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Note

Gas chromatographic and gas chromatographic—mass spectrometric studies on α-keto-γ-methylthiobutyric acid in urine following ingestion of optical isomers of methionine

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In current gas chromatographic (GC) analysis, the flame photometric detector (FPD) has come into frequent use in the specific detection of sulfur compounds. In 1977, Favier and Caillat [1] reported an analytical method of determining urinary α-keto-γ-methylthiobutyric acid by the use of a GC—FPD system for patients with hypermethioninemia. In our earlier studies using this

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analytical system, a remarkable difference in dimethyl sulfide exhalation was observed after the administration of optical isomers of methionine [2, 3].

In the present investigation, a quinoxalinol derivative of α-keto-γ-methylthiobutyric acid was analyzed using GC and gas chromatography—mass spectrometry (GC—MS) with the view to study first the metabolic difference between the optical isomers of methionine and, secondly, the urinary excretion of α-keto-γ-methylthiobutyric acid after the ingestion of L- or D-methionine.

MATERIALS AND METHODS

Extraction and derivatization

α-Keto-γ-methylthiobutyric acid (ACMTB) was obtained from Sigma (St. Louis, MO, U.S.A.). A 5-ml standard solution of ACMTB or urine samples of 5 to 25 ml were acidified by hydrochloric acid to pH 1.0 and extracted five times with diethyl ether. The pooled ether phase was concentrated to 5 ml under vacuum and was further extracted three times with a 5% aqueous solution of $NaHCO_3$. The water phase was re-acidified by hydrochloric acid to pH 1.0, and ACMTB was converted to the quinoxalinol derivative with *o*-phenylenediamine hydrochloride using the method of Favier and Caillat [1].

Apparatus and analytical conditions

A gas chromatograph (Model GC-6APFp, Shimazu, Kyoto, Japan) equipped with FPD was used for the present investigation. The glass column (3 m × 3 mm I.D.) was packed with 2% SP-2250 on Chromosorb W AW DMCS 100—120 mesh. The column temperature was initially isothermal at 180°C for 5 min, then increased to 240°C at the rate of 2°C/min with a hold at 240°C. The FPD with a 394-μm filter was operated at 750 V; the detector temperature was 300°C. Nitrogen was the carrier gas at a flow-rate of 50 ml/min.

The quinoxalinol derivative of ACMTB was identified with a gas chromatograph—mass spectrometer (Hitachi RMU-6M, Hitachi, Tokyo, Japan) equipped with a 002B Datalyzer. Electron energy was 20 eV and 60 μA; accelerating potential, 3200 V; ionization source temperature, 220°C and pressure, $1.2 \cdot 10^{-7}$ torr. Since the background mass spectra of SP-2250 had *m/e* 220, SE-30 was used as the liquid phase for the analytical column in GC—MS analyses.

Methionine loading test

Both L- and D-methionine were obtained from Wako (Osaka, Japan). After overnight fasting, three healthy subjects (one male and two females) ingested 3 g of L-methionine or 3 (or 1) g of D-methionine and urine samples were collected every hour for 4 h. After the determination of creatinine concentration (Technicon AA-II, Technicon, Tarrytown, NY, U.S.A.), urine samples, spiked with thymol, were stored at —20°C. The interval between each test per person was three weeks or more.

RESULTS AND DISCUSSION

The retention time of the quinoxalinol derivative of ACMTB by GC analysis

was 22.75 min. This derivative was quantitated by absolute calibration on logarithmic paper and its detection limit was 5 ng. As stated above, this derivative was further analyzed with GC—MS using SE-30 as the liquid phase for the analytical column (Fig. 1).

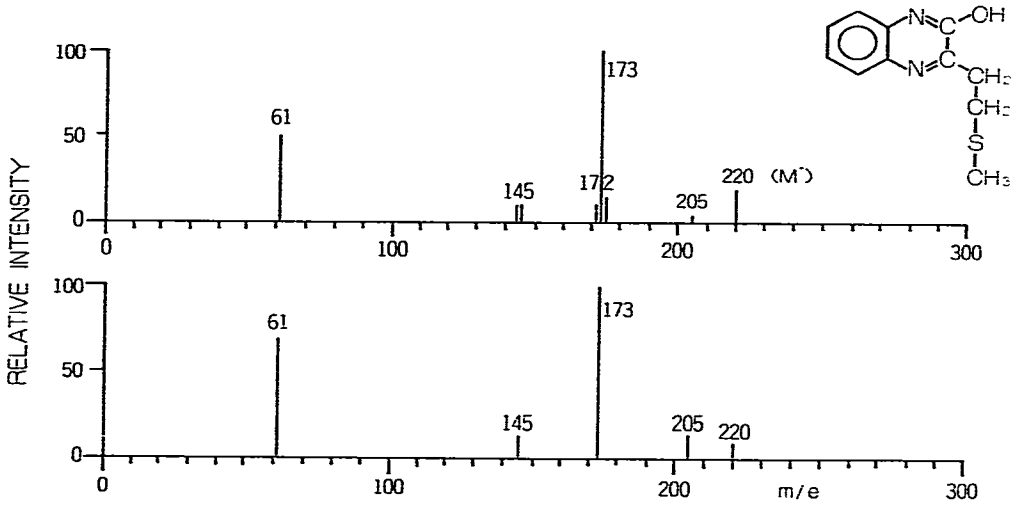

Fig. 1. Mass spectra of the quinoxalinol derivative of α-keto-γ-methyl-thiobutyric acid (ACMTB). Top, standard ACMTB solution; bottom, urine sample of 36-year-old female after ingestion of 3 g of D-methionine.

TABLE I

URINARY EXCRETION OF α-KETO-γ-METHYLTHIOBUTYRIC ACID AFTER THE INGESTION OF D- OR L-METHIONINE

Experimental subjects			Methionine ingested (g)	Urinary α-keto-γ-methylthiobutyric acid (μg/mg Cr)		
Subject	Sex and	age (years)		Before ingestion	Pooled urine A (1st and 2nd hour)	Pooled urine B (3rd and 4th hour)
A	female	36	D-methionine 3	0.11	578.1	766.6
B	male	42	D-methionine 3	0.05	155.8	81.5
B	male	42	D-methionine 1	0.29	272.0	63.5
B	male	42	L-methionine 3	0.21	0.2	0.4
C	female	36	L-methionine 3	0.09	29.7	58.5

The urinary ACMTB concentration per ml was converted into μg per mg of creatinine (μg/mg Cr) (Table I). The concentration of ACMTB before ingestion of the methionines was 0.15 ± 0.10 (mean ± S.D.) μg/mg Cr (n = 5) in all the exprimental subjects. In the case of 3 g of D-methionine ingestion, the concentration increased noticeably in pooled urine A (1st and 2nd hour) and pooled urine B (3rd and 4th hour) and this tendency was also observed even in case of 1 g of D-methionine ingestion. In the case of 3 g of L-methionine ingestion,

however, the increase in concentration was apparently smaller than in the case of D-methionine ingestion.

In our earlier studies in healthy subjects, it was demonstrated that the concentration of dimethyl sulfide in expired alveolar gas (alv-DMS) increased markedly from the fasting level of 2.0 ± 1.9 to the maximum concentration of 66.0 ± 42.1 ng/dl (mean ± S.D.) (n = 6) following the ingestion of 1 g of D-methionine. In contrast, alv-DMS increased only slightly after the administration of 2 g of L-methionine; the fasting level was 2.7 ± 1.3 and the maximum concentration was 4.6 ± 2.6 ng/dl (n = 4). In spite of the marked changes in alv-DMS concentration, the amount of methyl mercaptan in expired alveolar gas (alv-MM) did not always increase and it did not behave parallel to alv-DMS [2, 3].

It is believed that deamination, followed by reamination to the L-isomer, is the method by which the body uses the so-called unnatural isomer of many amino acids [4]. However, an alternate pathway for methionine metabolism has been recently demonstrated in vitro in rat and monkey liver homogenate, independent of S-adenosylmethionine formation. This pathway is thought to involve conversion of methionine to its keto analogue which then is oxidatively decarboxylated to 3-methylthiopropionic acid [5]. If 3-methylthiopropionic acid is further methylated to dimethyl β-propiothetin, dimethyl sulfide may be produced via this pathway. Dimethyl sulfide will be also produced by the methylation of methyl mercaptan, which has been stated to arise from methionine itself or from ACMTB [6, 7], or it may be formed from α-keto-γ-methylthiobutyric acid-δ-dimethyl sulfonium produced beforehand by the methylation of ACMTB.

In view of our results with urinary ACMTB and alv-DMS, it seems likely that a significant amount of D-methionine may be metabolized via the proposed alternative pathway and L-methionine may be metabolized via the normal trans-sulfuration pathway which involves formation of homocysteine. Further studies are in progress to investigate the metabolism of methionine isomers and their intermediates.

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