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Note

Gas chromatographic retention indices of twenty metabolically important acylglycines as trimethylsilyl derivatives

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Conjugation with glycine is an important mechanism for excretion of aromatic carboxylic acids in normal human metabolism. These aromatic acids are either produced in endogenous metabolism or derived from exogenous sources such as drugs. For instance, hippuric acid (benzoylglycine) is a major constituent of acid extracts of normal human urine. Salicylate is excreted in part as a glycine conjugate, *o*-hydroxyhippuric acid (*o*-hydroxybenzoylglycine) [1]. Glycine conjugates of other hydroxysubstituted aromatic acids have been found in human urine [2].

Recently glycine conjugates of carboxylic acids with short aliphatic chains have been found in the urine of patients with certain inborn errors of organic acid catabolism. These glycine conjugates include: isovalerylglycine in isovaleric acidemia [3], β-methylcrotonylglycine in β-methylcrotonyl CoA carboxylase deficiency [4], propionylglycine in propionic acidemia [5], and suberylglycine in dicarboxylic aciduria [6]. In addition, tiglylglycine has been detected in three inborn errors of isoleucine metabolism [7]. *n*-Hexanoylglycine has been identified in urine from patients with Jamaican vomiting sickness [8, 9] and urine from a patient with ethylmalonic aciduria [10]. *n*-Butyrylglycine has been identified in urine of hypoglycin-treated rats [11]. The detection of these unusual glycine conjugates is essential in the diagnosis of these organic acidurias. Therefore, it is of clinical importance to provide chromatographic and mass spectroscopic data on glycine conjugates of normal and pathological carboxylic acids for profiling urinary organic acids in normal subjects and in patients with metabolic diseases. The possibility exists that a new organic aciduria may be discovered in the future by identification of "new" acylglycines.

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In profiling urinary organic acids, two types of derivatization can be utilized. One is the methylation of carboxylic groups [9, 12] and the other is trimethylsilation [13, 14]. Both of them have been extensively used. We have previously published gas chromatographic (GC) retention indices [15] and mass spectral data [16] of the methyl esters of twenty metabolically important acylglycines. More recently, however, the trimethylsilylation procedure is being more extensively utilized than the methylation method because of the carcinogenic and explosive nature of reagents for the methylation method.

We report here methylene unit values on OV-1 and OV-17 columns of trimethylsilyl (TMS) derivatives of twenty acylglycines of known or potential biological significance. Seventeen of them were synthesized in our laboratory. Mass spectroscopic studies of TMS derivatives of these acylglycines will be published elsewhere.

MATERIALS

Acetylglycine and hippuric acid were obtained from Sigma (St. Louis, Mo., U.S.A.). *o*-Hydroxyhippuric acid was purchased from Aldrich (Milwaukee, Wisc., U.S.A.). The other acylglycines were synthesized in our laboratory as described previously [15]. Anakrom U, Anakrom ABS, OV-1 and OV-17 were purchased from Analabs (North Haven, Conn., U.S.A.). The *n*-hydrocarbon standards were obtained from Analabs and Eastman Organic Chemicals (Rochester, N.Y., U.S.A.). Tri-Sil BSA Formula P was from Pierce (Rockford, Ill., U.S.A.).

METHOD

Trimethylsilation

The method used is similar to that used for urinary organic acid analysis [13]. Under these conditions, two peaks are detected for each acylglycine due to mono- and di-TMS derivatives or di- and tri-TMS derivatives in the case of the hydroxylated aromatic glycine conjugates. Since we intended to report the methylene unit values for both potential TMS derivatives, we did not use conditions that gave a single derivative for each acylglycine [17].

Gas chromatography

Two GC columns, 5% OV-1 and 10% OV-17 columns were used in this study. The support used for these columns was Anakrom (80—90 mesh) that had been base washed, acid washed and silanized either by ourselves or by the vendor. OV-1 and OV-17 were used in 6 ft. × 2 mm silanized glass columns in a Varian 1800 gas chromatograph. Nitrogen was the carrier gas at a flow-rate of 10 ml/min. The column oven temperature was programmed at 4°/min from a starting temperature of 80°.

For the determination of methylene unit (MU) values, the TMS-acylglycines were mixed with hexane solutions of either even- or odd-chain *n*-hydrocarbons. MU values were calculated by linear interpolation from two adjacent hydrocarbon peaks. For the two columns used, when hydrocarbon retention times were plotted against carbon number, curves were obtained which deviat-

TABLE I

METHYLENE UNIT VALUES FOR TRIMETHYLSILYL DERIVATIVES OF ACYLGLYCINES

Compound	5% OV-1 column		10% OV-17 column	
	Mono-TMS derivative	Di-TMS derivative	Mono-TMS derivative	Di-TMS derivative
Acetylglycine	12.53	13.57	14.86	14.86
Propionylglycine	13.34	14.17	15.37	15.37
n-Butyrylglycine	14.16	14.79	16.24	15.88
n-Valerylglycine	15.14	15.59	17.23	16.67
n-Hexanoylglycine	16.11	16.48	18.17	17.48
Isobutyrylglycine	13.72	14.08	15.50	15.16
Isovaleryiglycine	14.64	15.10	16.56	16.02
α-Methylbutyrylglycine	14.51	14.91	16.41	15.80
Acrylylglycine	13.33	14.04	15.55	15.35
Methacrylylglycine	13.88	14.42	15.92	15.52
Crotonylglycine	14.82	15.13	17.18	16.52
Vinylacetylglycine	14.02	14.71	16.23	15.96
Tiglylglycine	15.49	15.49	17.69	16.76
β-Methylcrotonylglycine	15.39	15.63	17.60	16.97
2-Furoylglycine	16.17	16.47	18.97	18.33
Hippuric acid	18.06	17.85	21.10	20.04
Phenylacetylglycine	18.66	18.42	20.67	21.74
	Di-TMS	Tri-TMS	Di-TMS	Tri-TMS
o-Hydroxyhippuric acid	20.47	19.54	23.03	21.01
p-Hydroxyhippuric acid	22.02	21.25	24.56	22.78
p-Hydroxyphenylacetylglycine	21.82	21.82	24.72	23.48

ed insignificantly from linearity for any 2-MU range. MU values shown in Table I are the average of triplicate runs. A typical chromatogram is shown in Fig. 1.

Gas chromatography—mass spectroscopy

Gas chromatography—mass spectroscopy (GC—MS) was done with a Varian MAT 111 mass spectrometer coupled to a Varian 1400 gas chromatograph. Silanized glass columns (6 ft. × 2 mm) filled with the same batches of packing as described above were used. Helium was the carrier gas at a flow-rate of 15 ml/min. The injector, separator and inlet line were kept at 250°.

RESULTS

MU values for TMS derivatives of the acylglycines are listed in Table I. All of the acylglycines gave two peaks on at least one of the columns used. The two peaks were found to be mono- and di-TMS (di- and tri-TMS for the hydroxylated aromatic acids) derivatives by GC—MS analysis. The carboxylic and phenolic groups apparently react rapidly during the derivatization reaction while the amide nitrogen is trimethylsilated more slowly.

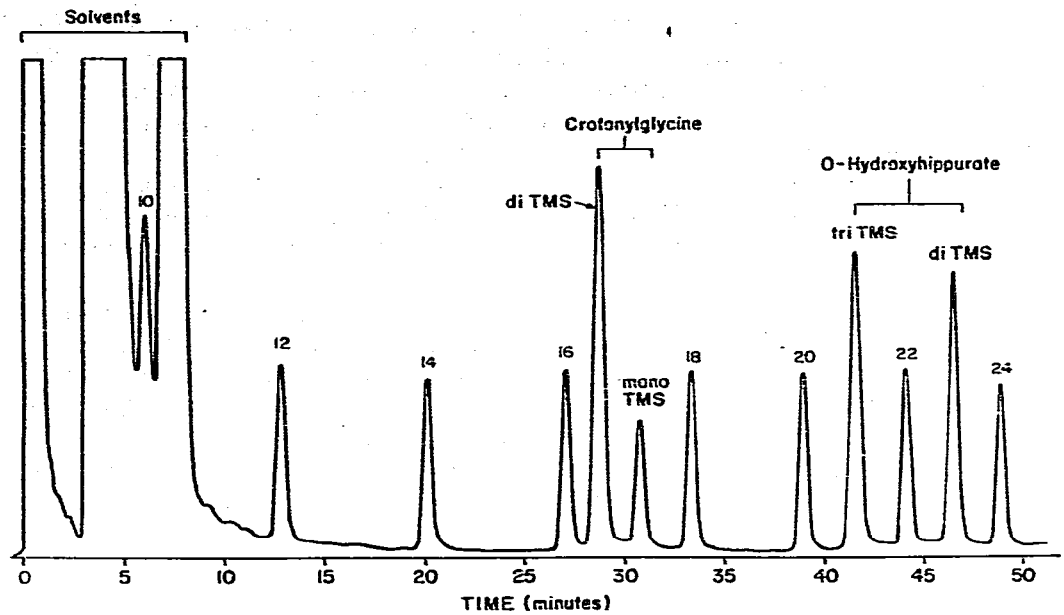

Fig. 1. Gas chromatogram of a mixture of crotonylglycine-TMS, *o*-hydroxyhippurate-TMS and even-number *n*-hydrocarbon standards. The column (2 mm x 180 cm) was packed with 10% OV-17. Temperature was programmed from 80° at a rate of 4°/min.

Di- (or tri-) TMS derivatives of some acylglycines are poorly resolved on either column. For instance, di-TMS butyrylglycine and di-TMS vinylacetylglycine elute close together on both columns and so do di-TMS valerylglycine and di-TMS tiglylglycine. The separations are better for these pairs as mono-TMS derivatives. In contrast, tiglylglycine and β-methylcrotonylglycine are better separated as di-TMS derivatives than as mono-TMS derivatives. *o*-Hydroxyhippuric and *p*-hydroxyhippuric acids are well resolved both as di- and tri-TMS derivatives on either column.

DISCUSSION

GC analysis of urinary acid extracts plays a key role in the detection of patients with organic aciduria. GC—MS analysis is usually used to identify unusual peaks. Occasionally, however, the mass spectral data are insufficient for identification due to incomplete separation of the components of an acid extract of urine. In such cases, precise GC characterization of the peak of interest can be of great value, if data for authentic standards are available as presented in this report. These GC retention indices are particularly useful for laboratories which are not equipped with GC—MS apparatus. Since the identification of the first organic aciduria, isovaleric acidemia, in 1966 [18], urinary organic acids in normal subjects [19] and in patients with various metabolic diseases [7] have been well characterized. It is our experience that with an extensive list of accurate GC retention indices of these normal and pathological metabolites on OV-1 and OV-17 columns, most of the known

diseases of organic acid metabolism could be readily identified without GC–MS. In these metabolic diseases, acylglycines are often the key metabolite for the diagnosis, but few of these acylglycines are commercially available. Previously, O'Neil-Rowley and Gerritsen [17] reported MU values of TMS derivatives of eight acylglycines. Mono- and di-TMS derivatives, however, were not identified in their study. As the first stage of a systematic study, we report here the MU values of TMS derivatives of twenty acylglycines. An extensive list of other organic acids is currently being compiled in our laboratory to be reported in the near future.

Trimethylsilylation is a commonly used derivatization method in urinary organic acid analysis. TMS derivatives are made by simple procedures using relatively less hazardous reagents. Especially when preceded by oxime formation, trimethylsilylation is applicable to acidic urinary metabolites with keto or aldehydic group [20]. The formation of two peaks with acylglycines is a problem, however. Formation of a single peak (di- or tri-TMS) is possible using a longer reaction time [17]. This reduces the complexity of chromatograms of samples containing acylglycines.

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