Journal of Chromatography, 106 (1975) 218–224

CHROM. 8054

Note

New approach to the separation and identification of some methylated histamine derivatives by gas chromatography

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(Received October 11th, 1974)

Recently, interest has developed in the identification and quantitation of chain-methylated metabolites of histamine in view of their normal occurrence in various species and their potent biological activities. They have been successively identified in man[1], dogs[2], guinea-pigs[3], amphibians[4], and sponges[5]. Their pharmacological actions have also been recognized on gastric secretion[6,7], smooth and striated muscle[8], methyl transferases[9], and cyclic AMP[10]. The compounds have been identified by paper and thin-layer chromatography. These methods have generally been inadequate for quantitative purposes, since it is difficult to successfully resolve all metabolites by the above-mentioned procedures.

Quantitative determination of N^{t}-methyl-histamine* (ref. 11) has been achieved[12,13]. However, in this case, it is possible that N^{π}-methyl-histamine could interfere with the determination.

To date, little work has been done on the use of gas–liquid chromatography (GLC) for the separation or quantitation of imidazoles[14–16]. The present technique adequately separates histidine and histamine and the naturally occurring methyl-histamines. Allied with proper purification procedures, the method could offer a basis for a precise and sensitive quantitative approach commensurate with their expected low concentration in living systems. The method could also be useful in evaluating the relationships between precursors and products when studying the metabolism of histamine.

MATERIAL AND METHODS

Chemicals and reagents

Histidine, histamine, N^{t}-methyl-histamine, and N^{π}-methyl-histamine were obtained from Calbiochem (San Diego, Calif., U.S.A.); trifluoroacetic anhydride, N,O-bis(trimethylsilyltrifluoroacetamide) (BSTFA), trimethylchlorosilane (TMCS), and hexamethyldisilazane (HMDS) from Chromatographic Specialties (Brockville, Ontario, Canada); OV-17 (dimethyl silicone), OV-22 (65% phenyl–phenylmethyl-diphenyl silicone) on 60–80 Gas-Chrom Q from Applied Science Labs. (State College,

* Throughout the text, the nomenclature for histamine derivatives is that proposed in ref. 11.

Pa., U.S.A.), and OV-11 (35% phenyl–phenylmethyldimethyl silicones) on 110–120 Supelcoport from Supelco (Bellefonte, Pa., U.S.A.). Phenanthrene was obtained from Canlab (Montreal, Canada).

N^{α}-methyl-histamine and N,N^{α}-dimethyl-histamine were gifts from Dr. C. F. Code (Mayo Clinic, Rochester, Minn., U.S.A.) or Dr. W. A. W. Duncan (Smith Kline and French Labs., Welwyn Garden City, London, Great Britain). All solvents were redistilled before use and obtained from Fisher (Montreal, Canada).

Chromatography

A Hewlett-Packard Model 7610A gas chromatograph (Hewlett-Packard, Montreal, Canada) equipped with a flame ionization detector was used in conjunction with a multi-level temperature programmer (Model 7660A), a strip chart recorder (Model 7128A), an electronic integrator and printer (Model 3370), and an automatic sampler (Model 7671A).

Temperatures. The injection port temperature was set at 250° and the detector temperature at 300°. The column temperatures were the following:

OV-11 —isothermal 120°, hold 1 min
- Level I: 6° min^{-1} to 170°, isothermal 4 min
- Level II: 1° min^{-1} to 195°, isothermal 6 min
- Level III: 10° min^{-1} to 250°, isothermal 4 min

OV-17 —isothermal 120°, hold 15 min
- Level I: 6° min^{-1} to 155°, isothermal 0 min
- Level II: 5° min^{-1} to 170°, isothermal 0 min
- Level III: 15° min^{-1} to 250°, isothermal 4 min

OV-22 —isothermal 120°, hold 1 min
- Level I: 1° min^{-1} to 142°, isothermal 4 min
- Level II: 2° min^{-1} to 170°, isothermal 4 min
- Level III: 15° min^{-1} to 250°, isothermal 4 min

Flow-rates. The gas flow-rates were: nitrogen, 40 ml/min; hydrogen, 60 ml/min; and air, 300 ml/min.

Columns. Silanized glass columns, 6 ft. × ¼ in. O.D., packed with the appropriate phase and support were used. All columns were conditioned for at least 24 h at 275° with a nitrogen flow-rate of 20 ml/min, except for OV-11, which was conditioned according to Gerhke and Leimer[17].

Procedures. All reactions were carried out in acid-washed, siliconized 1-ml Reacti-Vials (Pierce, Rockford, Ill., U.S.A.) or 100-µl vials (Hewlett-Packard, Model 4330-0549). Reaction mixtures were heated in a temperature-controlled heating block. Stock solutions of the compounds, containing phenanthrene as an internal standard, were prepared in 0.01 *N* HCl in concentrations of 1 nmole (calculated as the free base) per µl. Solutions were prepared fresh weekly and stored at 4°.

Reaction time and temperature were chosen after serial determination of maximum detector response with settings at an attenuation of 10×10^2 using a 1-µl injection size. Reactions were carried out in quadruplicates and each determination was done in triplicate. Methylene units were determined according to Butts[18]. Completeness of derivatizations was not evaluated.

TABLE I

CHROMATOGRAPHIC DATA

Nomenclature according to ref. 11. Data refer to the principal derivative. Temperature programmes as described in the text. RRT = retention time relative to phenanthrene; M.U. = methylene units.

Compounds	*OV-11 (10%)*						*OV-17 (3%)*				*OV-22 (5%)*			
	BSTFA		*HFBA*		*TFAA*		*BSTFA*		*HFBA*		*BSTFA*		*HFBA*	
	RRT	*M.U.*	*RRT*	*M.U.*	*RRT*	*M.U.*	*RRT*	*M.U.*	*RRT*	*M.U.*	*RRT*	*M.U.*	*RRT*	*M.U.*
Histidine	1.08	20.50		17.31	0.60	17.64	1.17	21.00	0.56	18.00	1.02	21.14	0.50	18.07
Histamine	0.94*	19.79*	0.64	18.00	0.67	18.19	0.95*	20.00*	0.67	18.58	0.87*	20.34*	0.67	19.16
N^{t}-Methyl-histamine	0.81	19.11	0.58	16.73	0.51	17.00	0.81	19.36	0.46	17.29	0.80	19.84	0.43	17.57
N^{τ}-Methyl-histamine	1.09	20.69	0.71	18.65	0.69	18.31	–	–	–	–	0.94	–	0.69	–
N^{α}-Methyl-histamine	0.62*	17.85*	0.70	18.40	0.74	18.63	0.51*	17.72*	0.75	19.47	0.52*	18.20*	0.74	19.53
N,N^{α}-Dimethyl-histamine	0.48	16.64	–	–	–	–	0.41	17.21	–	–	0.42	17.50	–	–
Phenanthrene retention time	30.24 min						41.85 min				36.93 min			

* Double derivatives.

Preparation of samples

Samples were prepared for derivatization in the following manner: 10 nmoles of the compounds were transferred to the vial and dried under nitrogen gas at 90°. 100 μl of methylene chloride were added and the sample dried again. This last step was repeated twice.

Trimethylsilyl derivatives. 10 μl of acetonitrile, 4 μl of TMCS (1% of TMCS calculated relative to the amount BSTFA added) and a 30 molar excess of BSTFA were added successively. The vial was capped under nitrogen before heating at 90° for 60 min.

Heptafluorobutyryl derivatives. 50 μl of HFBA were added and after capping under nitrogen the samples were heated at 90° for 30 min. They were then taken to dryness and diluted in 100 μl of ethyl acetate.

Trifluoroacetate derivatives. 50 μl of trifluoroacetic anhydride were added to the samples after sealing under nitrogen, the reaction was carried out at room temperature for 10 min.

RESULTS AND DISCUSSION

The results are summarized in Table I. Because of the diversity of the functional groups of the metabolites (primary, secondary and tertiary amine and imino nitrogen), it was not possible to find a single reagent giving only one derivative of each metabolite with adequate separation and eluting time. Trifluoroacetates (TFAA) and heptafluorobutyrates (HFBA) are known to be very useful derivatives in many GC applications because of their great volatility. TFAA have previously been used for

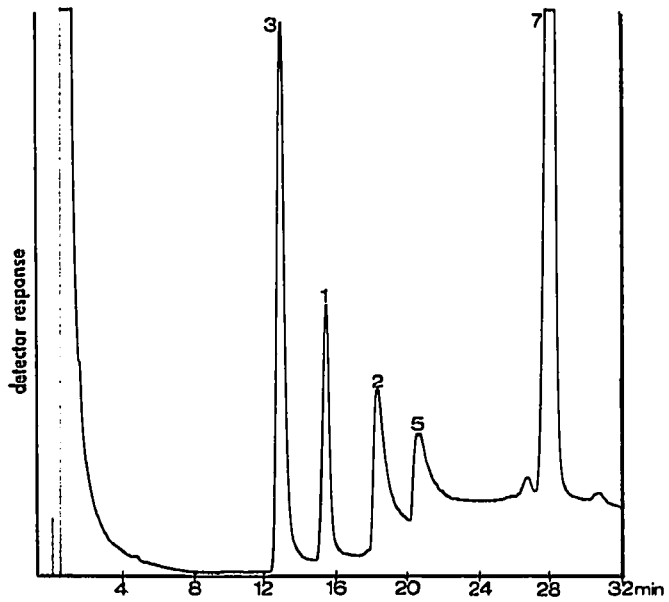

Fig. 1. Separation of HFBA derivatives of (1) histidine, (2) histamine, (3) N^1-methyl-histamine, (5) N$^{\alpha}$-methyl-histamine, and (7) phenanthrene on OV-11.

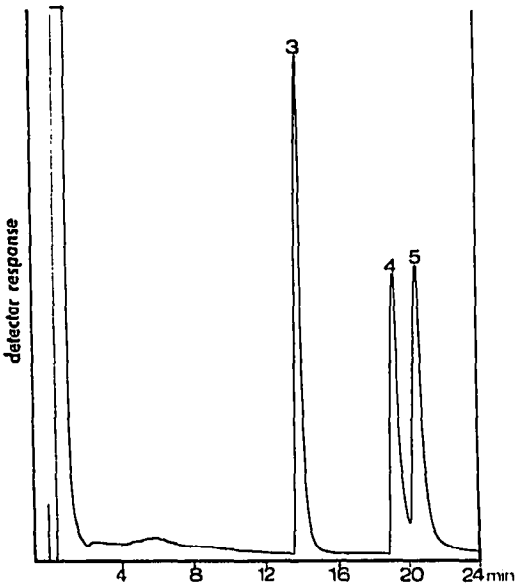

Fig. 2. Separation of HFBA derivatives of (3) N^t-methyl-histamine, (4) N^π-methyl-histamine, and (5) N^α-methyl-histamine on OV-11.

N-terminal protection in peptide synthesis[19]. HFBA have also been used to acylate primary and secondary groups[20].

HFBA and TFAA derivatives

Histidine, histamine, N^t-methyl-histamine, N^π-methyl-histamine and N^α-methyl-histamine gave good detector response and single derivatives, presumably at the primary amine (N) site, with HFBA (Figs. 1 and 2) and TFAA. These derivatives were suitable for the quantitative determination of these compounds. N,N^α-Dimethyl-histamine (TFAA) gave unsatisfactory detector response and eluted with considerable tailing under our experimental conditions. The derivatives are suitable for electron capture detection and preliminary experiments with histamine HFBA suggested a detectability of less than 150 pg/μl (ref. 20).

BSTFA

N-Trimethylsilyl derivatives are readily prepared by electrophilic attack at the multiply-bonded nitrogen[22]. Thus, all compounds studied reacted well with BSTFA (Fig. 3). N,N^α-Dimethyl-histamine and N^t- and N^π-methyl-histamine gave single derivatives with good detector response and appropriate separation from the other metabolites. However, histamine and N^α-monomethyl-histamine eluted as two peaks on all systems studied, as reactions probably occurred also at the N^α nitrogen and at the imino nitrogen. The minor peak of histamine-TFA was eluted with N^α-methyl-histamine TFA. N^α-Methyl-histamine gave an unsymmetrical peak, suggesting that both derivatives of N^α-methyl-histamine eluted closely together.

Overall, each liquid phase studied —OV-11, OV-17 and OV-22 —is useful and offers adequate separation of each metabolite. OV-11 shows some advantage in

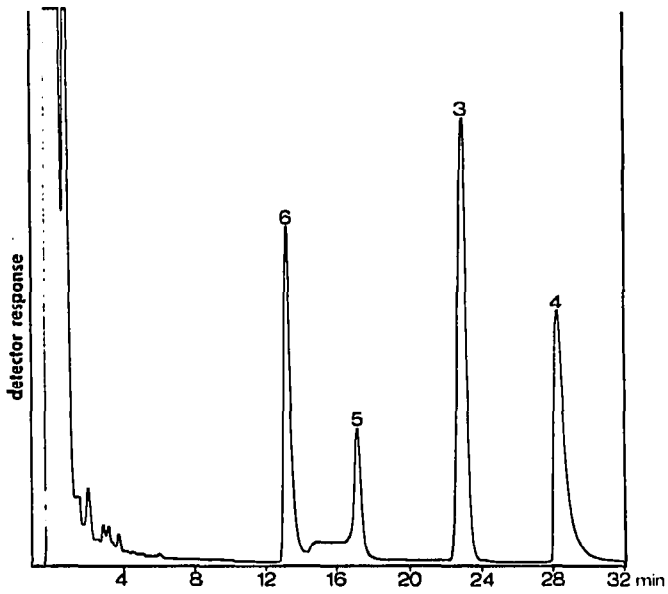

Fig. 3. Separation of BSTFA derivatives of (6) N,N$^{\alpha}$-dimethyl-histamine, (5) N$^{\alpha}$-monomethyl-histamine, (3) N^{1}-methyl-histamine, and (4) N$^{\pi}$-methyl-histamine on OV-11.

the case of BSTFA derivatives, while OV-17 and OV-22 give better separation of HFBA derivatives. Detectability, defined as three times the background noise, was of the order of 5 ng/μl. Under our experimental conditions, acetyl-histamine, a common metabolite of histamine, always gave multiple peaks and could not be chro-

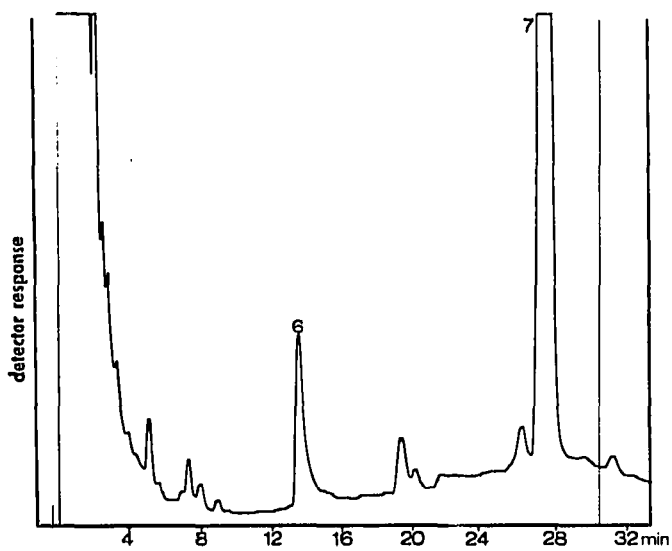

Fig. 4. Identification of BSTFA derivatives of (6) N,N$^{\alpha}$-dimethyl-histamine in human urine after preliminary purification of Amberlite CC50 and TLC on OV-11. 7 = Phenanthrene.

matographed adequately. Its determination could be achieved as a histamine derivative with preliminary acid hydrolysis of the acetyl group.

Hexane, carbon tetrachloride, carbon disulfide and tetrahydrofuran were useful solvents. Dinitrofluorobenzene, dansyl chloride and 7-chloro-4-nitrobenzofurazam (NBD cloride) were not useful derivatives as they showed considerable tailing under the present conditions.

Current work is tending towards the application of these methods to biological specimens. An example of the identification of N,N$^{\alpha}$-dimethyl-histamine in human urine is shown in Fig. 4.

ACKNOWLEDGEMENTS

The author wishes to acknowledge the technical assistance given by Miss C. Baril and the support of the Medical Research Council of Canada (grant No. 4240). The author is a scholar of the Medical Research Council of Canada.

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