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A New Synthesis of Calcium *N*-(5-Hydroxynicotinoyl)-L-glutamate and Its X-ray Diffraction Structure

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Abstract—A new synthesis of *N*-(5-hydroxynicotinoyl)-L-glutamic acid via a 5-hydroxynicotinic acid imidazolide intermediate has been developed. Its calcium salt (Ampasse) has been synthesized and its structure was studied by X-ray diffraction analysis. The reaction conditions for all stages of the process have been optimized and a method for the purification of the substance has been improved.

Keywords: Ampasse, calcium *N*-(5-hydroxynicotinoyl)-L-glutamate, *N*-(5-hydroxynicotinoyl)-L-glutamic acid, 5-hydroxynicotinic acid, diethyl L-glutamate, XRD

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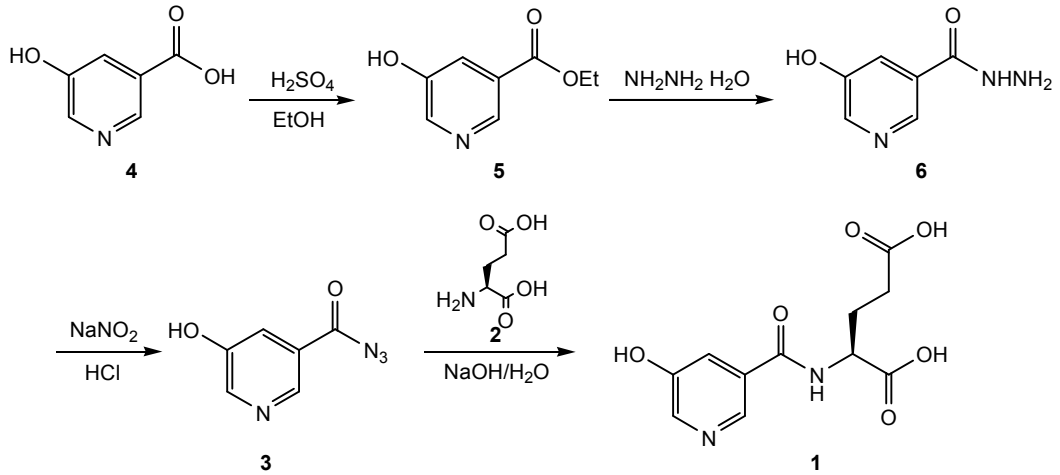
N-(5-Hydroxynicotinoyl)-L-glutamic acid, its mono- and divalent salts exhibit psychotropic (antidepressant and anxiolytic), neuroprotective, geroprotective, and anti-stroke action [1–6].

Until present, two methods of synthesis of *N*-(5-hydroxynicotinoyl)-L-glutamic acid **1** have been known.

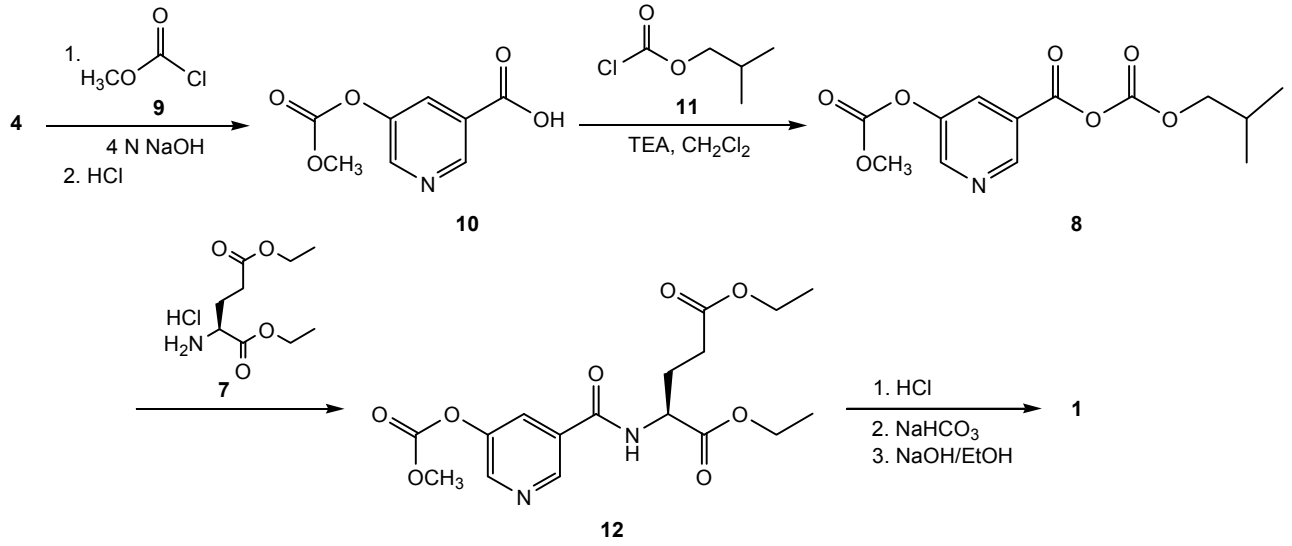
According to the first method [7], compound **1** is prepared by the reaction of L-glutamic acid **2** with 5-hydroxynicotinic acid azide **3** in the presence of NaOH in water at 20–23°C (Scheme 1).

5-Hydroxynicotinic acid **4** is converted to ethyl ester **5** in ethanol under reflux in the presence of H_2SO_4; treatment of ester **5** with aqueous hydrazine hydrate gives hydrazide **6**. The latter is reacted with sodium nitrite in the presence of aqueous HCl to obtain azide **3**, whose condensation with amino acid **2** in

Scheme 1.



Scheme 2.



aqueous alkali (pH 8–10) affords compound **1** (yield 35% per acid **4**).

The disadvantage of the described method is a low yield of compound **1**, as well as the necessity to use the unstable and explosive azide **3**, which undergoes tarring even when dried at 40°C and is impossible to use on an industrial scale.

According to the second method [8], compound **1** is synthesized by the reaction of diethyl L-glutamate hydrochloride **7** with mixed anhydride **8** (Scheme 2). Acid **4** is treated with ethyl chloroformate **9** to protect the OH group. The resulting 5-methoxycarbonyloxynicotinic acid **10** is treated with isobutyl chloroformate **11** in the presence of trimethylamine at –8 to –16°C to obtain mixed anhydride **12**, which is reacted with ester **7** in the presence of triethylamine. After completion of the reaction, the OH and COOH groups are deprotected, and target product **1** is isolated and recrystallized from ethanol (yield 56–62% per acid **4**).

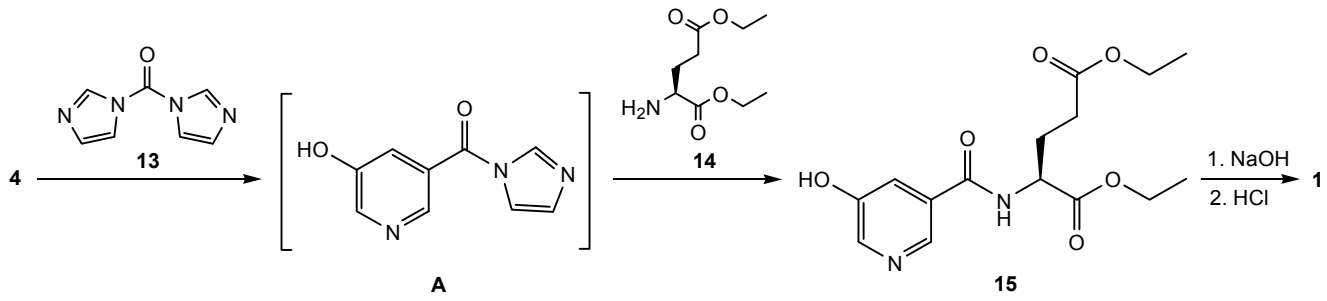
The disadvantages of the second method consist in the difficulty in controlling the degree of formation of mixed anhydride **8** and in the necessity to use costly chloroformates.

We developed a new synthesis of compound **1** (Scheme 3), which involves conversion of acid **4** into imidazolide **A** under the action of 1,1'-carbonyldiimidazole (CDI) **13** in toluene. Imidazolide **A** is reacted in situ with diethyl L-glutamate **14**. At the final stage, the COOH groups are deprotected. The yield of target product **1** after purification is 65–70% per acid **4**.

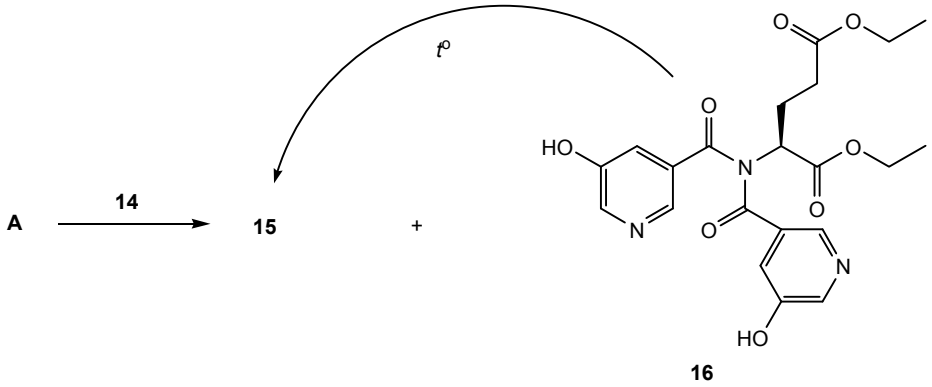
The first and second stages are accomplished in one pot without isolation of intermediate imidazolide **A**. After the second stage, the reaction mixture is concentrated. The third stage involves hydrolysis of the ester groups with simultaneous double purification of the product through the formation of disodium *N*-(5-hydroxynicotinoyl)-L-glutamate. To isolate the target product, the reaction mixture is acidified with conc. HCl to pH 4.

The reaction initially forms the target diethyl ester **15** (*M* 324.34) and by-product **16** (*M* 445.43) containing the second fragment of 5-hydroxynicotinic acid.

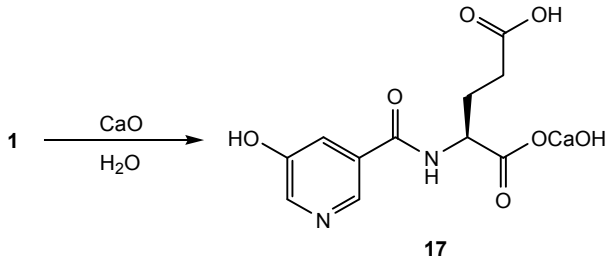
Scheme 3.



Scheme 4.



Scheme 5.



When heated at 70–80°C, by-product **16** (Scheme 4) spontaneously converts into ester **15**. Excess amino acid **14** favors this conversion. By varying the amino acid ester/starting amino acid ratio from 1.1 : 1 to 1.5 : 1 we found that the optimal ratio for the target reaction is 1.5 : 1. The time of heating at 80°C is 2 h (control by HPLC–MS).

Calcium *N*-(5-hydroxynicotinoyl)-L-glutamate (Ampasse) **17** (Scheme 5) was synthesized from acid **1**. To this end, CaO powder was added in portions to a suspension of acid **1**, and the resulting mixture was stirred at room temperature. In so doing, we observed liquefaction of the suspension. The undissolved solid material was filtered off. The transparent filtrate was heated to complete formation of the calcium salt. The product was precipitated from the aqueous solution with an organic solvent (methanol, ethanol, or acetone). The precipitate was filtered off, washed with the solvent, and dried to obtain pure compound **17**.

For X-ray diffraction structure analysis we grew crystals of calcium salt **17**.

The crystal of compound **17** (Fig. 1) contains two water molecules. One is incorporated into the calcium

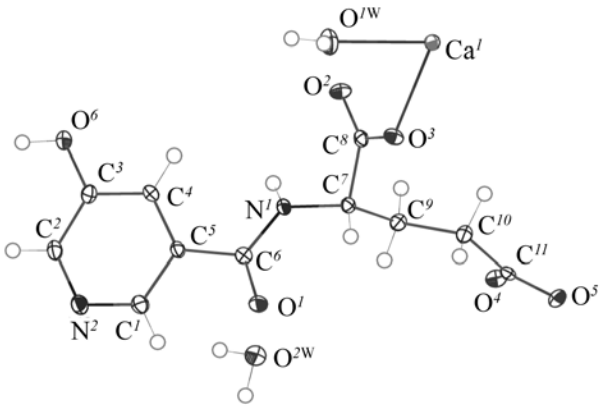


Fig. 1. Independent part of the unit cell of compound **17**. The atoms are drawn as thermal ellipsoids at the 50% probability level.

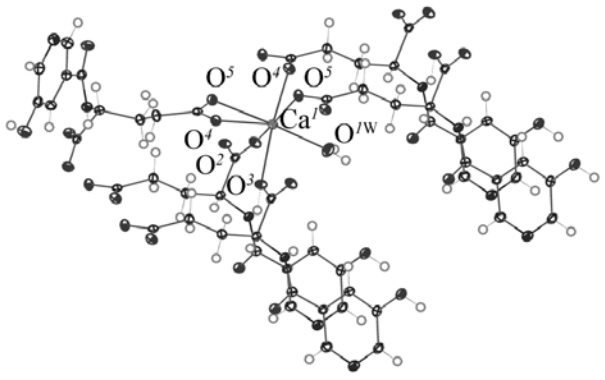


Fig. 2. Octahedral environment of the Ca^{2+} ion in the crystal of compound **17**. The atoms are drawn as thermal ellipsoids at the 50% probability level.

polyhedron, which the other no. One of the oxygen atoms of the COOH group "remote" from the aromatic ring coordinates to two calcium atoms, while the other oxygen, only to one calcium atoms. At the same time, in the "proximate" COOH group, one oxygen atom coordinates to one calcium atom, while the other coordinates to calcium and forms hydrogen bond with the NH group. The phenolic OH group forms hydrogen bond with the nitrogen atom in the aromatic ring (Fig. 2).

EXPERIMENTAL

Reaction progress and purity of compounds were controlled by HPLC−MS on a Thermo Acella 600 LC system coupled with an LCQ Fleet mass spectrometer; column Luna C18(2), 4.6 × 250 mm, was operated in a gradient mode (phase A: 0.1% TFA in water; phase B: 0.1% TFA in acetonitrile).

Diethyl *N*-(5-hydroxynicotinoyl)-L-glutamate (15). A suspension of 81 g (0.583 mol) of acid **4** and 89.1 g (0.550 mol) of CDI **13** in 600 mL of toluene was prepared in a 2-L flask equipped with a mechanical stirrer and reflux condenser stopped with $CaCl_2$ drying tube. The suspension was heated to 60–65°C (bath temperature) and stiired at this temperature for 30 min and, after bath temperature was elevated to 80–85°C, a solution of 176 g (0.867 mol) of diethyl L-glutamate **14** in 200 mL of toluene was added in one portion. The reaction mixture was stirred at 80–85°C for 1 h and then concentrated at 60°C in a vacuum of 20 mmHg to obtain 345 g of a raw product.

***N*-(5-Hydroxynicotinoyl)-L-glutamic acid (1).** A solution of 233 g (5.83 mol) of NaOH in 930 mL of water was added to 345 g of the raw diethyl ester **15** in a 3-L one-neck flask, and the mixture was stirred at room temperature for 2 h, after which 30 g of activated carbon was added, and stirring was continued for an additional 30 min and filtered on a Buchner funnel to remove the activated carbon. The filtrate was made acidic (pH 4) by adding 545 mL of conc. HCl and stirred for 1 h. The precipitate was filtered off (Schott filter no. 3), washed with water (3 × 600 mL), suspended in 750 mL of water, acidified to pH 4 with 65 mL of conc. HCl, and immediately neutralized to pH 8 with 90 mL of 40% NaOH. Activated carbon, 13 g, was then added, and the mixture was stirred for 30 min, the activated carbon was removed by filtration, and the filtrate was acidified to pH 4 with conc. HCl. The precipitate was filtered off, washed with water (3 × 500 mL), and dried at 55°C and a vacuum of 40 mmHg for 12 h to obtain 129 g (70%) of the target product, purity by HPLC 99.61%, main substance content by titration 80% (water 21%).

Calcium *N*-(5-hydroxynicotinoyl)-L-glutamate (17). A suspension of 109 g was prepared Powdered CaO, 16.7 g (0.297 mol), was added in portions to a suspension of 109 g (main substance content 83.93 g, 0.313 mol) of acid **1** in 800 mL of water in a 2-L beaker at room temperature. The suspension was stirred for 20 min, and solid materials was filtered off (Schott filter no. 4). The filtrate was transferred to a 4-L flask and stirred at 75–80°C for 1 h, after which 2400 mL of ethanol was added, and stirring was continued for an additional 30 min at 50–60°C. The reaction mixture was cooled to room temperature for 30 min, and the precipitate was filtered off (Schott filter no. 3), and washed with ethanol (2 × 200 mL), dried at 60°C in a vacuum of 20 mmHg for 12 h to obtain 77 g (80%) of the target product.

Single-crystal X-ray diffraction data for compound 17, $C_{11}H_{14}CaN_2O_8$ (M 342.32): orthorhombic, space group $P2_12_12_1$ (no. 19), a 5.1174(9) Å, b 8.0140(13) Å, c 33.969(6) Å, V 1393.1(4) Å^3, Z 4, T 100, μ(MoK_α) 0.495 mm^{-1}, D_{calc} 1.632 g/mm^3, 13909 measured reflection (2.4 = 2θ = 64.06), of which 4820 unique reflections (R_{int} 0.0670) were used in all computations. Final divergence factors: R_1 0.0437 [$I > 2\sigma(I)$] R_2 0.1086.

^{1}H NMR spectrum (D_2O), δ, ppm: 2.05–2.20 m (4H, glut H^{b2}), 2.34 m (2H, glut H^{g2}), 4.37 m (1H, glut H^{a}), 7.68 s (1H, pyr H^{4}), 8.25 s (1H, pyr H^{6}), 8.43 s (1H, pyr H^{2}), 8.64 s (1H, glut HN). ^{13}C NMR spectrum (D_2O), δ, ppm: 31 (glut C^{9}), 36.7 (glut C^{7}), 58.9 (glut C^{10}), 126 (pyr C^{4}), 133.7 (pyr C^{3}), 139.9 (pyr C^{2}), 142 (pyr C^{6}), 156.6 (pyr C^{5}), 170 (pyr C^{3}=O), 178.5 (glut C^{8}), 182.1 (glut C^{11}).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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