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Synthesis of pyrraline reference material

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Abstract A simplified and improved method is described for the preparation of pyrraline, a lysine derivative from the advanced Maillard reaction and potential indicator for heat treatment of foods. The compound was obtained in a high degree of purity and with a yield of 31% from N^{α}-*t*-butyloxycarbonyl-L-lysine after heating with 3-deoxy-D-*erythro*-hexos-2-ulose for 2 h at 70 °C in the dry state, preparative fractionation of the resulting N^{α}-*t*-butyloxycarbonyl pyrraline with reverse-phase liquid chromatography and final deprotection of the intermediate compound with acetic acid.

Key words Maillard reaction · 2-Amino-6-(2-formyl-5-hydroxymethyl-1-pyrrolyl)-hexanoic acid (registry no. 74509-14-1) · Pyrraline

Introduction

Pyrraline [lysyl-5′-hydroxymethylpyrrolaldehyde; 2-amino-6-(2-formyl-5-hydroxymethyl-1-pyrrolyl)-hexanoic acid, compound 4, Fig. 1] is an acid labile pyrrole compound, resulting from the reaction between the ε-amino group of lysine (compound 1, Fig. 1) and 3-deoxy-D-*erythro*-hexos-2-ulose (3-deoxyglucosulose) (compound 2, Fig. 1), which is a degradation product of reducing carbohydrates and aminoketoses [1–2]. Small amounts of the weak mutagenic [3] and antiproteolytic [4] free amino acid pyrraline have been found in aqueous extracts of processed foods [5]. Protein-bound pyrraline was first detected in alkaline hydrolysates of proteins that had been reacted with glucose [6–7]. Recently, various papers reported on the determination of pyrraline after total enzymic hydrolysis of the proteins, using either amino acid analysis with photodiode array detection [8–9] or RP-HPLC [10–11]. The lysine derivative could be quantified in a variety of foods, correlating with the intensity of heat treatment applied during food processing [9–10]. For the use of pyrraline as an indicator for the advanced stages of the Maillard reaction, an external standard of pure pyrraline is needed. Methods for the isolation of pyrraline published to date either provide only poor yield [1–2, 5, 11–12] or require the synthesis of numerous intermediate products with multiple extraction and purification steps [13–14]. In this paper, we describe a simple method for the preparation of pyrraline with a high yield and purity.

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Materials and methods

Chemicals. N^{α}-*t*-Butyloxycarbonyl-L-lysine (Boc-Lys-OH) was from Bachem (Heidelberg, Germany). All other chemicals were of highest purity available.

Amino acid analysis. Ion-exchange chromatography with photodiode array measurement and subsequent ninhydrin detection was performed as described previously [8–9].

Liquid chromatography. This was performed using a GradiFrac system with a HiLoad pump P-50, UV-monitor Uvicord SII, recorder REC-102 and a XK 26/100 chromatography column (all from Pharmacia, Freiburg, Germany). The column was filled with silica gel 100 C18-reverse-phase, particle size 15 to 35 μm, endcapped (Fluka, Neu-Ulm, Germany). Column effluent was monitored at 280 nm.

Spectroscopic analysis. [^{1}H]Nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz on a 500 AMX NMR spectrometer (Bruker, Rheinstetten, Germany) in D_2O. Values for ∂ are given relative to tetramethylsilane. Ultraviolet (UV) spectra were recorded on an Ultrospec III spectral photometer (Pharmacia).

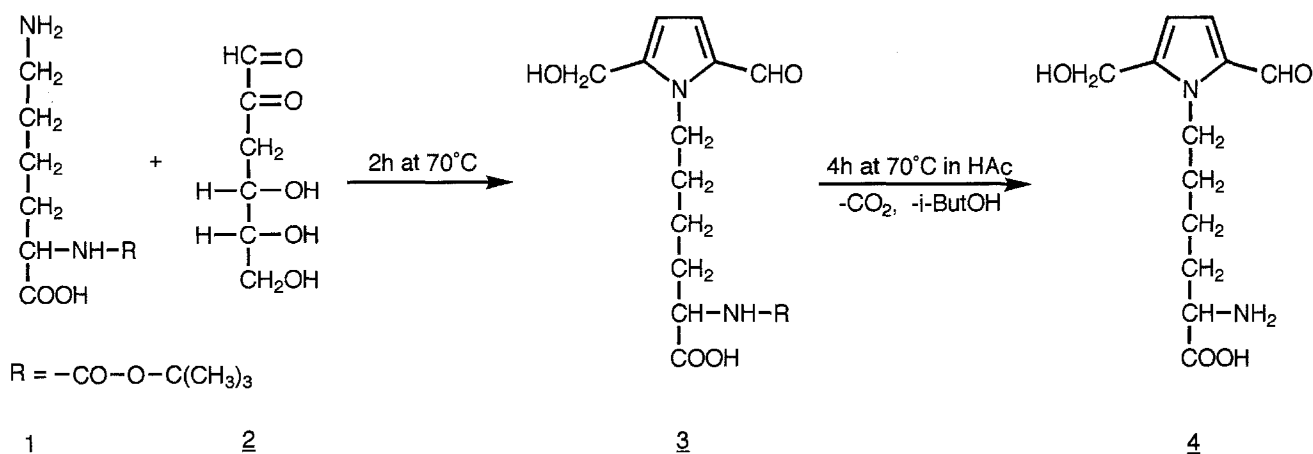

Fig. 1 Synthesis of pyrraline. Compound 1, lysine; compound 2, 3-deoxy-D-*erythro*-hexos-2-ulose (3-deoxyglucosulose); compound 3, 6-(2-formyl-5-hydroxymethyl-1-pyrrolyl)-2-(*N*-*t*-butyloxycarbonyl-amino)-hexanoic acid (N^{α}-Boc-pyrraline); compound 4, 2-amino-6-(2-formyl-5-hydroxymethyl-pyrrolyl)-hexanoic acid (pyrraline)

Synthesis of 3-deoxyglucosulose. The method of Madson and Feather [15] was used with some modifications. Amounts of 10 g glucose and 5.5 g *p*-toluidine were suspended in a mixture of 225 ml ethanol (95%) and 11 ml acetic acid and boiled under reflux for 30 min whilst being stirred continuously. To the solution, 16.5 g benzoylhydrazine were added, and the reflux was continued for 7 h. After cooling to room temperature whilst being stirred, the solution was kept at $-20\,°C$ overnight. Separated 3-deoxy-D-*erythro*-hexos-2-ulose bis(benzoylhydrazone) (= 3-deoxyglucosulose-benzoylhydrazone) was collected by filtration, washed with three portions each of 20 ml ice-cold ethanol and diethylether, and dried in air. The yield was 12.1 g.

To a suspension of 10 g 3-deoxyglucosulose-benzoylhydrazone, 300 ml ethanol (98.5%), 500 ml distilled water and 22 ml acetic acid, 16 ml benzaldehyde were added. While continuously being stirred, the mixture was boiled under reflux for 2 h. Then, the ethanol was evaporated off while, simultaneously, 500 ml water was added within 30 min. The solution was cooled in an ice bath and precipitated benzaldehydebenzoylhydrazone was removed by filtration. After adding 60 g of ion-exchanger Serdolyt MB3 (mixed-bed H^+/OH^-, Serva, Heidelberg, Germany) to the filtrate, the mixture was stirred in the dark for 15 min and filtered. The filtrate was concentrated under reduced pressure to about 100 ml, washed six times with 50 ml diethylether and evaporated to about 6 ml. The resulting yellow syrup was dissolved in a mixture of 5 ml water and 50 ml ethanol (95%). After adding 10 g Serdolyt MB3 and stirring in the dark for 10 min, the solution was filtered, evaporated to about 5 ml, frozen and freeze-dried to give an amorphous yellow powder of crude 3-deoxyglucosulose. The yield was 4.2 g. This was used without further purification.

Synthesis of 6-(2-formyl-5-hydroxymethyl-1-pyrrolyl)-2-(N-t-butyloxycarbonyl-amino)-hexanoic acid (= N^{α}-Boc-pyrraline). Amounts of 738 mg (3 mmol) Boc-Lys-OH and 2400 mg ($\approx$13 mmol) 3-deoxyglucosulose were dissolved in 250 ml of 0.1 N sodium acetate, pH 5.0. To the solution, 1200 mg cellulose (100 µm particle size) was added and the suspension was freeze-dried. The dry mixture was powdered and heated for 2 h at 70 °C in a drying oven. After cooling to room temperature, the brown powder was extracted five times, each time with 120 ml ethanol (98.5%). The pooled ethanol extracts were evaporated in vacuo at room temperature. The oily dark brown residue was dissolved in 20 ml water, membrane filtered (8 µm) and applied to the chromatography column, which had been previously equilibrated with 800 ml of a mixture of methanol and 50 mM acetic acid (3:7, v:v). The column was eluted isocratically with a mixture of methanol and 50 mM acetic acid (1:1, v:v) at a flow rate of 8 ml/min; 10-ml fractions were collected. The peak of N^{α}-Boc-pyrraline (compound 3, Fig. 1) eluted between 140 and 210 min. Corresponding fractions were pooled, methanol was evaporated off and the remaining solution was freeze-dried to yield brown N^{α}-Boc-pyrraline. The yield was 394 mg (950 µmol). Elementary analysis: $C_{19}H_{30}O_8N_2$ (mol. wt. = 414.45); calculated: C = 55.06%, H = 7.30%, N = 6.76%; found: C = 55.21%, H = 7.39%, N = 6.63%.

Synthesis of pyrraline. A solution of 390 mg (940 µmol) N^{α}-Boc-pyrraline in 1000 ml of 10% acetic acid was heated for 4 h at 70 °C whilst being continuously shaken in a water bath. After cooling to room temperature, 1000 ml water was added and the solution was freeze-dried. The residue was dissolved in 10 ml water and freeze-dried again. The yield was 253 mg (930 µmol). Elementary analysis: $C_{12}H_{18}O_4N_2 \cdot H_2O$ (mol. wt. = 272.31); calculated: C = 52.93%, H = 7.40%, N = 10.29%; found: C = 53.01%, H = 7.49%, N = 10.21%. UV spectroscopy: λ_{max} (methanol:water 4:1, v:v) 297 nm ($\log \varepsilon = 4.16$) and 265 nm (shoulder, $\log \varepsilon = 3.80$). 500-MHz NMR (D_2O): δ = 1.29–1.31 (m, 2H, β-CH_2), 1.65–1.68 (m, 2H, γ-CH_2), 1.75–1.78 (m, 2H, δ-CH_2), 3.60–3.62 (t, 1H, α-CH_2), 4.21–4.23 (t, 2H, ε-CH_2), 4.59 (s, 2H, 5-CH_2OH), 6.27 (d, 1H, 4-H), 7.04 (d, 1H, 3-H), 9.25 (s, 1H, 2-CHO).

Results and discussion

Pyrraline was originally prepared by Nakayama et al. [1] and since then with slight modifications by various authors [1–2, 5, 11–12] by a simple incubation of lysine with glucose and subsequent chromatographic isolation of pyrraline from the reaction mixture. This method is an ineffective and time-consuming procedure, leading only to very small amounts of pyrraline, as the lysine derivatization under such conditions ranges between 0.1 and 0.2%. Consequently, two methods for the direct synthesis of the lysine derivative are reported. According to Miller and Olsson [13], pyrraline can be prepared with an overall yield of 14% from 2-formylpyrrole via alkylation of the intermediate 2,5-diformylpyrrole with 6-bromonorleucine, reduction of one formyl group with sodium borhydride, followed finally by alkaline saponification and acid removal of the

N-protecting group. Seven intermediate products had to be prepared. In an alternative way proposed by Schüßler and Ledl [14], pyrraline was synthesized from N^{α}-trifluoracetyl lysine methyl ester and 2,9-dimethyl-deca-2,8-dien-4,7-dione, transformation of the pyrrole substituents by careful oxidation and final alkaline cleavage of the protection groups. In total, preparation of six intermediates and four chromatographic steps were necessary to improve the overall yield to 17%.

In contrast to these procedures, the method presented here is based on the direct reaction between the ε-amino group of Boc-Lys-OH with 3-deoxyglucosulose, the "natural" precursor of pyrraline in heated foods (Fig. 1). Preparation of 3-deoxyglucosulose was achieved according to [15] and was used without further chromatographic purification. Boc-Lys-OH is rather inexpensive and is available commercially. N^{α}-Boc-pyrraline is prepared by reacting Boc-Lys-OH with a fourfold molar excess of 3-deoxyglucosulose in the dry state, extraction of the reaction mixture and subsequent isolation of the pyrrole derivative by semipreparative isocratic reverse-phase liquid chromatography. N^{α}-Boc-pyrraline was clearly separated from resulting by-products, mainly 3-hydroxymethylfurfural and some minor compounds. Deprotection of the α-amino group was achieved in a large volume of diluted acetic acid to minimize size reactions at the pyrrole substituents. Under these conditions, no degradation of the lysylpyrrolealdehyde pyrraline was observed. Starting from 3 mmol of Boc-Lys-OH, 0.98 mmol pure pyrraline was obtained, corresponding to an overall yield of 31%. NMR data and UV spectra of the resulting product are in agreement with corresponding values reported in the literature [11, 13–14]. Correct elementary analysis was achieved. Amino acid analysis with photodiode array detection and ninhydrin derivatization showed only the peak related to pyrraline and no other UV-active or ninhydrin-positive compounds. Purity of the material is sufficient for it to be a reference material. Using this standard, we are presently investigating the formation of protein-bound pyrraline in foods and biological samples in order to prove whether this lysine derivative can be a useful indicator for advanced stages of the Maillard reaction.

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