A novel preparation of L-citrulline and L-homocitrulline

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Summary: L-citrulline was synthesized from L-ornithine monohydrochloride, which was treated with copper salt under alkaline conditions to protect α-NH_2, followed by formylation of δ-NH_2 with carbamide, and the removal of copper ion via the combination with sulfides or organic acids to release L-citrulline in satisfactory yield. L-homocitrulline was prepared from L-lysine monohydrochloride with the same method. This new method has the advantages of being environmentally friendly and suitable for industrial production.

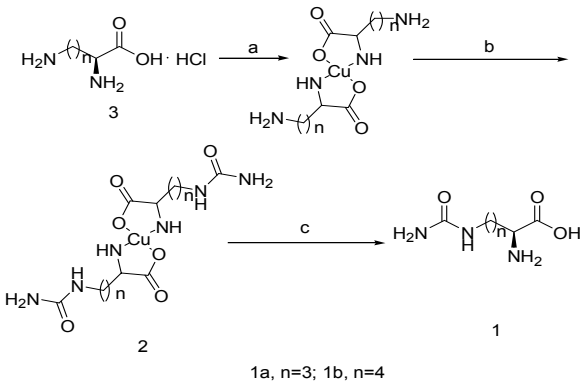
Key words: L-ornithine monohydrochloride, L-citrulline, L-lysine monohydrochlori- de, L-homocitrulline

Introduction

L-citrulline (**1a**) is a kind of ubiquitous amino acid in mammals [1, 2]. In the kidney, vascular endothelium and other tissues, L-citrulline can be readily converted to L-arginine, thereby providing a recycling pathway for the conversion of L-citrulline to nitric oxide (NO) via L-arginine [3, 4]. Recent studies have shown that oral administration of L-citrulline can decrease the proliferation of vascular smooth muscle cells, reduce blood pressure, protect myocardiocytes and prevent lipid peroxidation [5-7]. L-homocitrulline (**1b**), a structural analog of L-citrulline, has been widely used as a medical intermediate in the synthesis of polypeptides with promising application prospect [8, 9].

L-citrulline can be obtained by many methods, such as chemical synthesis [10, 11], extraction, enzymatic method [12-14] and microbial fermentation [15]. It is universally acknowledged that chemical synthesis is the only suitable approach to industrial production, by which L-citrulline is prepared by hydrolysis of L-arginine under alkaline conditions. However, due to the difficulty in concentration control of the alkali, several by-products, such as ornithine and urea, are produced by hydrolysis of L-arginine in the presence of alkali, leading to a tiny proportion of optical antipode D-citrulline and reduction of the purity product. Besides, the removal of copper from the copper-amino acid complex by the treatment with hydrogen sulfide is hazardous and may result in pollution to the environment. Thus, an efficient method for the synthesis of L-citrulline is required to improve the quality of the product and the reaction conditions.

Based on the previously published methods [16-19], we carried out the following route (Scheme-1) in our synthesis of L-citrulline. For the protection of α-NH_2, L-ornithine monohydrochloride was designated as the raw material to react with copper salts, such as copper sulfate pentahydrate, copper nitrate trihydrate, copper chloride dehydrate and copper carbonate, followed by formylation of δ-NH_2 with carbamide. Then the copper ions were removed via the combination with sulfides or organic acids to release L-citrulline in good yield. In addition, microwave irradiation was also applied in copper removal to investigate the effect on reaction duration and the product yield. L-homocitrulline was synthesized from L-lysine monohydrochloride with the same method.



Regents and conditions: a) copper salt, 100°C, alkalinity; b) carbamide, 100°C; c) sulfides or organic acids, 100°C.

Scheme 1: The synthetic route of L-citrulline and L-homocitrulline

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Results and Discussion

Normally, copper-compound amino acids, such as copper (α)-L-ornithine, copper (α)-L-arginine, copper (α)-L-lysine, are water-soluble. However, intermediates **2a** and **2b** are difficult to dissolve in water, probably due to the presence of intermolecular hydrogen bonds at the other end of the complexes. On the other hand, the insolubility of the intermediates facilitates the reaction and benefits the improvement of the yields (Table-1). Moreover, this method has the advantages that α-NH_2 protection by copper salt does not lead to the racemization of the end-products.

Table-1: Synthesis of copper (α)-L-citrulline and copper (α)-L-homocitrulline (**2a-b**)

Complexometric reagents	Molar ratio	Yield (2a) /%	Yield (2b) /%
Copper Carbonate	1:1	82.3	70.3
Copper Sulfate Pentahydrate	1:1	94.5	83.5
Copper Nitrate Trihydrate	1:1	87.3	76.2
Copper Chloride Dihydrate	1:1	84.9	88.5

The initial experiments to remove copper from copper-compound amino acids were performed using hydrogen sulfide. Despite the popularity of this canonical method, the problem of environmental pollution should be taken into account. In continuation of our efforts towards the decoppering reaction, by other sulfides and organic acids, we report herein a simple, environmentally friendly decoppering method. The treatment of **2** with other sulfides and organic acids in refluxing water led to the formation of **1** (Table-2). To our knowledge, this is the first report on copper removal from copper-compound amino acids using organic acids. This method of copper removal was inferior, in terms of yield, to that by sulfides, but was superior in environmental protection.

Table-2: Synthesis of L-citrulline and L-homocitrulline (**1a-b**)

Decoppering reagents	Yield (1a) /%	Reaction time (h)	Yield (1b) /%	Reaction time (h)
ferrous sulfide	92.9	0.8	78.9	1
sodium sulfide	76.0	1	68.1	1
zinc sulfide	37.1	5	30.4	2
hydrogen sulfide	97.0	1	95.0	1
ammonium sulfide	88.5	1	70.2	1
oxalic acid dihydrate	52.1	2	60.8	2
tartaric acid	15.0	4	10.0	4

Furthermore, microwave irradiation was involved, which has advantages in reducing reaction duration and increasing the product yield, compared with the conventional heating method (Table-3). However, profound research of microwave irradiation technology is required in further application in industrial production.

Table-3: Synthesis of L-citrulline and L-homocitrulline (**1a-b**) under microwave irradiation

	Reaction duration (min)	Yield (%)	radiation power (W)
1a	15	95.2	300
1b	20	83.5	500

Experimental

Reagents and Analysis

Melting points were measured on an XRC-1 melting point apparatus. Optical rotations were measured on a Perkin-Elmer model 341 automatic polarimeter. IR spectral analysis was carried out on a Perkin-Elmer Spectrum One spectrometer in KBr discs. Elemental analysis was performed using a Perkin Elmer CHNS analyzer. ^{1}H-NMR spectra were recorded in D_2O on a Bruker Avance 500 spectrometer; chemical shifts (δ) are reported in parts per million (ppm), with tetramethylsilane (TMS) as an internal standard. Solvents and reagents were purchased from their respective suppliers and used without further purification. Distilled water was prepared in laboratory.

General Procedures

General Procedures of Distilled Water Preparation

Distilled water was prepared in our laboratory using stainless steel electric water distiller (DZ-10) (Shijiazhuang Run Sun Hardware Co., Ltd.). Firstly, water was supplied until the water level exceeded heat wire. Secondly, the power was switched on and the condensed water tap was turned on. Then the distilled water was obtained when the vapour was encountered by condensed water, pH 6.9, A = 0.044.

General Procedure for the Synthesis of Copper (α)-L-Citrulline and –L-Homocitrulline(**2a-b**)

A mixture of **3** (0.059 mol), sodium bicarbonate (0.059 mol) and distilled water 60 ml was stirred in a round bottomed flask at ambient temperature. With the mixture dissolved, copper salt (0.059 mol) was added and then the mixture was hearted to reflux until TLC showed the disappearance of the starting material. When the reaction finished, urea was added and the resulting mixture was stirred

at 100°C for an additional 4 h (in case of insolubility of the copper salt in water, excess copper salt was filtered out). The resulting precipitates were isolated by filtration, washed with distilled water (4×50ml), and finally air-dried at 60°C for 5 h to obtain the intermediate **2**. The data correspond to the entries in Table-1.

General Procedures of the Synthesis of L-Citrulline and L-Homocitrulline (**1a-b**)

The intermediate **2** (0.02 mol), sulfide or organic acid (0.025 mol) and distilled water 100 ml were mixed together and refluxed in a round-bottomed flask or under microwave irradiation for the appropriate time (Table-2). Then the precipitates were filtered and the filtrate was decolorized by activated carbon and concentrated *in vacuo* to give white-like solid. Then the product **1** was isolated in its pure form by simple recrystallisation from the water-ethanol solution. The data corresponded to the entries in Table-2 and Table-3.

Spectral Date for Selected Compounds

L-citrulline **1a:** m.p. 220-222 °C (Lit.[20] m.p. 220-222 °C); $[\alpha]_D^{20}$ = + 22.0° (c = 2, 1 mol/L HCl); IR (KBr) ν cm^{-1}: 3454.15, 3333.25, 3095.41, 2940.20, 2869.13, 1652.15~499.31, 1405.19; Anal. Calc. for $C_6H_{13}N_3O_3$: C, 41.14 %, H, 7.48 %, N, 23.99 %. Found: C, 41.10 %; H, 7.52 %; N, 23.90 %. ^{1}H NMR (500 MHz, D_2O): δ = 3.75 (t, 1 H, C^2H), 1.80~1.86 (m, 2 H, C^3H_2), 1.52~1.58 (m, 2 H, C^4H_2), 3.14 (qu, 2 H, C^5H_2).

L-homocitrulline **1b:** m.p. 210-212 °C (Lit.[20] m.p. 210-212 °C); $[\alpha]_D^{20}$ = + 27.0° (*c* =1.8, 1 mol/L HCl); IR (KBr) ν cm^{-1}: 3439.45, 3343.25, 3080.41, 2938.20, 2865.13, 1628.20~1499.55, 1410.19; Anal. Calc. for $C_7H_{15}N_3O_3$: C, 44.43 %, H, 7.99 %, N,22.21 %. Found: C, 44.22 %, H, 7.93 %, N, 22.08 %. ^{1}H NMR (500 MHz□D_2O): δ = 3.80 (t, 1 H, C^2H), 1.77~1.83 (m, 2 H, C^3H_2), 1.35~1.40 (m, 2 H, C^4H_2), 1.49~1.54 (m, 2 H, C^5H_2), 3.10 (qu, 2 H, C^6H_2).

Conclusion

We have provided a convenient and novel method for the preparation of L-citrulline and L-homocitrulline, in which copper salts and sulfides (or organic acids) were used as protecting and deprotecting agents, respectively. This method can also be widely applicable to the synthesis of other amino acids and their derivatives [21-23].

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