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Note

Simple gas chromatographic analysis of 3-methylthiopropionate in human urine

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In our earlier studies with healthy volunteers, it was demonstrated that exhalation of dimethyl sulfide and urinary excretion of α-keto-γ-methiolbutyrate increased markedly following administration of D-methionine, and that, by contrast, these excretions were quite trivial following administration of L-methionine [1—3]. In order to study this metabolic difference between the optical isomers of methionine and to verify the precursors of dimethyl sulfide, it is essential to determine 3-methylthioproprionate as one of the intermediates of D-methionine metabolism.

In the present communication, a simple and sensitive gas chromatographic quantitation of urinary 3-methylthiopropionate without derivatization is presented.

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MATERIALS AND METHODS

Synthesis and identification of 3-methylthiopropionate

3-Methylthiopropionate was chemically synthesized from 3-methylthiopropionaldehyde (methional), which was obtained from Sigma (St. Louis, MO, U.S.A.), by a modification of the synthesis of 3-thiophenecarboxylic acid as described by Campaigne and LeSuer [4]. The identity of 3-methylthiopropionate was verified by element analysis, nuclear magnetic resonance spectroscopy, and mass spectrometry. Since the sodium salt of 3-methylthiopropionate was difficult to crystallize and the potassium salt was highly deliquescent, the lithium salt was used as the standard in the present investigation.

Extraction, preconcentration, and quantitation of 3-methylthiopropionate in standard aqueous solution and in urine

A 20-ml standard solution of the lithium salt of 3-methylthiopropionate or 20- to 150-ml urine samples were acidified by hydrochloric acid to pH 1.0 and extracted three times with chloroform. The pooled chloroform phases were evaporated to dryness and dissolved in 500 μl of methanol. Three microliters of the final methanol solution of 3-methylthiopropionate were directly injected into the injection port of the gas chromatograph.

Gas chromatography

A gas chromatograph equipped with a flame photometric detector (FPD) and a hydrogen flame ionization detector (FID) (Model GC-7AGPrFFP, Shimadzu, Kyoto, Japan) was used for the present investigation. The glass column (2.1 m × 3 mm I.D.) was packed with 10% SP-1200/1% H_3PO_4 on Chromosorb W AW 80–100 mesh (Wako, Osaka, Japan). The column temperature was initially isothermal at 140°C for 32 min, then increased to 195°C at a rate of 16°C/min with a hold at 195°C. The injection port temperature was 200°C. The FPD with a 394-μm filter was operated at 750 V. Nitrogen was the carrier gas at a flow-rate of 50 ml/min. The recorder responses were calculated by a Chromatopac C-RIA (Shimadzu).

RESULTS AND DISCUSSION

For the separation of 3-methylthiopropionate by gas chromatography, the operating conditions were determined as described in the Methods. The retention time of 3-methylthiopropionate was 19.36 min (Fig. 1). Reproducibility of analyses and linearity of the calibration curve were then studied by triplication at three different concentrations (Table I). The linear range for amount of 3-methylthiopropionate injected was 1 ng to 14.4 μg. The sensitivity of the FPD was about ten times higher than that of the FID and the detection limit was 1 ng. The recovery of 3-methylthiopropionate from urine was compared with that from distilled water solution at three different concentrations: 108% at 10 nmol/ml, 105% at 100 nmol/ml and 96% at 1 μmol/ml. Since the

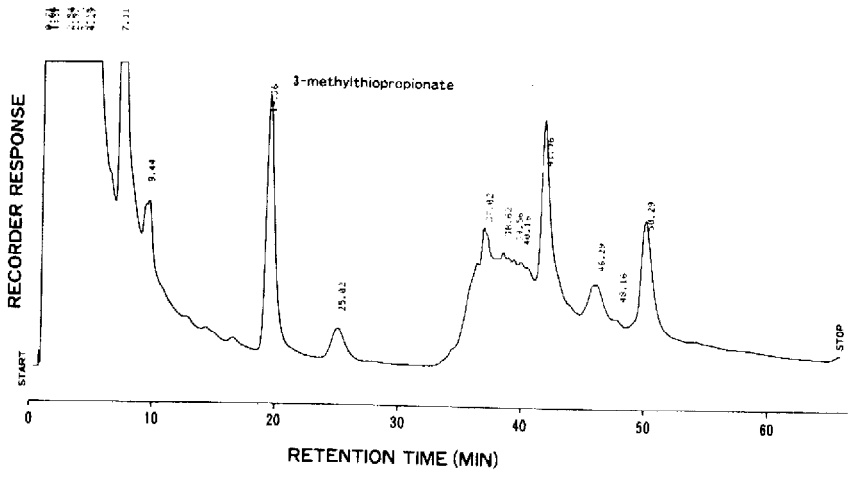

Fig. 1. Gas chromatographic analysis and flame photometric detection of 3-methylthiopropionate in a 2-h urine sample obtained from a 43-year-old healthy male following ingestion of 2 g of D-methionine. Retention time of 3-methylthiopropionate: 19.36 min.

TABLE I

REPRODUCIBILITY* OF THE DETERMINATION OF 3-METHYLTHIOPROPIONATE IN METHANOL SOLUTION BY GAS CHROMATOGRAPHY

Dose injected	Sensitivity and attenuation	Recorder response	Mean	S.D.
3.36 μg	$10^3 \times 32$	1322	1345	43.3
		1308		
		1406		
336 ng	10 × 8	2224	2179	127.4
		2005		
		2307		
33.6 ng	10 × 1	101	92	7.3
		83		
		92		

*$y = 4.9555 \cdot x^{0.42797}$ ($r = 0.9998$), where y = dose injected and x = recorder response.

creatinine and/or 3-methylthiopropionate concentration in urine varies over a wide range, it is difficult to specify in advance the sample volume of urine for measuring this acid. The detection limit of 3-methylthiopropionate was 1 ng per 3 μl final methanol solution. Because of its specificity and higher sensitivity, flame photometric detection was more useful.

In most studies on human methionine metabolism, methionine concentration has been directly measured in blood or urine [5—10]. In addition, there are other approaches using the quantitative analysis of several metabolites of methionine [11—13]. In our earlier studies, marked increases in exhalation of dimethyl sulfide and urinary excretion of α-keto-γ-methiolbutyrate following administration of the D-isomer of methionine were confirmed in

healthy volunteers [1—3]. Recently, the transamination pathway of L-methionine metabolism has been given attention and has been re-investigated in vitro in rat and monkey liver homogenate and in rat liver mitochondrial systems [14—17].

The simple gas chromatographic determination of 3-methylthiopropionate in urine presented here would seem to play an important role in the studies on the metabolism of methionine isomers.

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