Gas Chromatographic Mass Spectrometric Identification of *N*-Dicarboxylmonoglycines

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A number of *N*-dicarboxylmonoglycines of biological interest have been synthesized. They were characterized by means of mass spectrometry. Gas chromatography of the methyl esters of methylmalonyl-, succinyl-, glutaryl-, adipyl-, suberyl- and sebacylglycines showed a single sharp peak for each compound on Dexsil 300 and OV-17 columns. Methylene unit values and mass spectra of the six methyl esters are reported.

INTRODUCTION

The accumulation of short chain monocarboxylic acids in the body fluids of patients with certain types of organic acidurias has been shown to be accompanied by urinary excretion of *N*-monocarboxylglycines, i.e. conjugates between short chain monocarboxylic acids and glycine.[1–10] Therefore, both gas chromatographic and combined gas chromatographic mass spectrometric methods for the detection and identification of these types of acylglycines in urine have been developed, most recently by Rowley *et al.*[11] and Ramsdell *et al.*[12,13] Recently, we identified a hitherto unknown *N*-acylglycine, suberylmonoglycine, in the urine of a boy with C_6–C_{10}-dicarboxylic aciduria.[14] Other metabolic disturbances with urinary excretion of dicarboxylic acids, i.e. methylmalonic acid[15] and glutaric acid,[16,17] are known. However, *N*-dicarboxylmonoglycines (dicarboxylglycines) have so far not been detected in these dicarboxylic acidurias.

The aim of the present work has been to synthesize and to characterize methylmalonyl-, succinyl-, glutaryl-, adipyl-, suberyl- and sebacylglycines, and to measure some gas chromatographic and mass spectrometric properties of their methyl esters in order to develop a sensitive gas chromatographic mass spectrometric method for the detection of these compounds in biological fluids.

EXPERIMENTAL

Materials

Succinic acid (1,4-butanedioic acid), glutaric acid (1,5-pentanedioic acid), adipic acid (1,6-hexanedioic acid), suberic acid (1,8-octanedioic acid) and sebacic acid (1,10-decanedioic acid) were purchased from Koch-Light Laboratories Ltd (Buckingshamshire, England). Methylmalonic acid (2-methyl-1,3-propanedioic acid) was obtained from Gluka A.G. (Switzerland). Succinic anhydride and glycine were delivered by Merck A.G. (Darmstadt, Germany). Stationary phases for gas chromatography, Dexsil 300 and OV-17, were purchased from Analabs Inc. (North Haven, Connecticut, U.S.A.) and Pierce Chemical Co. (Illinois, U.S.A.), respectively. Reagent for methylation, Diazal® was obtained from EGA Chemical KG (Albuch, Germany). Column support, Chromosorb W (HP) was delivered by Pierce Chemical Co.

The adipyl, suberyl-, and sebacylglycines were synthesized in the following way: dicarboxylic acid (16 mmol) was treated with an equimolar amount of thionylchloride (16 mmol, 2.0 g) in 25 ml boiling dioxane for 5 h. The dioxane was then removed *in vacuo* and the dicarboxylmonochloride was mixed with 30 ml of a 2 mol l^{-1} NaOH solution containing glycine (120 mmol, 9.0 g). Stirring was continued for 1 h at room temperature before the reaction mixture was saturated with NaCl and pH was adjusted to 1 with HCl solution. The precipitate, consisting of a mixture of dicarboxylic acid and dicarboxylglycine, was removed by filtration and the filtrate was extracted with ether + ethylacetate. The organic phase was dried (Na_2SO_4), and removal of the solvent gave a crude mixture of dicarboxylic acid and dicarboxylglycine. This mixture, together with the first precipitate, was recrystallized from ethylacetate + hexane to give the pure dicarboxylglycine. Melting points were: adipylglycine 139–146 °C, suberylglycine 125.5–126 °C and sebacylglycine 123–126 °C. Elementary analysis for suberylglycine has been reported previously.[14]

Methylmalonylglycine was synthesized by the same procedure, except that the boiling of methylmalonic acid and thionylchloride was performed in 10 ml ether for 3 h. Melting point was 126–130 °C. Succinylglycine was synthesized from succinic anhydride (10 mmol) in a reaction at room temperature with glycine (40 mmol, 3.0 g) in 20 ml 1 mol l^{-1} NaOH solution for 1 h. The reaction mixture was treated as described above. Melting point was 145.4–146 °C. Elementary analysis: Found: C 41.05, H 5.45, N 7.76. Calculated for $C_6H_9NO_5$: C 41.13, H 5.18, N 8.00.

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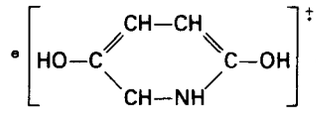
Table 1. Characteristic fragments in the mass spectra of dicarboxylmonoglycines. (Intensities relative to base peak are given in parentheses).

	$[M]^{\ddagger}$	$[M-18]^{\ddagger/a}$	$[M-45]^{+/b}$	$[M-74]^{+/c}$	$[M-102]^{+/d}$	*m/e* 112[e]	$[M-119]^{+/f}$	*m/e* 55[g]	Base peak
Succinylglycine	175	157(3)	130(4)	101(10)	73(9)	(64)	56(100)	(82)	56
Methylmalonylglycine	175	157(0.7)	130(4)	101(7)	73(50)	(0.4)	56(36)	(100)	55
Glutarylglycine	189	171(3)	144(4)	115(15)	87(15)	(2)	70(16)	(100)	55
Adipylglycine	203	185(0.2)	158(0.2)	129(5)	101(13)	(3)	84(6)	(100)	55
Suberylglycine	231	213(0.3)	186(0.5)	157(15)	129(1)	(100)	112(100)	(100)	55
Sebacylglycine	259	241(0.6)	214(1)	185(11)	167(2)	(11)	140(2)	(100)	55

[a] $[M-H_2O]^+$. [b] $[M-COOH]^+$. [c] $[M-NHCH_2COOH]^+$. [d] $[M-CONHCH_2COOH]^+$.

[e]  (tentatively).

[f] $[M-NHCH_2COOH-COOH]^+$. [g] $[CH_2CHCO]^+$ (tentatively).

Glutarylglycine was synthesized by the same method from glutaric anhydride, obtained by treating glutaric acid with acetylchloride.[18] Melting point was 111–113 °C. All six compounds were characterized by means of selected mass fragments in the mass spectra (Table 1).

Formation of methyl esters of the dicarboxylglycines was performed from approximately 1 mg compound by the method described previously.[14]

Instrumentation

The gas chromatographic analysis was performed on a Hewlett-Packard 5830 A gas chromatograph equipped with a flame ionization detector. The column was a 6 ft × 1/8 in glass coil packed with 3% Dexsil 300 or OV-17 on Chromosorb W (HP) 80/100 mesh. The column temperature was programmed from 60 °C at 4 °C min^{-1} to 260 °C. The helium carrier flow rate was 20 ml min^{-1}.

Electron impact mass spectra were obtained by means of an A.E.I. MS-30 double focusing mass spectrometer equipped with a Pye Unicam 104 gas chromatograph and a direct insertion probe. Ionizing and accelerating potentials were 70 eV and 4 kV, respectively.

The gas chromatographic conditions in the gas chromatographic mass spectrometric analysis of the dicarboxylglycine methyl esters were as follows: the 7 ft × 1/4 in glass column was packed with 3% Dexsil 300 on Chromosorb W (HP). The column temperature was programmed from 150 °C at 15 °C min^{-1}. The helium carrier flow rate was 40 ml min^{-1}.

RESULTS AND DISCUSSION

All the free dicarboxylglycines can be stored in a desiccator at room temperature for some months. However, adipylglycine is less stable than the other conjugates, and on recrystallization from hot ethylacetate will decompose to adipic acid and glycine. This decomposition is probably the reason for our difficulty in obtaining pure adipylglycine, and is reflected in the wide range of melting points (7 °C).

Characterization of the six dicarboxylglycines was performed mass spectrometrically by use of the fragment ions shown in Table 1. The accordance of the elementary analyses of succinylglycine and suberylglycine with the atomic composition of these compounds supports the legitimacy of this procedure.

The identification of *N*-monocarboxylglycines in biological fluids has employed gas chromatography combined with mass spectrometry of both trimethylsilyl esters and methyl esters.[1–13,19] We have chosen methyl esters because of the very good gas chromatographic behaviour of the methylated derivatives of the *N*-dicarboxylglycines. This has been demonstrated previously for suberylglycine,[14] and it is illustrated for succinylglycine and adipylglycine in Fig. 1. However, even on a well conditioned column, tailing of these very polar compounds cannot be avoided. Therefore, small amounts of dicarboxylglycines will result in small broad peaks, which may be overlooked in gas chromatographic analyses of metabolic profiles.

The methylene unit (U_m) values[20] for the dicarboxylglycines on two gas chromatographic columns coated with 3% Dexsil 300 and 3% OV-17, respectively, are shown in Table 2. It should be noted that the U_m values for glutarylglycine and benzoylglycine (hippuric acid) are similar. Hippuric acid has U_m value 17.79 on 3% Dexsil 300, and this compound will always be found in urine

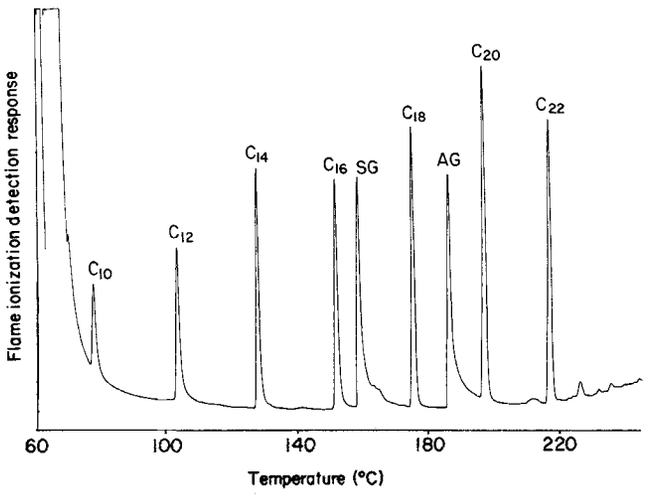


Figure 1. Gas chromatogram of methylated succinylglycine (SG) and adipylglycine (AG). The peaks C_{10}–C_{22} represents alkanes with carbon chain length C_{10}–C_{22} (g.c. conditions, see text).

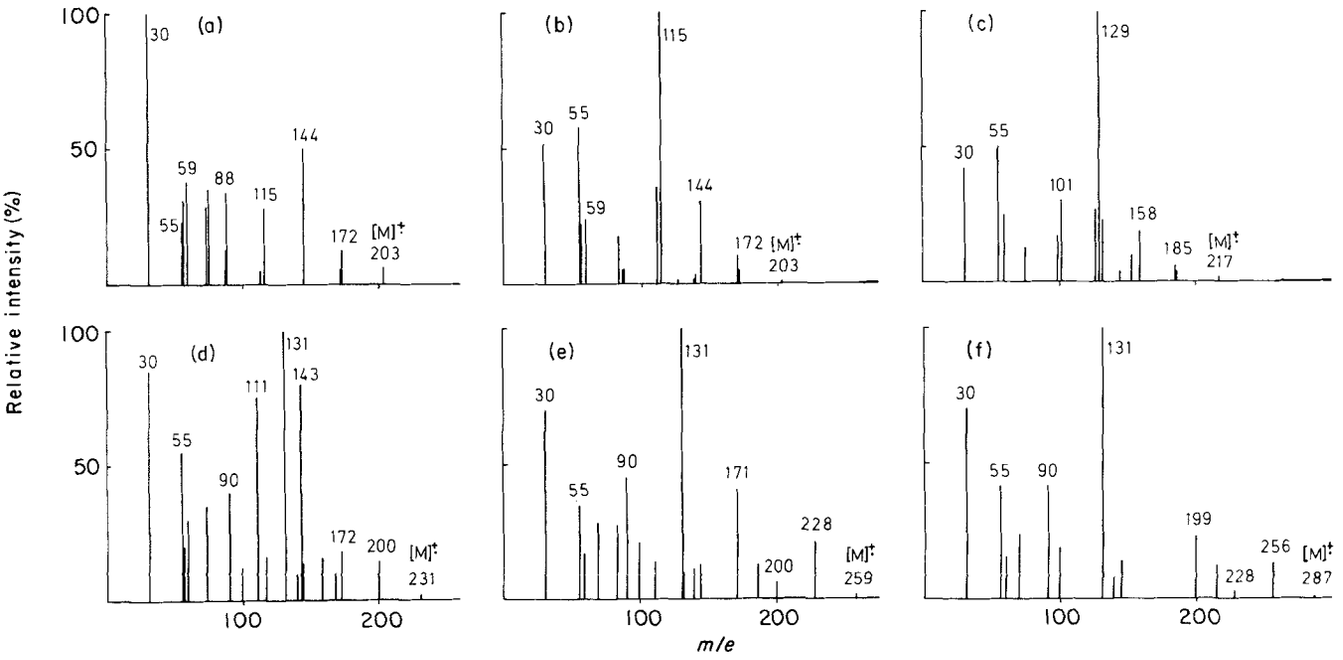
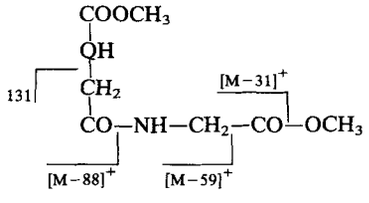


Figure 2. Mass spectra of methyl esters of: (a) methylmalonylglycine; (b) succinylglycine; (c) glutarylglycine; (d) adipylglycine; (e) suberylglycine; (f) sebacylglycine.

Table 2. Methylene unit values for dicarboxylglycine methyl esters

	Dexsil 300	OV-17
Methylmalonylglycine	15.47	17.58
Succinylglycine	16.62	19.07
Glutarylglycine	17.75	20.16
Adiplyglycine	18.97	21.49
Suberylglycine	21.07	23.5
Sebacylglycine	23.1	25.3

samples. The mass spectra of the methyl esters of the six synthesized *N*-dicarboxylglycines are shown in Fig. 2. The fragmentation patterns are similar for each compound. The most important fragments have the following origin:



$QH = CH_2, (CH_2)_2, (CH_2)_3, (CH_2)_5, (CH_2)_7$

The fragments $[M-31]^+$ and $[M-59]^+$ are ions which are common to all short and medium chain carboxylic acid methyl esters.[21,22] The ion at *m/e* 131 is very intense in the mass spectra of adipyl-, suberyl- and sebacylglycines. This fragment ion is equivalent to the ion *m/e* 74 from methylated carboxylic acids, where it represents the fragment $[HCH_2COOCH_3]^{\ddagger}$.[21] Therefore, the ion at *m/e* 131 is rather specific for medium and long chain mono- and dicarboxylglycines. As seen from the fragmentation pattern, the fragments at *m/e* 30 $[NH_2CH_2]^+$ and $[M-88]^+$ originate from the glycine part of the molecules. Therefore they can be considered as indicative for acylglycines when observed in mass spectra of compounds in metabolic profiles.

In screening programmes for metabolic disorders the profiles of organic acids in biological fluids are often analysed in methylated extracts by means of packed column gas chromatography.[14] Using such a system and the data presented in this report it has been possible to detect and obtain a positive identification of 200–500 μg suberylglycine/mg creatinine[14] and of 100–400 μg succinylglycine/mg creatinine[23] in the urine of a boy with C_6–C_{10}-dicarboxylic aciduria.

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