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GAS CHROMATOGRAPHIC SEPARATION OF HISTAMINE AND ITS METABOLITES*

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SUMMARY

A new, rapid and simple gas–liquid chromatographic method is described for the simultaneous determination of histamine, 1-methylhistamine, 4-imidazoleacetic acid and 1-methyl-4-imidazoleacetic acid. These compounds are derivatized at 60° in one fast 30-min reaction with bis(trimethylsilyl)acetamide–trimethylchlorosilane and are then separated on OV-17. No previous methylation of the acids is needed. A complete profile of the four metabolites can be obtained in 8 min with baseline resolution, and there are no tailing effects. The quantitative response curves are linear in the nanogram range, with detection limits of the order of 10 ng for the acids and 100 ng for the amines. The structures of these new trimethylsilyl derivatives have been verified by combined gas–liquid chromatography–mass spectrometry.

INTRODUCTION

The metabolism of histamine, although related to multiple physiological and pathophysiological processes[1–5], has received comparatively less attention than most of the other biogenic amines. This can be explained in part by the lack of an analytical method capable of providing the necessary specificity to cope with the relatively high levels of interferences present in biological extracts. Without special precautions, the bioassay and the chemical methods of detection may give false values for histamine[4].

Studies carried out by subcutaneous injections of [^{14}C]histamine[6] have demonstrated two main paths for the catabolism of histamine. One path involves the oxidative deamination of histamine to form 4-imidazoleacetic acid, and the other path, the most important quantitatively, involves the methylation of histamine to 1-methylhistamine followed by an oxidative deamination to 1-methyl-4-imidazoleacetic acid. There are some reports[7–11] of the determination of one or both of the two

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imidazoleacetic acid metabolites in urine samples, and recently the quantitative determination of these four metabolites by thin-layer chromatography (TLC) has been described[12]. However, to our knowledge, there is no suitable method for the concurrent qualitative and quantitative determination of all of the four metabolites, thus providing in one analysis a record of their metabolic profile in biological samples. Undoubtedly, such a capability would lead to a better definition of the metabolic path of histamine in various pathological states. The TLC method reported[12] is useful at the microgram level, but is rather complex and does not yield a true chromatographic profile as could be obtained by gas–liquid chromatography (GLC). Nevertheless, in spite of the obvious advantages of GLC methods in terms of their sensitivity, speed and extreme specificity when coupled with mass spectrometric (MS) detection, their application to the study of histamine metabolism has been mainly restricted to the acidic metabolites[7–11] because of the experimental difficulties associated with the GLC of histamine[13,14].

The present technique is based on a simple one-step simultaneous derivatization of histamine, 1-methylhistamine, 4-imidazoleacetic acid and 1-methyl-4-imidazoleacetic acid in combination with GLC separation and MS detection, as has already been described for other biogenic amines[15].

EXPERIMENTAL

Reagents

Acetone, methanol, N,O-bis(trimethylsilyl)acetamide (BSA), N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) were chromatography grades obtained from local commercial sources (Xpectrix Int., Barcelona, Spain). Histamine dihydrochloride (HT) was obtained from Sigma, St. Louis, Mo., U.S.A., and 1-methylhistamine (MHT), 4-imidazoleacetic acid (IMAA) and 1-methyl-4-imidazoleacetic acid (MIMAA) were obtained from Calbiochem., Los Angeles, Calif., U.S.A.

Gas–liquid chromatography

The glass column (2 m × 2.5 mm I.D.) was washed with acetone, methanol, water and methanol and silanized for 24 h with a 5% solution of hexamethyldisilazane (DMCS) in toluene. After washing with methanol, the column was dried and immediately packed with 3% OV-17 on Gas-Chrom Q (100–120 mesh). This column was usually conditioned for at least 48 h at 250–300° and silanized by repeated injections of BSTFA before use. The separations were achieved on a Perkin-Elmer Model 900 gas chromatograph equipped with dual flame ionization detectors. The samples were injected by use of 1-μl and 10-μl Hamilton syringes.

Gas chromatography–mass spectrometry

The mass spectra of the derivatives of histamine and its metabolites were obtained on a Hitachi RMU-6H mass spectrometer coupled, through a single-stage gold-jet-type molecular separator, to a Perkin-Elmer 3920 gas chromatograph. Mass spectra were recorded under the following conditions: chamber voltage, 70 eV; emission, 80 μA; current, 55–60 μA; acceleration voltage, 2400 V; source temperature, 180–200°.

Preparation of derivatives

The four histidine metabolites (HT, MHT, IMAA and MIMAA) were derivatized in one step with a mixture of BSA and TMCS (4%). For this purpose, 20 μl of a stock solution of the metabolites in methanol (1 μg/μl) were evaporated to dryness and allowed to react for 30 min at 60° with 40 μl of the BSA–TMCS mixture previously prepared, thus obtaining the corresponding silylated derivatives (TMS). The reaction mixtures were injected directly into the GLC columns.

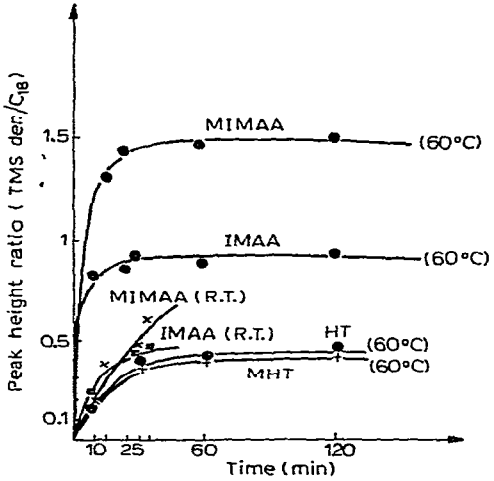

Fig. 1. Kinetic response curves obtained by GLC monitoring of the reaction of histamine (HT), 1-methylhistamine (MHT), 4-imidazoleacetic acid (IMAA) and 1-methyl-4-imidazoleacetic acid (MIMAA) with BSA–TMCS. The ordinate represents the value of the ratio between the peak height of the respective TMS derivatives and the peak height of a n-C_{18} alkane as internal standard.

RESULTS AND DISCUSSION

Reaction kinetics and detection limits

Fig. 1 shows the time responses obtained by reaction of HT, MHT, IMAA and MIMAA with BSA–TMCS at 60° and at room temperature using a flame ionization detector (FID). In accordance with these results, the derivatization of these compounds was carried out at 60° for 30 min, by which time the maximum reaction yields are obtained for the four metabolites. However, on an equivalent-weight basis, the FID responses of MIMAA and IMAA were about three and two times greater than those given by the amines. From a practical point of view this implies a lower limit of detection for the acidic metabolites as reflected by the respective response curves shown in Fig. 2. Both of these curves pass through the origin and are linear in the nanogram range. As indicated in Fig. 2, the lowest detection level achieved for MIMAA was 10 ng, and that for HT was 100 ng. In order to achieve maximum reaction yields it is important to avoid the use of solvents, and also to completely evaporate the methanol of the stock solutions, removing all traces of water before adding the BSA–TMCS mixture. Under these conditions the derivatives were stable for at least 3 h.

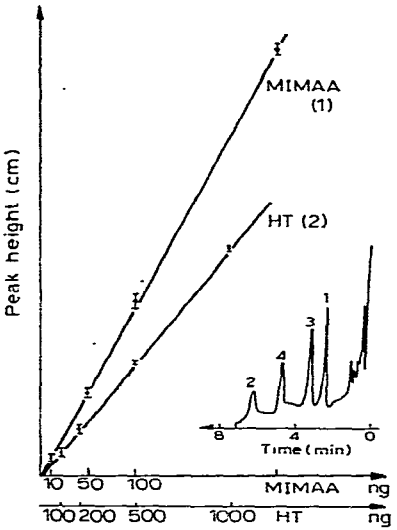

Fig. 2. FID response curves obtained with the TMS derivatives of 1-methyl-4-imidazoleacetic acid (1) and histamine (2). Each data point represents the mean value from three injections. The inset shows the GLC separation of the four metabolites: 1 = 1-methyl-4-imidazoleacetic acid; 2 = histamine; 3 = 4-imidazoleacetic acid; 4 = 1-methylhistamine. Conditions: glass column (2 m × 2.5 mm I.D.) packed with 3% OV-17 on Gas Chrom Q (100–120 mesh) at 160° with helium flow-rate of 35 ml/min; injector and detector temperatures, 200°; absolute amounts injected, 250 ng of 1 and 3 and 500 ng of 2 and 4.

Structures of the derivatives

The partial mass spectra of the trimethylsilyl (TMS) derivatives of the two amines and acids, metabolically derived from histidine, are given in Table I. The Table contains the most significant features observed in each of the mass spectral patterns obtained by GC–MS. The respective mass spectra demonstrate that, under the experimental conditions used for the derivatization, histamine incorporates 3 TMS groups [$HT(TMS)_3$], 1-methylhistamine and 4-imidazoleacetic acid incorporate two TMS groups each, giving $MHT(TMS)_2$ and $IMAA(TMS)_2$, respectively, while 1-methyl-4-imidazoleacetic acid gives a MIMAA(TMS) derivative. The amines (HT and MHT) were characterized by the base peak at *m/e* 174, which corresponds

TABLE I

PARTIAL MASS SPECTRA OF HISTIDINE METABOLITES (TMS DERIVATIVES)

	HT(TMS)₃		*MHT(TMS)₂*		*IMAA(TMS)₂*		*MIMAA(TMS)*		
	m/e	%	*m/e*	%	*m/e*	%	*m/e*	%	
M	327	2	269	1	270	91	212	57	M
$M-15_+$	312	17	254	20	255	67	197	90	$M-15$
$CH_2=N(TMS)_2$	174	100	174	100	226	100	168	100	$M-44$
$M-101$	226	17	168	17	225	20	167	20	$M-45$
β Cleavage + H	154	22	96	9	211	45	153	55	$(M-44)-15$
					154	59	96	19	β cleavage + H
					153	36	95	66	β cleavage

to a β cleavage with retention of the positive charge on the nitrogen of the side chain, thus giving rise to a bis(trimethylsilyl)ammonium ion, $CH_2{=}N^+[Si(CH_3)_3]_2$. There was also an M — 101 ion corresponding to a β cleavage with concomitant rearrangement of a TMS group on the imidazole moiety, giving an M — $[CH_2{=}N{-}Si(CH_3)_3]$ion.

The molecular ions of the acids (IMAA and MIMAA) were more prominent than those of the amines. However, in this case the base peak appeared at an *m/e* value corresponding to the loss of 44 units from *M*, which seems to involve an abstraction of CO_2 in both cases. There were also abundant ions at *m/e* values (211 and 153, Table I) corresponding to the base peak minus a methyl group, [(*M* —44) —15].

Chromatographic profiles

The GLC profiles of these derivatives are shown in Fig. 2, while their respective retention indices are given in Table II. It is important to point out that the two imidazoleacetic acids have been determined previously by GLC of their methyl esters[7,9], although in this case the elution of IMAA required times of the order of 100 min on an EGA column and 45 min on NGS, with chromatographic responses in the microgram range. A modification of Tham's method has also been described[11]. This method involves the determination of the ethyl ester of 1-methyl-4-imidazoleacetic acid (MIMAAE). However, the esterification procedure is rather elaborate and apparently requires 2–3 h. The retention time reported for MIMAAE is of the order of 35 min, with a chromatographic response also in the microgram range.

TABLE II

RETENTION INDICES OF HISTAMINE AND ITS METABOLITES AS TMS DERIVATIVES
Injector temperature, 250°; detector (FID) temperature, 250°; column temperature, 160°.

	3% OV-17
1-Methyl-4-imidazoleacetic acid (TMS)	1975
4-Imidazoleacetic acid $(TMS)_2$	2037
1-Methylhistamine $(TMS)_2$	2126
Histamine $(TMS)_3$	2180

In contrast, as shown by the chromatographic profile in Fig. 2, the four metabolites can be simply resolved under our conditions in less than 15 min after only 30 min of derivatization. In fact, excellent chromatograms have been obtained in 8 min. It is noteworthy that this method, allied with our procedure[15] for the recovery of amines and acids from biological fluids in a single fraction, would provide a complete metabolic profile of these compounds in samples representative of various metabolical conditions. We are currently evaluating the scope of the method in conjunction with simplified extraction procedures and combined GLC–multiple ion detection techniques, as well as its application to other less known metabolites of histamine.

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