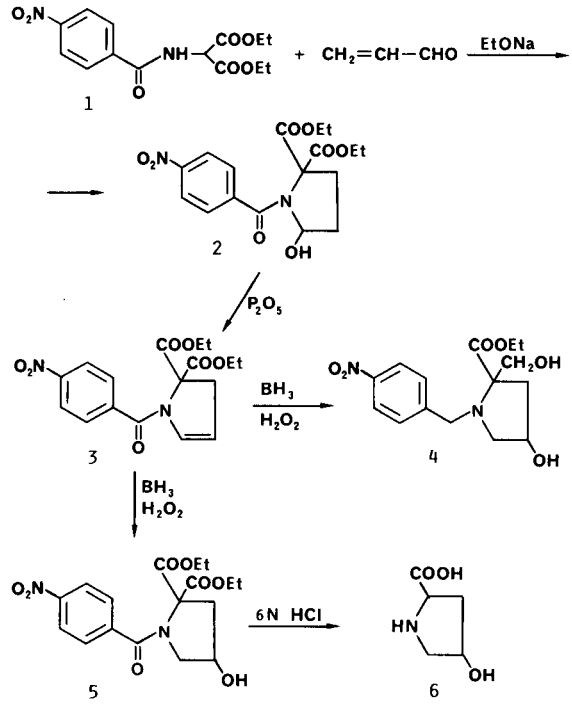
In view of the development of researches on pyrroline and pyrrolidine derivatives active on the cardiovascular apparatus and useful for preparation of structurally modified bioactive peptides, we have taken into consideration the possibility of setting up a synthetic method with a high degree of versatility which allowed us to obtain good results in the above cited domains. In this paper we describe the first results obtained with a new synthetic method which might have the required level of versatility.

In the case here considered such synthetic method was used in order to obtain 4-hydroxyproline. It has to be pointed out that this aminoacid was obtained from some natural sources. *trans*-4-Hydroxy-L-proline was obtained from collagen and gelatin hydrolysates [1-3]. *cis*-4-Hydroxy-L-proline is a constituent of phalloidine, the toxic polypeptide of *Amanita phalloides* [4]. Mixtures of diastereoisomeric *cis*- and *trans*-4-hydroxyproline were obtained with some synthetic methods [5-7], whereas in some cases 4-hydroxyproline was obtained by semisynthetic methods, starting from another aminoacid, such as proline or glutamic acid [8-10].

The method here described was suggested by previous researches carried out by one of us on the synthesis of some structural analogues of antitumor antibiotics anthramicine, tomaymicine and others. In the course of these studies 1-(2-nitrobenzoyl)-2,2-diethoxycarbonyl-2,3-dihydro-1*H*-pyrrole was shown to be an important key-intermediate giving easily both addition and substitution reactions on pyrroline double bond [11-13]. In particular, the hydroboration-oxidation reaction with boron hydride and hydrogen peroxide gave a 4-hydroxypyrrolidine derivative.

These results prompted us to elaborate a method for preparation of some proline and hydroxyproline analogues. The first application of this method has been a new synthesis of 4-hydroxyproline. Such synthesis (see Scheme 1) involved the preparation of diethyl 4-nitrobenzamidomalonate **1** by reaction of diethyl aminomalonate hydrochloride with 4-nitrobenzoyl chloride, applying the method reported by Wright *et al.* [14] with some modifications.

Compound **1** was then converted into 2,2-diethoxycarbonyl-5-hydroxy-1-(4-nitrobenzoyl)pyrrolidine **2** by Michael addition on acrolein followed by intramolecular cyclization. The critical intermediate **3**, namely 2,2-diethoxycarbonyl-2,3-dihydro-1-(4-nitrobenzoyl)-1*H*-pyrrole, was obtained at last from dehydration of **2** accomplished by treatment with phosphorus pentoxide in boiling benzene.



SCHEME 1

Hydroboration-oxidation reaction of compound **3** afforded both the desired 2,2-diethoxycarbonyl-4-hydroxy-1-(4-nitrobenzoyl)pyrrolidine **5** and 2-ethoxycarbonyl-4-hydroxy-2-hydroxymethyl-1-(4-nitrobenzoyl)pyrrolidine **4**. *cis*- And *trans*-4-hydroxyproline **6** were obtained from **5** by complete acidic hydrolysis.

The choice of *p*-nitrobenzoyl moiety as protecting group was suggested, according with our previous results obtained with 2-nitro analogues [12], by the facile course of the hydroboration-oxidation reaction leading to 4-hydroxy derivatives **4** and **5**.

EXPERIMENTAL

Precoated silica gel Whatman K6F plates were used for thin layer chromatography; detection of components was made by uv light or treatment with iodine vapors. Chromatographic separations were performed on columns packed with silica gel 60 from Merck (70-230 mesh ASTM). Melting points were determined with a Kofler hot stage microscope and are uncorrected. Elemental analyses were performed with a Perkin-Elmer Elemental Analyzer model 240. The ^1H and ^{13}C nmr spectra were recorded in deuteriochloroform on a Bruker WM250 instrument.

Diethyl 4-Nitrobenzamidomalonate (**1**).

A suspension of 21.2 g (0.1 mole) of diethyl aminomalonate hydrochloride in anhydrous tetrahydrofuran was added with 20 ml of triethylamine and then 18.6 g (0.1 mole) of 4-nitrobenzoyl chloride. The mixture was cooled in an ice-bath and stirred for 30 minutes. The reacted solution was filtered and the solid residue washed with tetrahydrofuran. The combined filtrates were evaporated *in vacuo* and the residue was recrystallized from benzene to afford 16.8 g (yield 62%) of colorless crystals, mp 135-136°; ^1H nmr: δ 8.33 (d, 2H) and 8.04 (d, 2H) (benzene protons, J = 8.5 Hz), 7.25 (broad, 1H, NH), 5.34 (d, 1H, CH, J = 7 Hz), 4.35 (two superimposed q, 4H, two CH_2), 1.35 (t, 6H, two CH_3).

Anal. Calcd. for $C_{14}H_{16}N_2O_7$: C, 51.85; H, 4.97; N, 8.64. Found: C, 51.62; H, 4.80; N, 8.70.

2,2-Diethoxycarbonyl-5-hydroxy-1-(4-nitrobenzoyl)pyrrolidine (**2**).

A suspension of 20 g (0.061 mole) of **1** in 100 ml of anhydrous benzene was added with 5 ml of 2% sodium ethoxide in anhydrous ethanol and then dropwise with 8 ml (0.12 mole) of acrylaldehyde dissolved in 10 ml of benzene. The mixture was kept at room temperature for 20 hours and then evaporated to dryness *in vacuo*. The crude oily residue was purified on a silica gel column eluting with diethyl ether. The solid residue obtained after evaporation of ether was recrystallized from benzene added with a small amount of chloroform to afford 13.4 g (yield 58%) of colorless crystals, mp 140°; ^1H nmr: δ 8.26 (m, 2H) and 8.02 (m, 2H) (benzene protons), 5.33 (m, 1H, H-5), 4.30 (q, 4H, two ethyl CH_2), 3.00 (broad d, 1H, OH), 2.62 (m, 1H) and 2.42 (m, 1H) (3-CH_2 geminal protons), 1.80 (m, 1H) and 1.54 (m, 1H) (4-CH_2 geminal protons), 1.33 (t, 6H, two ethyl CH_3).

Anal. Calcd. for $C_{17}H_{20}N_2O_8$: C, 53.68; H, 5.30; N, 7.37. Found: C, 53.45; H, 5.21; N, 7.40.

2,2-Diethoxycarbonyl-2,3-dihydro-1-(4-nitrobenzoyl)-1*H*-pyrrole (**3**).

A solution of 19 g (0.05 mole) of **2** in 200 ml of anhydrous benzene was refluxed for three hours in the presence of phosphorus pentoxide (7 g). After cooling, the solution was decanted, washed with a saturated solution of sodium hydrogen carbonate, dried on sodium sulfate, concentrated *in vacuo* up to a small volume and then chromatographed on a silica gel column eluting with diethyl ether-*n*-hexane (8:2). This procedure allowed us to isolate a product which was recrystallized from benzene to obtain 8.1 g (yield 45%) of colorless crystals, mp 67-68°; ^1H nmr: δ 8.33 (d, 2H) and 7.75 (d, 2H) (benzenic protons, J = 8.5 Hz), 6.32 (m, 1H, H-5), 5.18 (m, 1H, H-4), 4.35 (q, 4H, two ethyl CH_2), 3.31 (m, 2H, 3-CH_2), 1.35 (t, 6H, two ethyl CH_3); ^{13}C nmr: δ 167.68 (two carbethoxyl CO), 164.69 (CO-N), 149.04 (C-NO_2), 140.83 (benzene 1-C), 129.43 (5-CH), 128.66 and 123.76 (four benzene CH), 108.43 (4-CH), 72.48 (2-C), 62.47 (two ethyl CH_2), 40.81 (3-CH_2), 13.88 (two ethyl CH_3).

Anal. Calcd. for $C_{17}H_{18}N_2O_7$: C, 56.35; H, 5.01; N, 7.73. Found: C, 56.40; H, 4.95; N, 7.60.

2-Ethoxycarbonyl-4-hydroxy-2-hydroxymethyl-1-(4-nitrobenzyl)-pyrrolidine (**4**).

A solution of 3.6 g (0.01 mole) of **3** in 50 ml of anhydrous tetrahydrofuran was added with 1 ml of borane-dimethyl sulfide complex (4% of active hydrogen) and stirred for 6 hours at 50°. The reacted solution was added with 8 ml of water-tetrahydrofuran mixture (1:1), basified with sodium hydroxide (3*N* solution) and then added with 3 ml of 30% hydrogen peroxide solution. The mixture was kept at room temperature for two hours, then cooled in an ice bath and saturated with sodium chloride in order to obtain the separation of aqueous and organic layers. The tetrahydrofuran layer was dried on anhydrous sodium sulfate and examined by tlc on a silica gel plate eluting with diethyl ether: this procedure allowed us to evidence the presence of two products with different Rf values. The solution was concentrated *in vacuo* to a small volume and chromatographed on a silica gel column eluting with diethyl ether. The first product eluted from the column was **4** (Rf = 0.57), which was obtained as an oil (0.85 g, 26%); ^1H nmr: δ 8.10 (d, 2H) and 7.48 (d, 2H) (benzene protons, J = 8.5 Hz), 4.31* (m, 1H, H-4), 4.22 (q, 2H, ethyl CH_2; J = 7 Hz), 3.76 (s, 2H, phenyl-CH_2-N), 3.92 (d, 1H) and 3.65 (d, 1H) (CH_2OH geminal protons, J = 14.7 Hz), 3.40 (broad, 2H, two OH groups, exchanged with deuterium oxide), 2.95* (dd, 1H, J_{gem} = 13.3 Hz, $J_{5,4}$ = 3.5 Hz) and 2.83 (d, 1H, J_{gem} = 13.3 Hz) (5-CH_2 geminal protons), 2.51* (dd, 1H, J_{gem} = 14.0 Hz, $J_{3,4}$ = 6 Hz) and 2.10 (d, 1H, J_{gem} = 14.0 Hz) (3-CH_2 geminal protons), 1.30 (t, 3H, ethyl CH_3, J = 7 Hz); the asterisked assignments were confirmed by selective decoupling experiments; ^{13}C nmr: δ 173.34 (COO), 147.36 and 147.23 (C-NO_2 and benzene 1-C), 128.75 and 123.69 (four benzene CH), 71.15 (2-C), 69.69 (4-CH), 62.76 (ethyl CH_2), 61.20 and 60.88 (phenyl-CH_2-N and CH_2OH), 52.42 (5-CH_2), 43.33 (3-CH_2), 14.36 (ethyl CH_3); these assignments are based on DEPT sequence.

Anal. Calcd. for $C_{15}H_{20}N_2O_6$: C, 55.55; H, 6.22; N, 8.64. Found: C, 55.37; H, 6.17; N, 8.71.

2,2-Diethoxycarbonyl-4-hydroxy-1-(4-nitrobenzoyl)pyrrolidine (**5**).

The second product eluted from the above mentioned column was **5** (Rf = 0.30). Recrystallization from benzene/*n*-hexane mixture (1:1) afforded 0.9 g (24%) of colorless crystals, mp 81-82°; ^1H nmr: δ 8.20 (d, 2H) and 7.62 (d, 2H) (benzene protons, J = 8.5 Hz), 4.32 (m, 1H, H-4), 4.23 (two superimposed q, 4H, two ethyl CH_2), 3.75 (broad, 1H, 4-OH, exchanged with deuterium oxide), 3.59 (dd, 1H, J_{gem} = 11.5 Hz, $J_{5,4}$ = 3.5 Hz) and 3.42 (d, 1H, J_{gem} = 11.5 Hz) (5-CH_2 geminal protons), 2.62 (d, 1H, J_{gem} = 13.5 Hz) and 2.48 (dd, 1H, J_{gem} = 13.5 Hz, $J_{3,4}$ = 4 Hz) (3-CH_2 geminal protons), 1.25 (two superimposed t, 6H, two ethyl CH_3); ^{13}C nmr: δ 169.35, 167.86 and 167.74 (two carbethoxy CO and CO-N), 148.74 (C-NO_2), 141.72 (benzene 1-C), 127.97 and 123.64 (four benzene CH), 71.47 (2-C), 69.77 (4-CH), 62.57 and 62.27 (two ethyl CH_2), 58.54 (5-CH_2), 43.92 (3-CH_2), 13.81 and 13.73 (two ethyl CH_3).

Anal. Calcd. for $C_{17}H_{20}N_2O_8$: C, 53.68; H, 5.30; N, 7.37. Found: C, 53.55; H, 5.23; N, 7.35.

4-Hydroxyproline (**6**).

A mixture of 1.5 g (4 mmoles) of **5** and 10 ml of 6*N* hydrochloric acid was refluxed for three hours. The cooled solu-

tion was extracted with chloroform in order to remove *p*-nitrobenzoic acid. The aqueous layer was adjusted to *p*H 4.5 with sodium hydrogen carbonate and then evaporated *in vacuo* to dryness. The solid residue was dissolved in a small amount of water-methanol mixture (1:1) and then examined by tlc on a silica gel plate in comparison with authentical specimens of *trans*-4-hydroxy-L-proline and *cis*-4-hydroxy-L-proline (from Aldrich), eluting with chloroform-methanol-water mixture (2:4:1). The product under examination resulted to be a mixture of *cis*- and *trans*-4-hydroxyproline, which were separated by preparative tlc on a silica gel plate using the same eluting mixture. We obtained in this manner the same amount (0.15 g, yield 29%) of each component of the mixture. These two products resulted identical to the authentical specimens of *cis*- and *trans*-4-hydroxyproline, respectively, showing the same elemental composition, melting point, ir, ^{1}H and ^{13}C nmr spectra.

REFERENCES AND NOTES

[1] J. Kapfhammer and R. Ech, *Z. Physiol. Chem.*, **170**, 294 (1927).

[2] P. B. Hamilton and P. J. Ortiz, *J. Biol. Chem.*, **184**, 607 (1950).

[3] German Offen. 2,620,834, 25 Nov 1976; *Chem. Abstr.*, **86**, P155966 (1977).

[4] H. Wieland and B. Witkop, *Ann.*, **543**, 171 (1940).

[5] H. Leuchs and J. F. Brewster, *Ber.*, **46**, 986 (1913).

[6] R. Gaudry and C. Godin, *J. Am. Chem. Soc.*, **76**, 139 (1954).

[7] S. G. Ramaswamy and E. Adams, *J. Org. Chem.*, **42**, 3440 (1977).

[8] C. Eguchi, *Bull. Chem. Soc. Japan*, **47**, 17004 (1974).

[9] N. Kurokawa and Y. Ohfune, *J. Am. Chem. Soc.*, **108**, 6041 (1986).

[10] J. Hara and Y. Inouye, *Bull. Chem. Soc. Japan*, **54**, 3871 (1981).

[11] G. De Martino, S. Massa and R. Giuliano, *Farmaco, Ed. Sci.*, **31**, 785 (1976).

[12] G. De Martino, S. Massa and S. Selleri, *Eur. J. Med. Chem.*, **12**, 572 (1977).

[13] S. Massa, G. De Martino and F. Corelli, *J. Heterocyclic Chem.*, **19**, 1497 (1982).

[14] W. B. Wright, D. B. Cosulich, M. J. Fahrenbach, C. W. Waller, J. M. Smith and M. E. Hultquist, *J. Am. Chem. Soc.*, **71**, 3014 (1949).