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Note

Pentafluorobenzyl derivative of histamine for determination by gas chromatography–negative ion chemical ionization mass spectrometry

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Substantial inaccuracy and unreliability has been shown to be associated with currently available methods for quantification of histimine in biological fluids[1]. Such methods include both the widely used single and double isotope radioenzymatic assays[2–4] as well as manual and automated fluorometric methods[5–7]. With our own attempts to quantify histamine in urine by radioenzymatic assay, we also encountered severe inaccuracies with this method of analysis which could not be readily rectified. Because of the recognized high degree of accuracy associated with the method of stable isotope dilution and quantification by mass spectrometry (MS), we have directed our efforts recently towards the development of a mass spectrometric method for the determination of histamine in biological fluids.

There have been reports describing a mass spectrometric method for the analysis of histamine using electron impact (EI)[8,9]. However, the limits of sensitivity associated with the EI-MS method would not seem to allow the routine analysis of histamine in small volumes of certain biological fluids in which the concentration of histamine normally present is very low. Therefore, we have decided to examine the application of negative ion chemical ionization (NICI)-MS for the analysis of histamine because of the potential of enhanced sensitivity associated with NICI-MS compared to EI-MS. As an initial basis for the development of such a method for the quantitication of histamine in biological fluids, we examined the derivatization of histamine for a derivative that seemed to have favorable characteristics for determination by NICI-MS. We report here a potentially suitable derivative formed by treatment of histamine with pentafluorobenzyl bromide.

EXPERIMENTAL

Materials

Pentafluorobenzyl bromide and diisopropylethylamine were obtained from Pierce (Rockford, IL, U.S.A.). Histamine dihydrochloride was obtained from Sigma (St. Louis, MO, U.S.A.). Histamine-$\alpha,\alpha,\beta,\beta$-[$^2H_4$] dihydrochloride was obtained from Merck Isotopes (Montreal, Canada). SP2100 and SP2310 GC column packing was obtained from Supelco (Bellefonte, PA, U.S.A.) and Poly I-110 from Applied Science Labs. (State College, PA, U.S.A.).

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Gas chromatography–mass spectrometry

EI-MS analysis was performed using an LKB-9000 gas chromatograph mass spectrometer equipped with a PDP-12A data processing system. Conditions: electron energy 70 eV, interface temperature 25°C, trap current 60 μA, injection port temperature 250°C. Analysis was performed with a 3 ft. packed column of 3% SP2100 operated at approximately 230°C.

NICI-MS analysis was performed using a Hewlett Packard 5982A gas chromatograph–mass spectrometer modified to detect negative ions. Conditions: electron energy 250 eV, interface temperature 250°C, internal source temperature 225°C, direct inlet line, emission current 300 μA, methane as reagent and carrier flow gas, analyzer manifold pressure $1.2 \cdot 10^{-5}$ Torr, injection port temperature 250°C, conversion diode potential −3 KV. Analysis was performed using 2 ft. packed column of 3% SP-2100, 3% SP-2310 and 3% Poly I-110 operated at 230–250°C.

Formation of the pentafluorobenzyl derivative of histamine

Histamine dihydrochloride was dissolved in 30 μl of acetonitrile to which was added 10 μl of diisopropylethylamine and 15 μl of a solution of 25% pentafluorobenzylbromide in acetonitrile. The reaction was allowed to proceed for 30 min at room temperature. Excess reagents were then evaporated under a stream of nitrogen and the residue dissolved in ethyl acetate for injection and analysis by GC–MS.

RESULTS

In the previous reports describing the determination of histamine by EI-MS[8,9], a N^{α}-heptafluorobutyrl-N^{τ}-ethoxycarbonyl derivative was formed by sequential treatment of histamine with heptafluorobutyric anhydride and ethyl chloroformate[10]. For the naming of substituted histamines see ref. 11. We initially examined the NICI mass spectrum of this derivative of histamine. The NICI mass spectrum obtained for this derivative of histamine yielded essentially a single ion at *m/z* 306 which was interpreted to have been formed by the loss of the ethoxycarbonyl group (CH_3CH_2OOC), from the molecular ion at *m/z* 379 (data not shown). The NICI mass spectral characteristics of this derivative were not considered very favorable because of the fact that this derivative was very volatile and generated a base ion of relatively low mass which might be expected to be associated with enhanced likelihood of interference from co-eluting impurities in the selected ion monitoring analysis of histamine isolated from biological fluids.

We therefore attempted to form a less volatile derivative of histamine than N^{α}-heptafluorobutyryl-N^{τ}-ethoxycarbonylhistamine which would yield an intense negative ion of higher mass than *m/z* 306 to minimize the likelihood of co-eluting impurity peaks following extraction of histamine from biological fluids and analysis by selected ion monitoring with NICI-MS. A derivative with potentially favorable characteristics was found following treatment of histamine with pentafluorobenzyl bromide in the presence of diisopropylethylamine. This derivative of histamine was initially examined by GC–EI-MS. A single total ion current peak was observed and the mass spectrum obtained is shown in Fig. 1. The base ion in this mass spectrum is *m/z* 181, $[CH_2C_6F_5]^+$. Ions of lesser intensity were also present at *m/z* 390 and *m/z* 275. The formation of *m/z* 275 and *m/z* 390 as a result of α and β cleavage to the side

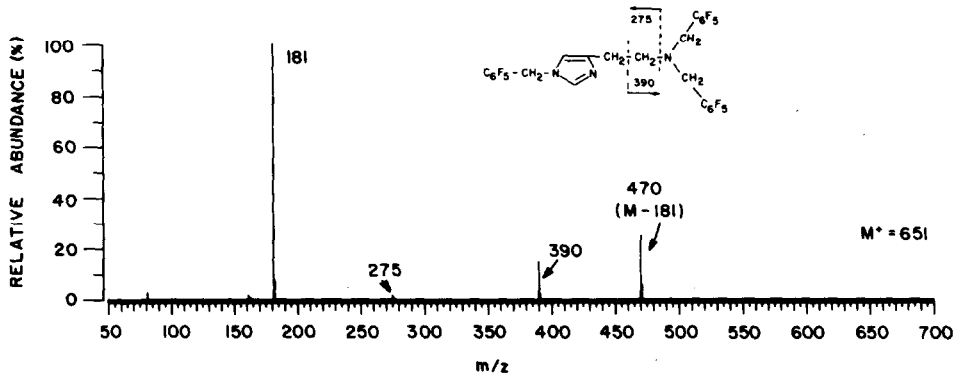

Fig. 1. EI mass spectrum of the derivative of histamine formed following treatment with pentafluorbenzyl bromide.

chain alpha-nitrogen as depicted in Fig. 1 was supported by mass spectral analysis of the same derivative of histamine-$\alpha,\alpha,\beta,\beta$-[$^2H_4$] dihydrochloride in which m/z 275 was shifted upwards four mass units and m/z 390 was shifted upwards two mass units. The ion at m/z 470 was interpreted to have been formed as a result of the loss of 181 mass units, ($CH_2C_6F_5$), from a molecular ion at m/z 651. Although a molecular ion at m/z 651 was not observed on complete mass spectral analysis, the presence of this ion was confirmed by selected ion monitoring. A molecular ion of m/z 651 indicated that in addition to the two pentafluorobenzyl groups attached to the side chain alpha-nitrogen, a third pentafluorobenzyl group was also attached, presumably to the imidazole ring tele-nitrogen. This was confirmed by analysis of histamine following sequential treatment with pentafluorobenzyl bromide and ethyl chloroformate. Ethyl chloroformate has previously been shown to derivatize the imidazole ring tele-nitrogen[10]. However, treatment of histamine with ethyl chloroformate following treatment with pentafluorobenzyl bromide yielded only a single total ion current peak with the same retention time and mass spectrum as was found following treatment of histamine with pentafluorobenzyl bromide alone. This data indicated that treatment of histamine with pentafluorobenzyl bromide resulted in derivatization of both the side chain alpha-nitrogen and the imidazole ring tele-nitrogen to tertiary nitrogens yielding $(CH_2C_6F_5)_3$-histamine.

$(CH_2C_6F_5)_3$-Histamine was then analyzed by NICI-MS. The mass spectrum of this derivative of histamine obtained under these conditions is shown in Fig. 2. This mass spectrum was characterized by a single high mass negative ion of high intensity at m/z 430. The formation of this ion was interpreted to occur by the loss of 181 + (2 × 20) mass units, $CH_2C_6F_5$ + (2 × FH), from the molecular ion at m/z 651. Additional ions of very small intensity were seen at m/z 611, 591, 410 and 390 which were interpreted to have formed by multiple combined losses of $CH_2C_6F_5$ and FH from the molecular ion as indicated in Fig. 2.

The GC characteristics of $(CH_2C_6F_5)_3$-histamine were also examined on columns of differing polarity. Using a 2-ft. column of 3% SP2100 at 230°C, considerable peak tailing was noted (Fig. 3A). However, when chromatographed on 2-ft. columns of more polar phases such as 3% SP2310 or Poly I-110 at 250°C, peak tailing was

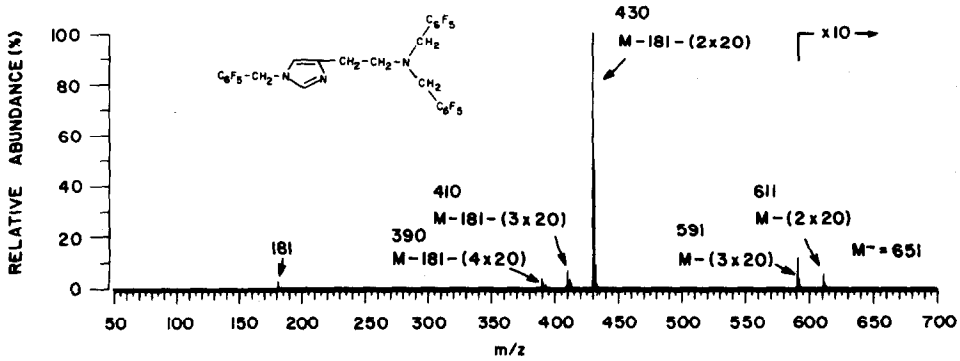

Fig. 2. NICI mass spectrum of $(CH_2C_6F_5)_3$-histamine.

substantially reduced (Fig. 3B). Thus, GC column phases such as SP 2310 and Poly I-110 seem most suitable for the analysis of this derivative of histamine.

The lower limits of detection of $(CH_2C_6F_5)_3$-histamine were then examined using a GC column of 3% Poly-I-110 with NICI selected ion monitoring of *m/z* 430. As shown in Fig. 4, a signal-to-noise ratio of approximately 10:1 was obtained with injection of subpicogram quantities in the range of 500 fg. Thus, NICI-MS is a method with remarkable sensitivity capable of detecting very small quantities of $(CH_2C_6F_5)_3$-histamine.

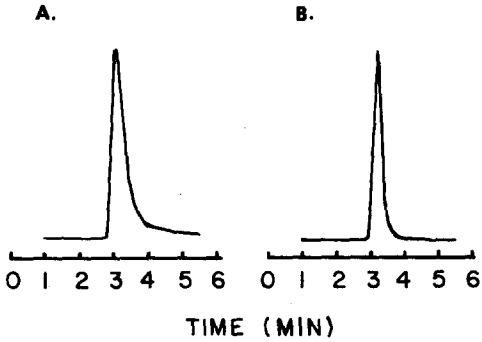

Fig. 3. Selected ion monitoring chromatogram of *m/z* 430 in the NICI mass spectrum of $(CH_2C_6F_5)_3$-histamine using a GC column of SP2100 (A) and Poly I-110 (B).

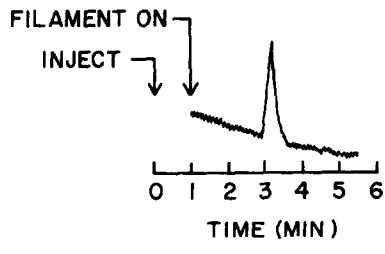

Fig. 4. Selected ion monitoring chromatogram of *m/z* 430 in the NICI mass spectrum of $(CH_2C_6F_5)_3$-histamine following injection of 500 fg, using a GC Column of Poly I-110.

DISCUSSION

Because of the potential of substantially enchanced sensitivity associated with NICI compared to EI-MS we have directed efforts towards the development of a mass spectrometric method using NICI for the determination of histamine. As a basis for the development of such a method, we have attempted to find a derivative of histamine with favorable characteristics for analysis using NICI-MS.

One of the most frequently used procedures for the derivatization of amines is acylation[12,13]. Treatment of histamine with the commonly used acylation reagent heptafluorobutyric anhydride has been previously shown to convert histamine to N^{α}-heptafluorobutyryl-histamine[10]. Heptafluorobutyric anhydride derivatizes the side chain alpha-nitrogen to a secondary nitrogen but does not derivatize the imidazole ring although this can be accomplished using chloroformate reagents[10]. Although pentafluorobenzoyl chloride is not uncommonly used to form acyl derivatives of amines, derivatization of amines using pentafluorobenzyl bromide has not apparently been widely used although reported for the derivatization of sulfonamides, barbiturates and the drugs clonidine and theophylline[12,13]. We found that treatment of histamine with pentafluorobenzylbromide formed a derivative with very favorable characteristics for analysis using NICI-MS. It was of interest to find that although treatment of histamine with the acylating reagent heptafluorobutyric anhydride only derivatizes the side chain alpha-nitrogen to a secondary nitrogen and does not derivatize the imidazole ring, treatment with pentafluorobenzyl bromide in the presence of diisopropylethylamine under mild conditions of room temperature for 30 min derivatized the side chain alpha-nitrogen to a tertiary nitrogen and also derivatized the imidazole ring tele-nitrogen yielding $(CH_2C_6F_5)_3$-histamine. This suggests the possible value of examining on a broader scale the potential usefulness of derivatization of other amine compounds using pentafluorobenzyl bromide.

In summary, treatment of histamine with pentafluorobenzyl bromide forms a derivative with very favorable characteristics for determination of histamine using GC–NICI-MS: (a) both the side chain alpha-nitrogen and imidazole ring ring tele-nitrogen are derivatized simultaneously under mild conditions, (b) the NICI mass spectrum of $(CH_2C_6F_5)_3$-histamine generates a base ion of relatively high mass at m/z 430, and (c) lower limits of detection with selected ion monitoring of m/z 430 were found to be remarkably low in the range of 100–500 fg injected on-column. With the commercial availability of deuterium-labelled histamine, this derivative of histamine seems potentially valuable as a basis for the development of very sensitive and accurate stable isotope dilution GC–NICI-MS methods for quantification of histamine in a variety of biological fluids.

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