Identification of 4-sulfaminobutanoic acid in urine of a patient with nonketotic hyperglycinemia

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4-Sulfaminobutanoic acid, a previously unknown 4-aminobutanoic acid conjugate, was found in urine of a nonketotic hyperglycinemia patient.

Key words: 4-aminobutanoic acid, 4-sulfaminobutanoic acid; nonketotic hyperglycinemia.

Nonketotic hyperglycinemia is an inborn deterioration of metabolism characterized by high concentrations of glycine in blood, urine, and cerebrospinal fluid, while the content of urinary organic acids is normal.[1] The disease is caused by a molecular defect in P, H, and T proteins of glycine-cleavage multienzyme complex. In the classic phenotype, the illness develops in neonates and leads to death of patients within first two weeks of life. The present study aimed to confirm the diagnosis "nonketotic hyperglycinemia" for a 3-week old girl and to identify the unknown organic acid excreted with her urine.

Results and Discussion

The diagnosis "nonketotic hyperglycinemia" was in agreement with a clinical picture and a high concentration of glycine found in urine, blood plasma, and cerebrospinal fluid of this patient (*cf.* Ref. 1).

HPLC of organic acids revealed a large amount of an unknown compound (5.25 mg mL^{-1}) in urine; this compound was isolated by that method.

The absence of absorption in the UV spectrum at $\lambda > 230$ nm indicates the absence of aromatic and heteroaromatic groups and conjugated double bonds. In the ^{13}C NMR spectrum, four signals of comparable intensity (18.46, 31.62, 46.57, and 172.31 ppm) are present, allowing one to assume the presence of four carbon atoms in the simplest case. The signal at 172.31 ppm located in the area characteristic for carboxyl along with IR spectroscopy data (bands at 1675 and 1580 cm^{-1}) allow one to conclude that the isolated substance is a salt of carboxylic acid.

The analysis of the published data on the ^{13}C NMR spectra for four-carbon compounds containing a carboxylic group revealed that 4-aminobutanoic acid (**1**)[2] and 2-pyrrolidone (**2**)[3] have similar spectra.

The signals at δ 2.08, 2.49, 3.51, and 4.0 (the ratios of intensities 2 : 2 : 2 : 1) are present in the ^1H NMR spectrum of the isolated compound (see below). On the basis of these data, one can assume that the studied compound is a derivative of 4-aminobutanoic acid with an electron-withdrawing substituent at the nitrogen atom. It can be the N-sulfate (sulfamate) group (see the lowfield shift of H(4) in the spectra of **2** (3.44 ppm) and **3** (3.51 ppm) *vs.* that of **1** (2.99 ppm)). In fact, the MALDI negative ion mass spectrum of this compound (as NH_4^+ salt) shows one peak at m/z 182 corresponding to the pseudomolecular anion $HOOC(CH_2)_3NHSO_3^-$ (see structure **3**).

The IR and ^1H NMR spectra for compound **3** from Ref. 4 are in accordance with those obtained in the present work.

To our knowledge, isolation of 4-sulfaminobutanoic acid (**3**) from biological sources has not been reported previously. The female patient, from the urine of which 4-sulfaminobutanoic acid **3** was isolated, took 4-hydroxybutanoic acid (**4**). The amount of compound **3** excreted with urine is approximately equal to that of acid **4** injected. The latter is the normal metabolite of 4-aminobutanoic acid **1** and it is produced in the brain and kidney.[5] It is reasonable to assume that with loading

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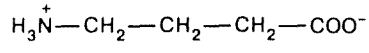

δ ^{1}H	2.99 (t)	1.88 (q)	2.88 (t)	
δ ^{13}C	40.6	24.8	35.6	182.7

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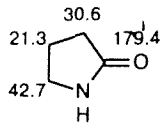

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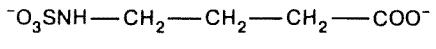

δ ^{1}H	4.0 (s)	3.51 (t, 2 H)	2.08 (m, 2 H)	2.49 (t, 2 H)
δ ^{13}C		46.57	18.46	31.62	172.31

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of an organism with 4-hydroxybutanoic acid, the equilibrium may be shifted to the opposite direction, although we find in the literature no reports recording such a transformation. Sulfatase-catalyzed sulfation of 4-aminobutanoic acid in kidney tissue has not been reported either, although sulfation of various substances is a well-known detoxification pathway. Thus one may conclude that the isolated **3** formed in the patient's organism from acid **4** through dehydration of the latter into succinic semialdehyde affording compound **1** transformed further to **3** through *N*-sulfation. However, experimental evidence of these speculations is beyond the scope of this paper.

Experimental

The reagents and solvents used were of HPLC grade. The eluents were filtered through PTFE membrane filters (0.5 μm) (Advantec Toyo, Japan) before use and degassed on ultrasonic bath.

The UV spectra were measured with a Specord UV-VIS spectrometer (Carl Zeiss). The IR spectra were obtained with a Perkin-Elmer 577 instrument in KBr pellets. The ^{1}H and ^{13}C NMR spectra were recorded with a Jeol FX 90Q spectrometer at 89.55 and 22.50 MHz, respectively, for solutions in D_2O (the residual signal of HDO was taken as δ 4.8); for ^{13}C NMR spectra, MeOH was used as the internal standard (δ 50.15 from tetramethylsilane). Mass spectra were run with a Kratos Kompact instrument in MALDI TOF negative ion mode.[6] Sinapinic acid was used as the matrix.

Deproteinization of urine. Urine (200 μL) was added to anhydrous ethanol (200 μL) and the mixture was allowed to stay for 1 h at −20 °C, and then centrifuged for 10 min at 14000 g. The supernatant was lyophilized, the residue was dissolved in water (400 μL), and the sample was stored at −20 °C.

Chromatography of urinary organic acid was performed with a Jasco 800 liquid chromatograph consisting of two 880-PU pumps, an 820SC controller, a Chromatocorder 12 integrator, an 865CO thermostat, and an 875UV photometric detector tuned at λ = 210 nm (System Instrument Company, Japan). Separations were carried out at ambient temperature on an Aminex HPX 87H, 7 μm (300×7.8 mm) column (Bio-Rad, USA) protected with a Separon C_{18}, 5 μm (50×4 mm) precolumn using isocratic elution with aqueous 6 m*M* H_2SO_4, pH 2.5, 0.4 mL min^{-1} flow. The other conditions are as described previously.[7] The collected fractions were treated with an excess of $BaCO_3$ to remove H_2SO_4. The spectra were measured for barium or ammonium salts; the latter was obtained by passing the Ba salt solution through a small column with Dowex WX-4 (H^+) and neutralization of the eluate with aqueous ammonia.

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