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Note

A method for the gas chromatographic determination of urinary 1,4-methyl-imidazoleacetic acid

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1,4-Methyl-imidazoleacetic acid (MeIAA) is a major metabolite of histamine in man[1] and its urinary excretion is thought to reflect the endogenous release of histamine[2–4]. The role of histamine release in various physiological and pathological conditions may be facilitated by measurement of urinary MeIAA[5]. This metabolite has been determined previously by gas chromatography (GC) based on the formation of a methyl ester[4,6] and by thin-layer chromatography (TLC)[2]. The GC method described here is an adaptation of the TLC determination and involves the separation of MeIAA from urine by ion-exchange chromatography followed by the formation of the ethyl ester (MeIAAEt). This method has been applied to the study of histamine release in normal human subjects who have inhaled cotton dust[7].

EXPERIMENTAL

Reagents

All chemicals were of analytical reagent or spectrosol (S) grade. Doubly distilled water was used where appropriate. The buffer solutions[2] used were: Buffer I — 22.2 ml of 4 *M* $K_3PO_4 \cdot H_2O$ plus 77.8 ml of 1.5 *M* citric acid with 0.1% (v/v) toluene as a preservative; buffer II — 2.17 *M* $K_3PO_4 \cdot H_2O$. Dowex 1-X10 anion-exchange resin (200–400 mesh) was prepared in the acetate form by washing several times with 3 *N* HCl followed by 3 *N* NaOH and finally with 8 *N* acetic acid. Between each treatment the resin was washed with CO_2-free distilled water. After the acetic acid treatment washing was continued until the pH of the eluate had reached 8.8. Ethanolic HCl was prepared by saturating super-dry ethanol with dry HCl gas. After determining the strength of the solution by titration, the ethanolic HCl was diluted to 1 *N* with super-dry ethanol.

Extraction of MeIAA from urine

24-h urine samples were collected from normal adult male subjects in bottles containing 10 ml of 11 *N* HCl. Urine was stored at −20° until analysed. Four 50-ml aliquots were taken from each urine specimen. To two of these samples authentic MeIAA (100 μg) was added in order to assess the recovery in the assay procedure.

Each 50-ml sample was concentrated under reduced pressure at 50° to 10–15 ml using a rotary film evaporator. The pH of the concentrated urine was taken to

8.8 with 2 *N* NaOH and the volume was adjusted to 25 ml with water. The urine sample was then centrifuged at 1000 × *g* for 15 min and the supernatant was applied onto a 15-cm column of Dowex 1-X10 in the acetate form (I.D. 0.6 cm). The column was operated at room temperature (20°) and the flow-rate was regulated to 12–15 ml/h. Following the urine sample, the column was washed with 25 ml of CO_2-free distilled water. MeIAA was then eluted with 60 ml of 0.5 *M* acetic acid. The eluate was evaporated to dryness under reduced pressure at 50°.

Esterification of MeIAA

100 ml of 1 *N* HCl in super-dry ethanol was added to the dried residue obtained from the extraction step. The mixture was then refluxed at 100°, the open end of the reflux condenser being fitted with a $CaCl_2$ drying tube. After 2 h the mixture was cooled under cold water and neutralized by the addition of 35 g of Na_2CO_3. On standing for 30 min, the mixture was filtered through a Whatman No. 1 filter paper and the solid retained by the filter was washed several times with small volumes of super-dry ethanol. The filtrate and washings were combined and evaporated under reduced pressure at 40° to yield a small quantity of a brown oil. This was dissolved in 25 ml of buffer I and 10 ml of buffer II. The pH of the resulting solution was adjusted to 7.5 with either 1.5 *M* citric acid or 4.0 *M* potassium phosphate.

Extraction of esterified MeIAA

The solution from the esterification procedure was placed in a continuous liquid–liquid extractor and was extracted with 40 ml of dry diethyl ether at 40° for 2 h. The ether extract was concentrated under reduced pressure at 40° to a volume of about 3 ml. This solution was then quantitatively transferred to a 10-ml tapered tube containing the internal standard (methyl docosanoate, 300 μg) and evaporation was continued to dryness. The residue was stored in the stoppered tube at −20° until required for GC when it was dissolved in 300 μl of $CHCl_3$ (S).

GC conditions

The gas chromatograph used was a Pye Model 104 equipped with a hydrogen flame ionization detector and operated isothermally at 175°. The stationary phase was 6% ethylene glycol adipate on Diatoport S (80–100 mesh) coated with 1% polyvinylpyrrolidone and packed into a 2.7-m glass column (I.D. 0.4 cm). The flow-rate of the carrier gas (nitrogen) was 60 ml/min and the injection point was maintained at 200°. All compounds and urine extracts were dissolved in 300 μl of $CHCl_3$ (S) prior to GC.

RESULTS

Methyl docosanoate was chosen as the internal standard for identification and calculation of MeIAAEt. On the GC column employed the internal standard had a retention time of 45 min compared with 35 min for MeIAAEt (Fig. 1). It was impracticable to use an internal standard with a retention time shorter than 30 min since peaks due to other compounds present in the extracts of urine were being eluted during this earlier time period. As both peaks of methyl docosanoate and MeIAAEt

were symmetrical, the product (P) of the height and width of the peak at half height was employed for quantitation.

A calibration curve was prepared by adding various amounts of authentic MeIAAEt in $CHCl_3$ (S) to a series of tubes containing 300 μg of the internal standard. The solutions were evaporated to dryness under reduced pressure at 40° and then reconstituted in 300 μl of $CHCl_3$ (S) for GC analysis. A linear relationship between amount of MeIAAEt and ratio of P of ester/internal standard was obtained over the range studied (Fig. 2).

A known amount of authentic MeIAA was added to samples of each urine

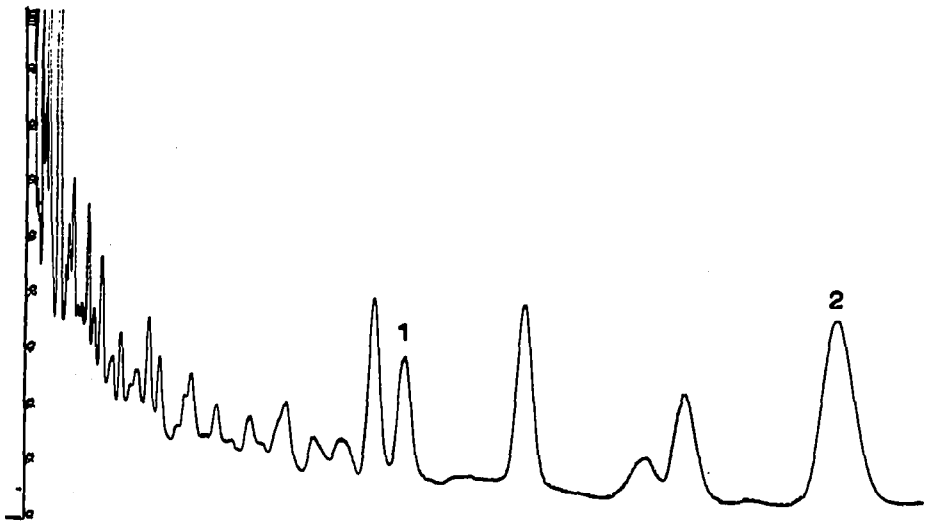

Fig. 1. Gas chromatogram of a urine extract. 1 = Ethyl ester of 1,4-methyl-imidazoleacetic acid; 2 = methyl docosanoate (internal standard).

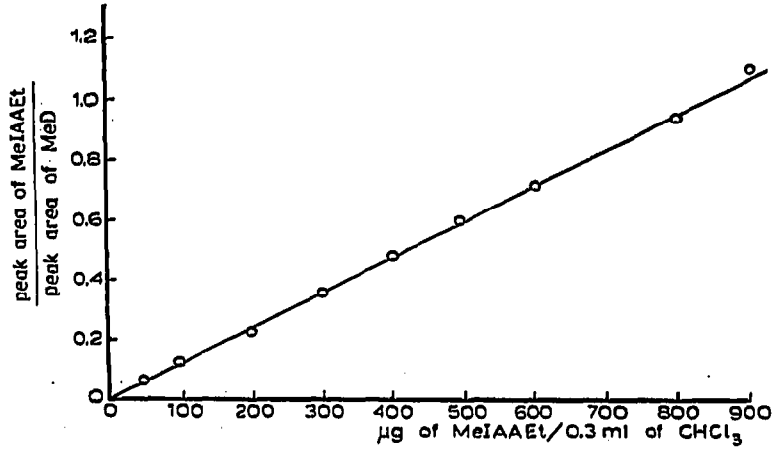

Fig. 2. The standard calibration graph of the ratio of the peak areas of the ethyl ester of 1,4-methyl-imidazoleacetic acid (MeIAAEt) and methyl docosanoate (MeD) against the number of μg of MeIAAEt in the extract.

specimen to estimate recovery during the various stages of the procedure described above. For each urine, duplicate analyses were made. In ten experiments the recovery of MeIAA added to urine was 76.7 ± 2.9% (mean ± S.E.). The recovery of MeIAA added to water was 82.3 ± 0.9% (mean ± S.E.). The recovery was found to be independent of the amount of MeIAA added (50–100 μg). Recoveries of 81.6 ± 0.8%[4] and 59.2 ± 1.5%[6] for MeIAA added to urine have been reported previously.

Using the above procedure a comparison of urinary excretion of MeIAA in four male subjects was made under varying dietary conditions. The 24-h excretion of MeIAA was 14.7 ± 3.4 mg (mean ± S.E.) when an unrestricted high-protein diet was allowed. On a low-protein low-histamine diet[8] a significantly ($P < 0.05$) lowered excretion (5.8 ± 1.2 mg) of MeIAA occurred. These results are closely in agreement with those of Granerus[9] and indicate the importance of performing investigations of histamine metabolism in man under dietary control. Using this technique, it has been found that normal human subjects on a controlled diet excreted increased amounts of MeIAA following the inhalation of cotton dust[7]. These results have been taken to indicate that cotton dust releases histamine in the lungs and that the latter agent is responsible for acute changes in lung function seen after inhalation of the dust.

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