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Identification of 1,4-thiomorpholine-3-carboxylic acid (TMA) in normal human urine

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The sulfur-containing cyclic imino acid 1,4-thiomorpholine-3-carboxylic acid (TMA) has been identified in normal human urine. After the enrichment procedure with ion-exchange chromatography, the urine extracts were reacted with diazomethane. Gas-liquid chromatography revealed the presence of two peaks with the same retention times exhibited by authentic TMA after the same derivatization. The two compounds have been identified by mass-spectrometry as the monomethylated and dimethylated derivatives of TMA. This result represents the first indication of the occurrence of TMA in a mammalian sample.

Thiomorpholine-3-carboxylic acid, 1,4-; Sulfur compound; Cyclic imino acid; Urine; (Human)

1. INTRODUCTION

Recent papers from this laboratory reported the occurrence of 1,4-thiomorpholine-3,5-dicarboxylic acid (TMDA) in the bovine brain [1] and in normal human urine [2]. Glutamine transaminase [3] and a specific ketimine reductase [4] are assumed to be involved in the biosynthetic pathway of this cyclic imino acid starting from L-lanthionine. The decarboxylated form of L-lanthionine, *S*-aminoethyl-L-cysteine, is also actively transaminated by glutamine transaminase and therefore the occurrence in vivo of the monocarboxylated analogue of TMDA, i.e. 1,4-thiomorpholine-3-carboxylic acid (TMA); is reasonable. In this paper we report evidence of the presence of this new imino acid in normal human urine.

2. EXPERIMENTAL

2.1. *Materials*

Samples of normal urine were collected in the morning from fasting laboratory personnel, males and females, 20–40 years old, usual mixed diet, and submitted to the extraction procedure as soon as possible. A sample was stored for creatinine analysis performed by the Jaffe reaction using the test combination from Boehringer-Mannheim. Authentic TMA was prepared according to [5].

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2.2. *Extraction*

Urine samples (250 ml) were shaken with one spoon of Norite A, particle size 4–7 μm (Serva), filtered, adjusted to pH 2 with 4 M HCl, and introduced into a Dowex 50x8, 200–400 mesh (H^+ form), 2 × 18 cm column which was washed with H_2O to neutrality and eluted with 4 N NH_4OH. The fractions between 25 ml before and 60 ml after the alkaline front were collected and dried under reduced pressure at 50°C. The residue, dissolved in 10 ml of water, was introduced into a 2 × 18 cm Dowex 1x4, 200–400 mesh (OH^- form) column, which was washed with water to neutrality and eluted with 5 M CH_3COOH. The fractions between 25 ml before and 60 ml after the acid front were collected and dried under reduced pressure at 50°C. The residue was then solubilized in a minimum amount of water and stored at −20°C until analyzed.

2.3. *Identification*

An aliquot of extract was carefully dried in a desiccator under reduced pressure and submitted to the earlier reported methylation procedure [6]. The gas-chromatographic method for the identification of TMA was the same as reported for cyclothionine and TMDA determination [1,2] with the exception that the column temperature, for determination of TMA, was 150°C.

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GC-MS analyses were done on a GCD Pye Unicam gas-chromatograph connected to a LKB 2091 low resolution mass spectrometer, fitted with a digital PDP 11 computer. Chromatographic separations were performed on a 25 m × 0.25 mm i.d. Supelco SPB1 fused silica capillary column. The flow rate of He carrier gas was 1 ml/min. The column temperature was programmed from 80 to 200°C at a rate of 8°C/min; injector temperature was 170°C; the molecular separator temperature was 250°C. Mass spectrometer experimental conditions were ionization mode, electron impact, electron energy, 70 eV; ion source vacuum 1×10^{-7} mmHg.

3. RESULTS

Fig.1A reports part of a typical gas-liquid chromatogram of the urine extract prepared as indicated in section 2. Two peaks with the same retention times expected for TMA are present in the chromatogram. Authentic TMA added to the sample (fig.1B) coincides with both peaks. As reported in the earlier paper [6], treatment of TMA with diazomethane produces a double peak (note that in that paper TMA was abbreviated TMC), due to the carboxyl methylation for the slower running derivative, and to the methylation of both the carboxyl and the imino-nitrogen for the faster running derivative. GC-MS of the two TMA derivatives gave the profile illustrated in fig.2. The

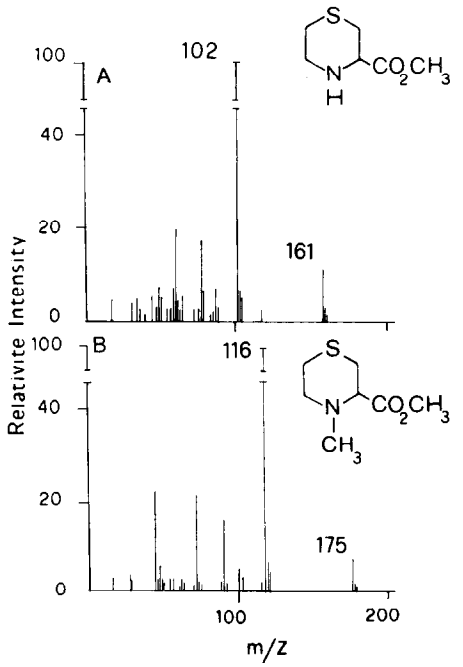

Fig.2. Mass spectra of (A) the slower running monomethylated derivative and (B) the faster running dimethylated TMA derivatives, extracted from normal human urine. The spectra should be compared with those reported in [6] for authentic TMA.

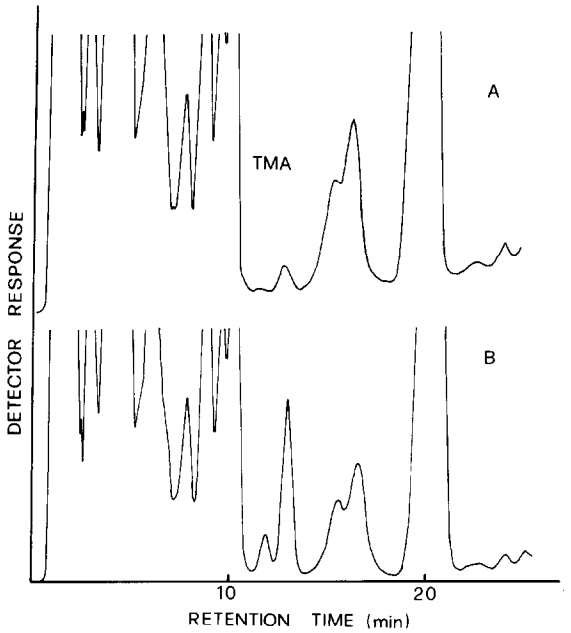

Fig.1. Gas liquid chromatography using a sulfur detector of enriched human urine extract. (A) Urine extract. (B) A + 0.5 μg authentic TMA. See text for details.

molecular ion (m/z = 161) for the monomethylated compound and (m/z = 175) for the dimethylated compound is seen in the diagrams. Furthermore the fragmentation pattern of the two compounds is almost identical to the fragmentation of the two derivatives of TMA reported in the previous paper [6]. The decarboxylated fragments with m/z 102 (161 minus $-CO_2CH_3$) and m/z 116 (175 minus $-CO_2CH_3$) are among the main features of the fragmentation patterns which further establish the identity of the compound detected in human urine with authentic TMA.

4. DISCUSSION

TMA is a compound detected in the form of sulfoxide in marine algae and named chondrine [7]; it has been obtained by reduction of chondrine [7] and prepared also chemically [3,8,9]. The present work represents the first indication of the occurrence of TMA in a mammalian sample. The precise quantitation of the content of TMA in nor-

mal urine requires the control of various physiological parameters and will be done later. The preliminary data obtained in the present work indicate the excretion of TMA in the order of 1–2 μmol per g creatinine. Apart from the possible alimentary source, the most plausible mechanism of TMA formation in mammals could be the monodeamination of aminoethylcysteine to the respective ketimine followed by the enzymatic reduction of the C=N bond. The enzymatic production of aminoethylcysteine by OH-serine exchange with SH-cysteamine by cystathionine-β-synthase (EC 4.2.1.22) has been reported earlier [10] and confirmed more recently [11]. Glutamine transaminase (EC 2.6.1.15) from various sources has been found to deaminate aminoethylcysteine producing the respective ketimine [3,12,13]. The reduction of the ketimine by a NAD(P)H requiring reductase has also been established recently [4]; this enzymatic route could account for the production of TMA detected in urine. An alternative mechanism of TMA production could be the monodecarboxylation of TMDA, i.e. a compound already known to be present in mammals [1,2]. Which one of these paths is the preferred one remains to be established.

Apart from the mechanism of its production, the presence of TMA in human urine appears a relevant finding. TMA is another cyclic sulfur containing imino acid to be added to the group of similar natural cyclic compounds recently identified in our laboratory in human urine [2] and in a mammalian brain [1]. In addition TMA is related structurally to pipecolic acid which represents the sulfur containing analogue. Since a functional role for pipecolic acid has been proposed [14] it could be of interest to compare the biochemical role of these two compounds in vivo and in vitro in mammals.

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