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Note

Improved chromatographic characteristics of histamine and some of its analogues by ring acetylation

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The gas chromatographic behavior of imidazoles, with unsubstituted ring nitrogens, is usually unsatisfactory even on the most polar liquid phases. This is manifested by severe tailing, leading to inadequate quantification. To overcome this problem, double derivatization has been used to improve the chromatographic characteristics of imidazoles such as histidine [1—5]. A similar approach to decrease the tailing of histamine was followed by Mita et al. [6]. They used heptafluorobutyric anhydride (HFBA) and chloroformic acid ethyl ester to prepare the N^{τ}-acetylated ester of histamine. We report data regarding the preparation of stable acetylated heptafluorobutyric derivatives which not only reduce tailing of histamine but also of other histamine analogues of biological interest. In addition, the chromatographic characteristics are such that it allows convenient elution of these metabolites for simultaneous quantitation.

EXPERIMENTAL

Reagents

N^{τ}-Methylhistamine (N^{τ}MeHi) and histamine were obtained from Calbiochem-Behring; N^{α}-methylhistamine (N^{α}MeHi), 2-methylhistamine (2-MeHi), 4-methylhistamine (4-MeHi), N^{π}-methylhistamine (N^{π}MeHi) were gifts from Smith Kline and French Labs. Heptafluorobutyric anhydride and

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column packing, 3% OV-275 on Chromosorb W HP, 80—100 mesh, were purchased from Chromatographic Specialities. All solvents used were of the highest purity available. Ethyl acetate was redistilled. In this report the nomenclature proposed by Black and Ganellin [7] was used for all imidazole derivatives.

Gas chromatography

Chromatographic analyses were carried out with a Hewlett-Packard Model 5730A instrument equipped with a nitrogen—phosphorus detector. The column was a 2 m × 2 mm glass coil packed with 3% OV-275 on Chromosorb W HP. Helium was used as carrier gas at a flow-rate of 40 ml/min. The chromatography was carried out isothermally at 170°C. The injection and detector port temperatures were maintained at 200 and 300°C, respectively.

Procedure

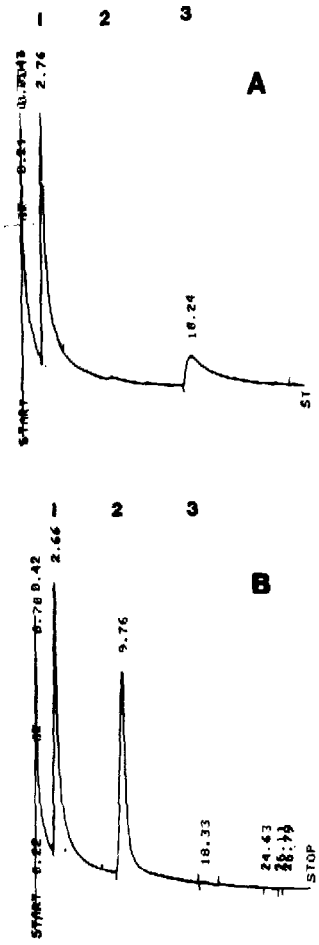
Histamine and its basic analogues (25 nmol) were derivatized with HFBA as previously described [8]. After the dried material was taken up in 250 μl ethyl acetate, a few crystals of sodium acetate were added followed by 2 μl of acetic anhydride. The vial was immediately capped, the contents were mixed and allowed to stand for 40 min at room temperature, after which 2 μl were injected into the chromatograph. For the evaluation of short reaction times and stability of the derivatives, the samples were kept at room temperature. To assess stability for longer periods (24—48 h), samples were stored at −10°C.

RESULTS AND DISCUSSION

The conditions used in the present experiments for the derivatization of histamine and its analogues with HFBA result in monoheptafluorobutyric derivatives [9]. The chromatographic characteristics of N^{τ}-methyl-N^{α}-heptafluorobutyrylhistamine (HFB-N^{τ}MeHi) and N^{α}-heptafluorobutyrylhistamine (HFB-Hi) are shown in Fig. 1A. Following the reaction of HFB-Hi with acetic anhydride, the HFB-Hi peak shifted from a broad peak with an elution time of 18.24 min and a tailing factor* of 8.5 to a narrow peak eluting at 9.76 min with a tailing factor of 2.0 (Fig. 1B). Although, the tailing was not completely eliminated, reproducible quantitation of histamine and its analogues was possible and was used successfully in an assay procedure [10]. HFB-N^{τ}MeHi, which possesses a methyl group on one of the ring nitrogens, was not modified by the acetylation reaction, as suggested by its unchanged chromatographic characteristics. Thus, it is likely that acetylation occurs on one of the ring nitrogens. Mita et al. [6] came to a similar conclusion after mass spectrometric analyses to determine the position of the carbethoxy group of the N^{α}-heptafluorobutyrylcarbethoxyhistamine derivative.

Acetylation of the HFB derivatives was carried out at room temperature. The extent of acetylation was improved by the addition of a small amount of sodium acetate, as shown in Fig. 2 for HFB-Hi. In the lower panel, the acetyla-

*This symmetry factor compares the base of the second half of the chromatographic peak to the first half. The base is measured at 10% of the peak height.



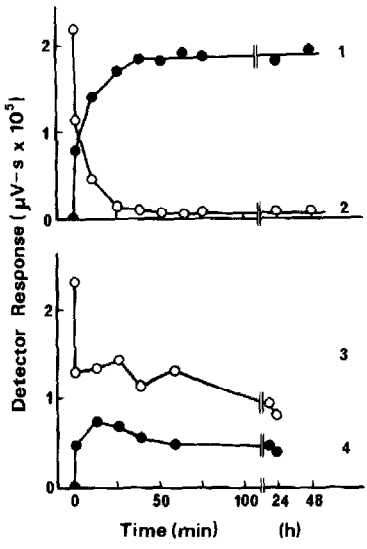


Fig. 1. Chromatograms of N^{τ}-methylhistamine and histamine derivatives (0.2 nmol per injection) (A) before and (B) after the acetylation reaction. Peaks: 1 = HFB-N^{τ}MeHi; 2 = acetylated HFB-Hi; 3 = HFB-Hi.

Fig. 2. Effect of sodium acetate on the acetylation of HFB-Hi and on the stability of acetyl HFB-Hi. Acetylation of a constant amount of HFB-Hi was carried out in the presence (upper panel) and in the absence (lower panel) of sodium acetate. Curves 1 and 4 represent acetyl HFB-Hi; curves 2 and 3 represent HFB-Hi at a given time period.

tion was allowed to proceed without sodium acetate, and only a partial conversion of HFB-Hi (graph 3) to the acetylated HFB-Hi (graph 4) occurred, whereas in the presence of sodium acetate (top panel) only 4% of the starting compound remained unconverted after 40 min of reaction (graph 2). Thus it can be assumed that 96% of HFB-Hi was in the acetylated form (graph 1). This derivative remained stable for at least 48 h at −10° C.

The acetylation procedure is simple and improves the quantitation not only of histamine but also of other histamine analogues with no substitution on the ring nitrogens. In fact the spacing of the elution peaks from packed columns after acetylation of the analogues allows the combined chromatographic separation of N^{τ}MeHi, 2-MeHi, 4-MeHi, N^{α}MeHi, histamine and N^{π}MeHi in one single run (Table I). Preliminary results on capillary columns (30 m × 0.32 mm,

TABLE I

CHROMATOGRAPHIC DATA OF HEPTAFLUOROBUTYRYL DERIVATIVES OF HISTAMINE AND HISTAMINE ANALOGUES

Samples were prepared as described in the text. Acetylation was carried out in the presence of sodium acetate. The retention times were obtained on a 3% OV-275 column, 2 m × 2 mm, maintained at a constant temperature of 170°C and with helium as carrier gas (40 ml/min).

Heptafluorobutyryl derivative	Retention time (min)	
	Before acetylation	After acetylation
N^{τ}-Methylhistamine	2.58	2.58
2-Methylhistamine	8.72	5.20
4-Methylhistamine	13.48	7.53
N^{α}-Methylhistamine	13.49	6.52
Histamine	15.82	9.41
N^{π}-Methylhistamine	25.64	25.64

DB-225) have shown a 2.6-fold increase in peak height for histamine when the acetylation step was included, indicating a potential usefulness for capillary column chromatography as well [11]. Other imidazoles with various functional groups like imidazoleacetic acid can also be easily acetylated following esterification of the carboxylic group to improve their chromatographic characteristics. The acetylation reaction is applicable to biological extracts [10, 11].

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