Journal of Chromatography, 338 (1985) 187—191
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2409

Note

Urinary excretion of N-acetylamino acids

HARTMUT M. LIEBICH* and CLAUDIA FÖRST

Medizinische Universitätsklinik, 7400 Tübingen (F.R.G.)

(Received August 23rd, 1984)

N-Acetylation is a pathway in the metabolism of amino acids, to which little attention has been paid. There are only a few reports on single N-acetylamino acids detected in urine. Small amounts of N-acetyltryptophan [1], N^{α}-acetyllysine [2] and N-acetylhistidine [3] were found to be excreted in urine. Increased urinary N^{α}-acetyllysine was observed in a case of hyperlysinaemia and lysinuria [2], and elevated urinary N-acetylhistidine in patients with histidinaemia [3]. The N-acetyl derivative of a more unusual amino acid, aminooctanoic acid, has also been described [4]. During our analysis of organic acids a series of N-acetylamino acids was found in normal urine.

EXPERIMENTAL

Materials and samples

Alanine, valine, leucine, aspartic acid and glutamic acid from E. Merck (Darmstadt, F.R.G.) were used to prepare N-acetyl derivatives. The reference substance N-acetyl-O-methyltyrosine methyl ester was purchased from Sigma Chemie (Taufkirchen, F.R.G.).

Several 24-h urine samples were collected from healthy individuals and analysed directly after the collection period.

Analysis of organic acids in urine

The sample preparation and the gas chromatographic (GC) and gas chromatographic—mass spectrometric (GC—MS) studies were carried out according to the procedure previously described [5]. The reference compounds were analysed by the same GC and GC—MS conditions as the urinary constituents.

0378-4347/85/$03.30

Synthesis of N-acetylamino acid methyl esters

One milligram of each of the reference amino acids was allowed to react in screw-capped vials (Macherey-Nagel, Düren, F.R.G.) with 1 ml of a solution of 10% acetyl chloride in methanol for 10 min at 110° C. The reaction mixture was evaporated to dryness and 1 ml of acetic anhydride was added. After a reaction of 30 min at 110° C, the solution was evaporated to dryness again and the resulting N-acetylamino acid methyl ester was redissolved in 1 ml of methanol.

RESULTS AND DISCUSSION

Detection of N-acetylamino acids within the profile of the organic acids

The N-acetyl metabolites of alanine, valine, leucine, aspartic acid and glutamic acid are constituents of fraction 3d, N-acetyltyrosine a component of fraction 4 of the urinary organic acids. Both are polar fractions. Fig. 1 demonstrates that the N-acetylamino acids of fraction 3d belong to the compounds of medium to low concentration. The same is observed for N-acetyltyrosine. Their recognition and identification in urine by GC—MS is facilitated by the pre-fractionation technique, through which they are enriched in the subfractions. Quantification has not yet been attempted.

Mass spectrometric fragmentation and identification of the N-acetylamino acid methyl esters

The MS fragmentation of the N-acetylamino acid methyl esters is characterized by a number of general features. For the substances identified as urinary constituents these characteristics are systematically described in Table I, even though the spectra of the methyl esters of N-acetylleucine [6] and N-acetylglutamic acid [7] have been published.

The molecular ion can be distinguished as a small peak only in the methyl esters of N-acetylalanine and N-acetyltyrosine. A major fragment in all of the mass spectra is the ion $(M—COOCH_3)^+$. By further loss of ketene from the acetyl group resulting in $(M—COOCH_3, —CH_2CO)^+$, another ion with high

TABLE I

MASS SPECTROMETRIC FRAGMENTATION OF THE METHYL ESTERS OF THE N-ACETYLAMINO ACIDS IDENTIFIED IN URINE

The values represent the relative intensities (%) of the fragments listed.

Substance	MW*	M^+	$(M—COOCH_3)^+$	$(M—COOCH_3, —CH_2CO)^+$	*m/e* 43
N-Acetylalanine	145	4	69	100	45
N-Acetylvaline	173	—	60	100	53
N-Acetylleucine	187	—	51	100	47
N-Acetylaspartic acid	203	—	53	100	64
N-Acetylglutamic acid	217	—	19	27	54
N-Acetyltyrosine**	251	2	75	11	29

*Molecular weight.

**Methylated at the phenolic —OH group.

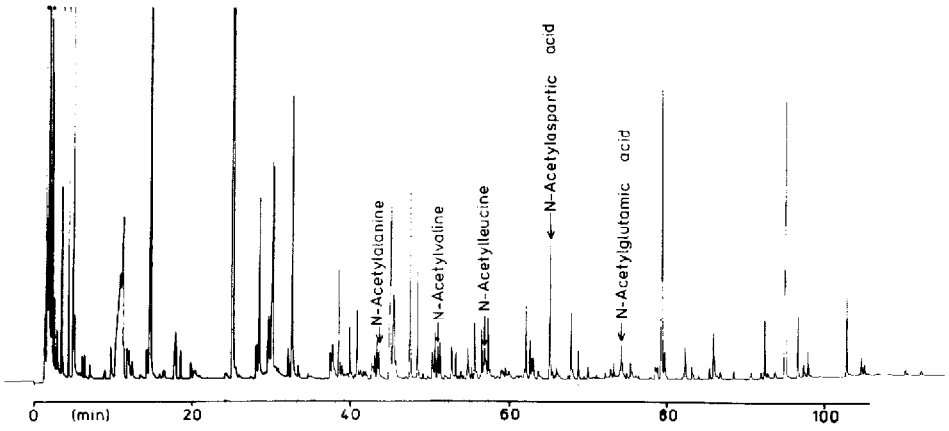

Fig. 1. Gas chromatogram of fraction 3d of the methyl esters of the organic acids in urine of a healthy individual. The N-acetylamino acids are labelled.

abundance, in some cases the base peak, is formed. An intense fragment in all of the spectra is also the peak at *m/e* 43, corresponding to the acetyl ion. The loss of the side-chain R of the amino acid derivative and of a ketene molecule leads to an ion at *m/e* 88, which has its highest abundance in the spectrum of N-acetylvaline methyl ester with R being an isopropyl group. A fragment of low intensity, however, always present, is $(M-CH_3CO)^+$.

McLafferty rearrangement at the carbomethoxy group leads to *m/e* 131 in the fragmentation of N-acetylvaline and N-acetylleucine. As in the spectra of regular dicarboxylic acid methyl esters, the fragmentation of the N-acetylaspartic acid (Figs. 2 and 3) and N-acetylglutamic acid methyl esters includes the ion $(M-COOCH_3, -CH_3OH)^+$. By additional loss of ketene the fragment $(M-COOCH_3, -CH_3OH, -CH_2CO)^+$ is formed which is the base peak of glutamic acid methyl ester. Further characteristic peaks of low abundance in the spectra of the aminodicarboxylic acid methyl esters are $(M-CH_3O)^+$ and $(M-CH_3OH)^+$. In the N-acetyltyrosine methyl ester in which the phenolic —OH group is methylated as well by the procedure applied to the urinary acids, the base peak corresponds to the aromatic ion *m/e* 121.

m/e 88	$(M-CH_3CO)^+$	*m/e* 131	$(M-COOCH_3, -CH_3OH)^+$	$(M-COOCH_3, -CH_3OH, -CH_2CO)^+$
11	3	—	—	—
42	1	2	—	—
24	2	11	—	—
10	2	—	3	26
9	2	—	35	100
6	2	—	—	—

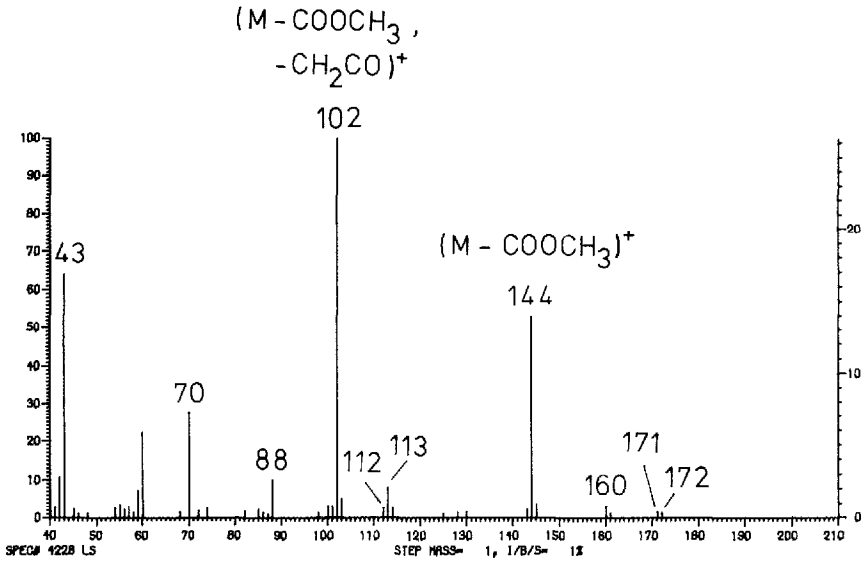

Fig. 2. Mass spectrum of the methyl ester of N-acetylaspartic acid identified in urine of a healthy individual.

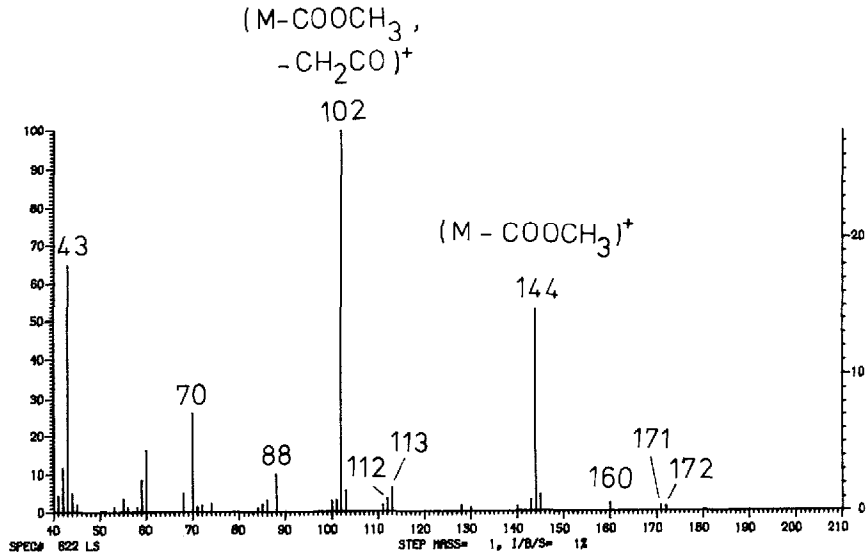

Fig. 3. Mass spectrum of the reference N-acetylaspartic acid methyl ester.

On the basis of the discussed general characteristics of the MS fragmentation of the N-acetylamino acid methyl esters, the recognition of possibly occurring further metabolites should be feasible.

N-Acetylamino acids in urine

The six to our knowledge not so far reported N-acetylamino acids are regularly found in normal urines. Together with N-acetyltryptophan, N^{α}-acetyllysine and N-acetylhistidine, nine acetyl derivatives of common amino acids are now identified. We conclude from these findings that N-acetylation of the

normal amino acids is a metabolic pathway of minor extent but occurring regularly. The biochemical mechanism, the role of enzymes in the N-acetylation and the location where this metabolic reaction takes place, remain to be investigated.

REFERENCES

1 M.D. Armstrong, K.N.F. Shaw, M.J. Gortatowski and H. Singer, J. Biol. Chem., 232 (1958) 17.
2 M.D. Armstrong and M. Robinow, Pediatrics, 39 (1967) 546.
3 S.K. Wadman, P.K. de Bree, C. van der Heiden and F.J. van Sprang, Clin. Chim. Acta, 31 (1971) 215.
4 A. Grupe and G. Spiteller, J. Chromatogr., 226 (1981) 301.
5 H.M. Liebich and C. Först, J. Chromatogr., 309 (1984) 225.
6 W. Vetter, in G.R. Waller (Editor), Biochemical Applications of Mass Spectrometry, Wiley, New York, 1972, pp. 387—404.
7 E. Lerch and M. Hesse, Helv. Chim. Acta, 57 (1974) 1584.