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GAS–LIQUID CHROMATOGRAPHY AND MASS SPECTRAL ANALYSIS OF MONO-, DI- AND TRICARBOXYLATES AS THEIR *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES

THOMAS P. MAWHINNEY*, R. S. ROBIN ROBINETT, ASMARE ATALAY and MICHAEL A. MADSON

Departments of Child Health and Biochemistry, University of Missouri-Columbia, 322-A Chemistry Building, Columbia, MO 65211 (U.S.A.)

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SUMMARY

A gas–liquid chromatographic (GLC) procedure is described which permits the baseline separation and quantitation of twenty saturated monocarboxylates, sixteen dicarboxylates (both alkanedioic and alkenedioic), and the tricarboxylates citrate and aconitate in a single GLC analysis. The carboxylic acids, as their *tert.*-butyldimethylsilylated derivatives, are readily separable on a SPB-1 (bonded) capillary column. Separation data on an OV-17 (bonded) capillary column and a SP-2250 packed column of these carboxylates, in addition to fifteen unsaturated carboxylates, are also given. The *tert.*-butyldimethylsilylation of carboxylic acids in their free or ammonium salt forms is accomplished in a single derivatization step with N-methyl-N-(*tert.*-butyldimethylsilyl)-trifluoroacetamide. Retention data and responses for all mono-, di- and tricarboxylic acids are given. Mass spectral analysis of all *tert.*-butyldimethylsilylated carboxylic acids is presented and each displays a prominent and characteristic [M − 57] fragment ion.

INTRODUCTION

Short and long chain monocarboxylic acids, unsaturated long chain monocarboxylates, dicarboxylates and tricarboxylates play many important roles in biochemistry, biological processes and in many facets of industry. Because of this, there has been extensive developments over the past two decades in the utilization of gas–liquid chromatography (GLC) for the separation and analysis of carboxylic acids. Reviews of many of these methods have appeared in the literature and provide some scope of the research in this area[1,2].

For GLC analysis, carboxylic acids have been generally separated as esterified compounds or in their free-acid form. The esters of carboxylic acids have been shown to be readily produced in a variety of ways. Some of these methods include the use of hydrochloric acid–methanol, boron trifluoride–methanol, sulphuric acid–methanol and diazomethane to form the methyl esters[3]. A wider range of esters, from

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methyl to *tert.*-butyl, can also be readily produced with N,N-dimethylformamide dialkyl acetal as the catalyst[4]. In addition, the trimethylsilyl esters (TMS)[5] and, more recently, the acetonyl esters[6] of carboxylic acids have been employed for GLC analysis of many carboxylates. As noted above, separation of some carboxylic acids in their free-acid form has also been studied and these methods are currently in use in many areas of research.

Most of these methods provide excellent separation and quantitation of specific groups of carboxylic acids on either packed or capillary columns employing flame ionization GLC detectors. Of themselves, though, few procedures permit the simultaneous analysis of monocarboxylates, dicarboxylates and tricarboxylates via a single derivatization and a single chromatographic run.

Recently, it has been reported that the *tert.*-butyldimethylsilyl (tBDMS) function is considerably more stable than the TMS group[7]. Furthermore, N-methyl-N-(*tert.*-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) has been shown to be a very powerful tBDMS silyl donor capable of *tert.*-butyldimethylsilylating active protic functions (*i.e.*, hydroxyl, amino, carboxylic and thiol moieties)[8]. The utility of this reagent to *tert.*-butyldimethylsilylate inorganic acids[9] and amino acids[10] and allow their separation by GLC has been demonstrated. MTBSTFA has also been shown to be useful in the GLC analysis of C_2–C_5 volatile fatty acids[11] in silage.

The present investigation reports on an analytical method which employs the tBDMS derivatives of carboxylic acids for their separation and quantification. This procedure allows the baseline separation of twenty saturated monocarboxylates, sixteen dicarboxylates (both alkanedioic and alkenedioic), and the tricarboxylates citrate and aconitate in a single GLC analysis. Additionally, a number of unsaturated monocarboxylates can be separated even though complete separation of C_{18} unsaturates was not achieved. The derivatization of the free carboxylic acids is accomplished in a single step with MTBSTFA, with or without an aprotic solvent. Dissolved in dimethylformamide, the carboxylic acids in their ammonium salt forms can also be derivatized via this procedure. As the tBDMS-carboxylic acid derivatives, the mono-, di- and tricarboxylic acids are stable for over 24 h. Separation of the tBDMS-carboxylic acids is readily accomplished on a SPB-1 (bonded) capillary column with each tBDMS-carboxylic acid displaying a single chromatographic peak, except for malonate which gradually showed a second peak. Chromatographic results are also given for a OV-17 (bonded) capillary and a packed SP-2250 GLC columns.

Lastly, as is commonly observed for many compounds possessing a tBDMS function when analyzed by mass spectrometry (MS)[7–11], the mass spectrum for each tBDMS-carboxylic acid is relatively simple being dominated by a unique and unambiguous mass minus 57 [M − 57] fragment ion which, for many of the carboxylic acids, serves as the base fragment ion.

EXPERIMENTAL

Materials

All dicarboxylic acid and saturated monocarboxylic acid standards were obtained from PolyScience (Niles, IL, U.S.A.). Unsaturated monocarboxylates were purchased from Sigma (St. Louis, MO, U.S.A.). Tetrahydrofuran (THF), N,N-dimethylformamide (DMF), acetonitrile, dimethylsulfoxide (DMSO), ethyl acetate and

chloroform were purchased from Aldrich (Milwaukee, WI, U.S.A.) and were redistilled prior to use. MTBSTFA, with and without 1% *tert.*-butyldimethylsilyl chloride (tBDMS-Cl), was either synthesized in this laboratory[8] or was purchased from Regis (Morton Grove, IL, U.S.A.).

Gas chromatography

Direct capillary GLC analysis was performed with a Varian GLC, Model 3700 (Varian, Park Ridge, IL, U.S.A.) equipped with dual flame ionization detectors. Chromatographic columns employed were a 25 m × 0.32 mm I.D., fused-silica capillary column with 0.25-μm bonded OV-17 (Quadrex, New Haven, CT, U.S.A.) and a 30 m × 0.32 mm I.D. capillary column with 0.25-μm bonded SPB-1 (Supelco, Bellefonte, PA, U.S.A.). The helium flow-rate was 5 ml/min, with injector and detector temperatures of 300°C. After an initial hold of 1 min at 60°C the column was temperature programmed at 4°C/min to 260°C. Packed-column GLC was performed using a Perkin-Elmer Sigma 3 instrument equipped with dual flame ionization detectors. The glass chromatographic column was 6 ft. × 1/8 in. O.D. (1.8 mm I.D.), packed with 3.0% SP-2250 (Supelco) on Supelcoport, 100–120 mesh. The nitrogen flow-rate was 18 ml/min, with injector and detector temperatures of 300°C. After an initial hold of 1 min at 60°C the column was temperature programmed at 4°C/min to 260°C. Peak areas and retention times were recorded using a Shimadzu C-R3A Chromatopac integrator (Shimadzu, Columbia, MA, U.S.A.).

Mass spectrometry

Mass spectra were obtained on a Kratos MS 50 S mass spectrometer (Kratos, Urmston, Manchester, U.K.) interfaced with a Carlo Erba Model 4160 gas chromatograph. Mass spectra were recorded at 70 eV with an ionization current of 50 μA, a source temperature of 250°C, and a transfer temperature 218°C.

Carboxylic acid standard solution

Two standard solutions were employed. One contained the carboxylates in their free acid form and the other contained the carboxylates as their ammonium salts. Both standard solutions contained 0.5 mg/ml of each carboxylate in THF. Hexadecane was included at the same concentration as the internal standard. For GLC and gas–liquid chromatography–mass spectrometry (GLC–MS) analysis 0.1-ml aliquots of the respective stock solution were used.

Derivatization of carboxylic acids

All monocarboxylic and dicarboxylic acids in their free acid forms were derivatized by the following procedure. To the respective reactivial, equipped with a small PTFE-coated stir bar and a PTFE-faced silicone septum, 10 μl of DMF was added to the sample to be derivatized. Then 250 μl of MTBSTFA, without tBDMS-Cl, was added at room temperature with stirring. The ammonium salts of the monocarboxylic and dicarboxylic acids were derivatized in the same manner with the exception that 40 μl of DMF was used and after the addition of 250 μl of MTBSTFA, containing 1% tBDMS-Cl, the samples were heated at 60°C with constant stirring until complete dissolution of the sample was achieved. For packed and capillary column GLC analysis 1.5 and 0.1 μl were injected per analysis, respectively. In other experiments, THF, DMSO, acetonitrile or no solvent was substituted for DMF.

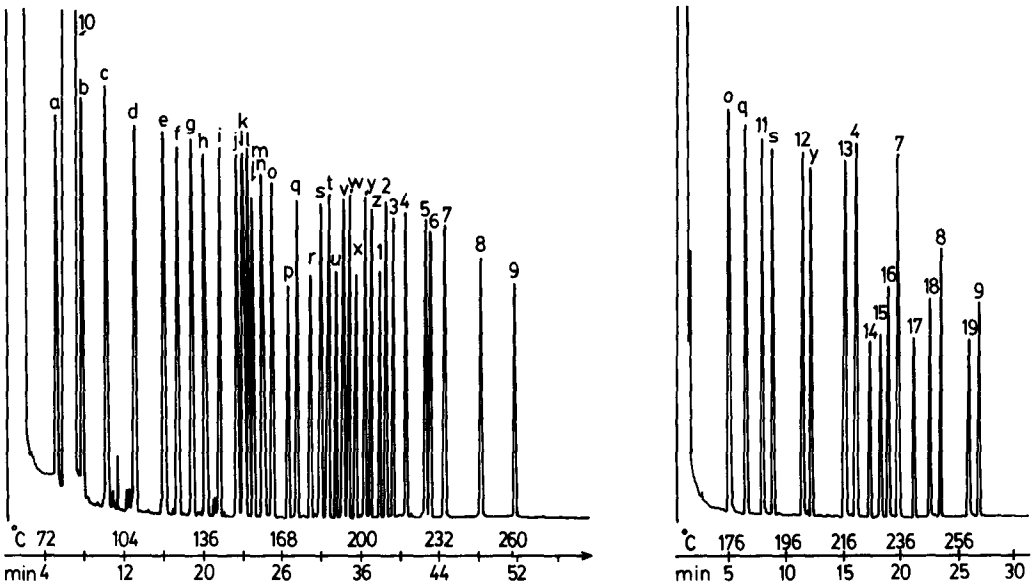

Fig. 1. Gas–liquid chromatogram of the tBDMS derivatives of 35 carboxylic acids. Separation was performed on a 30 m × 0.32 mm I.D., SPB-1 (bonded) capillary GLC column with a film thickness of 0.25 μm. Injected sample contained 5.0 nmol of each tBDMS carboxylate. Program: after an initial hold of 1 min at 60°C the column was temperature programmed at 4°C/min to 260°C. Helium was employed as the carrier gas at a flow-rate of 5 ml/min. Peaks: a = butyric; b = valeric; c = caproic; d = oenanthic; e = caprylic; f = oxalic; g = pelargonic; h = malonic; i = capric; j = maleic; k = succinic; l = pelargonic; m = fumaric; n = glutaric; o = lauric; p = adipic; q = tridecanoic; r = pimelic; s = myristic; t = phthalic; u = suberic; v = pentadecylic; w = isophthalic; x = azelaic; y = palmitic; z = terephthalic; 1 = sebacic; 2 = margaric; 3 = aconitic; 4 = stearic; 5 = *n*-nonadecylic; 6 = citric and dodecanedioic; 7 = arachidic; 8 = behenic; 9 = lignoceric. Peak labeled 10 is the derivatizing reagent N-methyl-N-*tert.*-butyldimethylsilyltrifluoroacetamide (MTBSTFA).

Fig. 2. Gas–liquid chromatogram of the tBDMS derivatives of eight saturated and nine unsaturated monocarboxylic acids. Separation was performed on a 30 m × 0.32 mm I.D., SPB-1 (bonded) capillary GLC column with a film thickness of 0.25 μm. Injected sample contained 5.0 nmol of each tBDMS carboxylate. Program: after an initial hold of 1 min at 160°C the column was temperature programmed at 4°C/min to 260°C. Helium was employed as the carrier gas at a flow-rate of 5 ml/min. Peaks: o = lauric; q = tridecanoic; 11 = myristoleic; s = myristic; 12 = palmitoleic; y = palmitic; 13 = oleic; 4 = stearic; 14 = arachadonic; 15 = 11,14-eicosendioic; 16 = 11-eicosenoic; 7 = arachidic; 17 = docosahexaenoic; 18 = erucic; 8 = behenic; 19 = nervonic; 9 = lignoceric.

RESULTS AND DISCUSSION

Derivatization

tert.-Butyldimethylsilylation, in DMF, of all the carboxylates in this study, in their free acid form, was complete at room temperature upon the addition of 250 μl of MTBSTFA. Substitution of acetonitrile, DMSO, THF, ethyl acetate or chloroform for DMF as the carboxylate solvent did not affect the derivatization rate. Furthermore, *tert.*-butyldimethylsilylation directly with MTBSTFA went to completion in the absence of any organic solvent. This is probably related to the observation that all the tested carboxylates were readibly soluble in the derivatizing reagent itself.

Complete *tert.*-butyldimethylsilylation of the carboxylates in their ammonium salt form was accomplished only when MTBSTFA containing 1% tBDMS-Cl as a catalyst was used. With constant stirring and heating at 60°C, derivatization was complete for all carboxylates upon dissolution (< 15 min).

GLC separations

The GLC separation of the tBDMS-derivatives of a standard mixture of carboxylic acids on a SPB-1 (bonded) fused-silica capillary column is presented in Fig. 1. With the exception of malonate, for which a second slowing growing peak was observed after 1 h, each mono-, di- and tricarboxylic acid derivative displayed a single sharp symmetrical chromatographic peak with no significant peak tailing. Notably, with the exception of citrate and dodecanedioic acid which displayed the same retention time, complete baseline separation was achieved in a single chromatographic run for all of the saturated monocarboxylates and dicarboxylates in the standard. This also includes the dicarboxylates malic and tartaric acids which are not shown in Fig. 1.

Fig. 2. shows the separation of eight saturated monocarboxylates and nine unsaturated monocarboxylates in a single GLC run employing a SPB-1 (bonded) fused-silica capillary column. Though not shown, no useful separation of the C_{18} unsaturates, *i.e.*, oleic, vaccenic, elaidic, petroselinic and linolenic acids, could be achieved using this column.

Separation of the above standard mixtures on an OV-17 (bonded) capillary column generally provided the same elution pattern as seen on the SPB-1 column, though several compounds demonstrated overlapping peaks. Also, the use of this phase of intermediate polarity exhibited no advantage in the separation of the unsaturated fatty acids other than that achieved on the SPB-1 column.

Retention data

Retention times and relative retention times for each tBDMS-carboxylic acid derivative on a 3.0% SP-2250 packed GLC column and on an OV-17 (bonded) and SPB-1 (bonded) capillary columns are given in Table I. As might be expected from the fact that OV-17 and SP-2250 are very similar phases, the elution order of the carboxylic acids on the 3.0% SP-2250 packed-column and on the OV-17 (bonded) capillary column was the same. Typically, for each group of carboxylates, the carboxylic acids emerged from the non-polar SPB-1 capillary column primarily in the order of the molecular weight of the carboxylic acid derivatives. This is also true for the results achieved on the intermediate polarity columns (SP-2250 and OV-17).

Stability of the tBDMS-carboxylic acids as a function of time

In order to study the stability of each tBDMS-carboxylic acid derivative, a carboxylate standard, in DMF, was *tert.*-butyldimethylsilylated by adding 250 μl of MTBSTFA and mixing at room temperature. Following an immediate GLC analysis, aliquots of this standard were then injected at 12-h intervals for 48 h with the results being presented in Table II. As is shown, with the exception of malonic acid, all the saturated monocarboxylates, dicarboxylates, tricarboxylates and unsaturated monocarboxylates demonstrated excellent stability during the 48-h period. The gradual decrease from the initial malonic acid relative weight ratio is complemented with an

TABLE I

GAS CHROMATOGRAPHIC RETENTION DATA AND RELATIVE WEIGHT RESPONSE (RWR) FOR CARBOXYLIC ACIDS AS THEIR *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES

Gas chromatographic retention data are expressed in methylene units. The chromatographic conditions for each column employed are described in the Experimental section.

	Packed column 3% SP-2250	*Capillary column*		*RWR**		*Packed column 3% SP-2250*	*Capillary column*		*RWR**
		OV-17	*SE-30*				*OV-17*	*SE-30*	
Saturated monocarboxylates									
Propionic	10.32	10.57	9.94	0.96	Glutaric	19.16	19.23	18.46	1.29
Butyric	11.60	12.00	11.20	0.95	Adipic	20.29	20.37	19.59	1.27
Valeric	12.30	12.66	11.87	0.97	Pimelic	21.33	21.43	20.60	1.32
Caproic	13.22	13.43	12.78	0.83	Suberic	22.38	22.45	21.57	1.27
Oenanthic	14.26	14.32	13.83	0.96	Phthalic	22.64	22.90	21.25	1.38
Caprylic	15.28	15.27	14.81	0.93	Isophthatic	23.56	23.73	21.97	1.40
Pelargonic	16.23	16.27	15.78	0.81	Terephthalic	23.62	23.58	22.95	1.32
Capric	17.22	17.29	16.75	0.95	Azelaic	23.44	23.48	22.60	1.22
Pelargonic	18.19	18.39	17.73	0.83	Sebacic	24.47	24.49	23.64	1.12
Lauric	19.21	19.55	18.77	1.00	Dodecanedioic	26.52	26.66	26.00	1.01
Tridecanoic	20.19	20.45	19.77	0.89					
Myristic	21.21	21.25	20.81	0.87	*Tricarboxylates*				
Pentadecylic	22.20	22.00	21.80	0.81	Citric	26.00	26.60	26.00	1.61
Palmitic	23.32	23.18	22.81	0.85	Aconitic	24.54	24.61	24.01	1.30

Margaric	24.22	24.30	23.82	0.84	*Unsaturated monocarboxylates*				
Stearic	25.29	25.29	24.81	0.61	Myristoleic	21.18	21.27	20.56	0.63
n-Nonadecylic	26.24	26.29	25.84	0.81	Palmitoleic	23.31	23.21	22.51	0.77
Arachidic	27.28	27.36	26.88	0.84	Petrosalinic	25.24	25.14	24.48	0.65
Behenic	29.30	29.38	28.90	0.53	Elaidic	27.28	25.35	24.55	0.72
Lignoceric	31.36	31.31	30.82	0.46	Oleic	27.21	25.20	24.47	0.52
					Vaccenic	27.28	25.24	24.52	0.64
Dicarboxylates					*cis*-Linoleic	27.31	25.30	24.34	0.62
Oxalic	15.83	16.19	15.42	1.05	Linolenic	27.59	25.54	24.41	0.59
Malonic (I)	17.04	17.02	16.35	1.52	11-Eicosenoic	27.16	27.22	26.48	0.45
Malonic (II)	24.92	20.72	20.04	1.85	11,14-Eicosadienoic	29.39	27.40	26.38	0.43
Maleic	16.36	18.35	17.31	1.41	*Homo* γ-linolenic	29.33	27.34	26.00	0.48
Fumaric	18.18	18.31	18.00	1.64	Arachidonic	29.22	27.12	25.78	0.33
Malic	21.38	21.42	20.93	1.04	Docosahexaenoic	31.41	29.51	27.72	0.41
Tartaric	24.00	24.30	24.12	1.59	Erucic	29.24	29.21	28.54	0.37
Succinic	18.17	18.47	17.46	1.85	Nervonic	31.17	31.19	30.51	0.30

* The RWR represents the mean relative weight response of ten different sample injections of each carboxylate relative to the relative weight response of the internal standard hexadecane; [RWR = (carboxylate/hexadecane)]. The standard relative deviation for each RWR was less than 2% in all cases (not shown).

TABLE II

STABILITY OF THE *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES OF CARBOXYLIC ACIDS

Data expressed as the mean relative weight response (RWR) of five different sample injections of each respective carboxylic acid relative to the relative weight response of the internal standard hexadecane. Samples were injected on a 30 m × 0.32 mm I.D., SPB-1 (bonded) capillary GLC column. The chromatographic conditions are described in the Experimental section.

	RWR (carboxylate/hexadecane)						*RWR (carboxylate/hexadecane)*				
	0	*6 h*	*12 h*	*18 h*	*24 h*		*0*	*6 h*	*12 h*	*18 h*	*24 h*
Saturated monocarboxylates											
Propionic	0.93	0.93	0.92	0.92	0.92	Glutaric	1.29	1.30	1.27	1.27	1.25
Butyric	0.95	0.94	0.92	0.92	0.93	Adipic	1.27	1.25	1.26	1.24	1.24
Valeric	0.97	0.96	0.97	0.95	0.95	Pimelic	1.32	1.35	1.30	1.28	1.27
Caproic	0.83	0.85	0.82	0.81	0.81	Suberic	1.27	1.30	1.30	1.26	1.25
Oenanthic	0.96	0.94	0.91	0.92	0.91	Phthalic	1.38	1.35	1.34	1.32	1.30
Caprylic	0.93	0.93	0.91	0.90	0.91	Isophthatic	1.40	1.38	1.38	1.36	1.33
Pelargonic	0.81	0.83	0.80	0.80	0.79	Terephthalic	1.32	1.30	1.28	1.29	1.62
Capric	0.95	0.93	0.94	0.93	0.91	Azelaic	1.22	1.21	1.20	1.18	1.18
Pelargonic	0.83	0.84	0.81	0.81	0.80	Sebacic	1.12	1.14	1.12	1.12	1.10
Lauric	1.00	1.01	1.00	0.99	0.99	Dodecanedioic	1.01	1.04	1.00	1.01	1.00
Tridecanoic	0.89	0.91	0.88	0.89	0.87						
Myristic	0.87	0.85	0.86	0.85	0.84	*Tricarboxylates*					
Pentadecylic	0.81	0.80	0.80	0.78	0.79	Citric	1.61	1.64	1.60	1.59	1.60
Palmitic	0.85	0.86	0.84	0.85	0.83	Aconitic	1.30	1.27	1.29	1.27	1.26
Margaric	0.84	0.82	0.82	0.82	0.81						
Stearic	0.91	0.92	0.90	0.90	0.89	*Unsaturated monocarboxylates*					
n-Nonadecylic	0.81	0.81	0.80	0.78	0.78	Myristoleic	0.63	0.65	0.62	0.62	0.60
Arachidic	0.84	0.82	0.82	0.81	0.80	Palmitoleic	0.77	0.78	0.77	0.77	0.76
Behenic	0.53	0.55	0.52	0.52	0.51	Petroselinic	0.65	0.65	0.64	0.62	0.62
Lignoceric	0.46	0.46	0.44	0.42	0.43	Elaidic	0.72	0.70	0.69	0.70	0.68
						Oleic	0.52	0.50	0.47	0.48	0.47
Dicarboxylates						Vaccenic	0.64	0.62	0.62	0.61	0.59
Oxalic	1.05	1.03	1.03	1.02	1.01	*cis*-Linoleic	0.62	0.62	0.63	0.61	0.60
Malonic I	1.52	1.50	1.47	1.42	1.39	Linolenic	0.59	0.59	0.59	0.57	0.56
Malonic II	0.00	0.07	0.12	0.18	0.21	11-Eicosenoic	0.45	0.45	0.46	0.43	0.41
Maleic	1.41	1.39	1.38	1.36	1.37	11,14-Eicosadienoic	0.43	0.40	0.39	0.38	0.36
Fumaric	1.64	1.62	1.62	1.60	1.58	*Homo* γ-linolenic	0.48	0.45	0.42	0.41	0.41
Malic	1.04	1.05	1.03	1.04	1.02	Arachidonic	0.33	0.32	0.31	0.30	0.28
Tartaric	1.59	1.57	1.56	1.56	1.55	Docosahexaenoic	0.41	0.40	0.39	0.39	0.38
Succinic	1.85	1.84	1.85	1.83	1.83	Erucic	0.37	0.35	0.33	0.32	0.32
						Nervonic	0.30	0.29	0.28	0.27	0.27

TABLE III

INTERPRETATION AND RELATIVE INTENSITIES OF THE MAJOR FRAGMENT IONS IN THE MASS SPECTRA OF THE *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES OF SATURATED MONOCARBOXYLIC ACIDS

The intensities of fragment ions of each derivatized carboxylate are given relative to the indicated base fragment ion, *i.e.*, *m/z* (100), observed in the respective mass spectrum. M^+ were recognized for each derivative and each had relative intensities that were less than 1.0%. Interpretations: M − 15, M − $-CH_3$; M − 57, M − $-C(CH_3)_3$; *m/z* 131, *tert.*-butyldimethylsiloxide, $(TBDMS)O^+$; *m/z* 129, $CH_2=CH-CO-O^+=Si(CH_3)_2$; *m/z* 117, *tert.*-butylmethylsilanol, $H-O^+=Si(CH_3)C(CH_3)_3$; *m/z* 115, *tert.*-butyldimethylsilane, $^+Si(CH_3)_2C(CH_3)_3$; *m/z* 75, dimethylsilanol, $H-O^+=Si(CH_3)_2$; *m/z* 73, *tert.*-butyloxide, $^+O-C(CH_3)_3$.

Saturated monocarboxylic acid	*m/z (relative intensity, %)*								
	M^+	*M−15*	*M−57*						
Butanoic	202	187 (1.6)	145 (100)	131 (0.2)	129 (0.9)	117 (0.6)	115 (5.7)	75 (91)	73 (22)
Pentanoic	216	210 (1.7)	159 (93)	131 (0.3)	129 (1.9)	117 (2.1)	115 (6.5)	75 (100)	73 (25)
Hexanoic	230	215 (1.6)	173 (100)	131 (10)	129 (2.8)	117 (3.2)	115 (6.0)	75 (81)	73 (25)
Heptanoic	244	229 (1.8)	187 (86)	131 (13)	129 (5.8)	117 (5.4)	115 (6.4)	75 (100)	73 (27)
Octanoic	258	243 (1.8)	201 (85)	131 (14)	129 (8.9)	117 (6.2)	115 (5.9)	75 (100)	73 (27)
Nonanoic	272	257 (1.9)	215 (88)	131 (16)	129 (13)	117 (8.3)	115 (6.5)	75 (100)	73 (29)
Decanoic	286	271 (1.8)	229 (87)	131 (15)	129 (14)	117 (9.2)	115 (6.1)	75 (100)	73 (28)
n-Hendecanoic	300	285 (1.8)	243 (89)	131 (14)	129 (16)	117 (11)	115 (6.2)	75 (100)	73 (29)
10-Hendecenoic	298	283 (1.7)	241 (100)	131 (12)	129 (13)	117 (5.1)	115 (5.1)	75 (88)	73 (31)
Dodecanoic	314	299 (1.7)	257 (94)	131 (13)	129 (17)	117 (12)	115 (5.9)	75 (100)	73 (22)
Tridecanoic	328	313 (1.1)	271 (88)	131 (12)	129 (14)	117 (12)	115 (5.6)	75 (100)	73 (27)
Tetradecanoic	342	327 (1.5)	285 (100)	131 (13)	129 (24)	117 (9.8)	115 (5.1)	75 (92)	73 (25)
Pentadecanoic	356	341 (1.5)	299 (100)	131 (8.4)	129 (11)	117 (8.9)	115 (3.3)	75 (36)	73 (10)
Hexadecanoic	370	355 (1.7)	313 (100)	131 (12)	129 (20)	117 (15)	115 (5.9)	75 (94)	73 (29)
Heptadecanoic	384	369 (1.2)	327 (100)	131 (9.7)	129 (14)	117 (13)	115 (4.6)	75 (74)	73 (25)
Octadecanoic	398	383 (1.3)	341 (96)	131 (12)	129 (26)	117 (17)	115 (6.5)	75 (100)	73 (18)
Nonadecanoic	412	397 (1.3)	355 (100)	131 (13)	129 (23)	117 (22)	115 (7.5)	75 (87)	73 (32)
Eicosanoic	426	411 (1.4)	369 (100)	131 (3.5)	129 (19)	117 (5.1)	115 (1.2)	75 (65)	73 (26)
Docasanoic	454	439 (1.8)	397 (100)	131 (4.4)	129 (21)	117 (6.9)	115 (1.7)	75 (20)	73 (22)
Tetracosanoic	482	467 (1.5)	425 (100)	131 (1.3)	129 (19)	117 (3.7)	115 (2.2)	75 (52)	73 (19)

TABLE IV

INTERPRETATION AND RELATIVE INTENSITIES OF THE MAJOR FRAGMENT IONS IN THE MASS SPECTRA OF THE *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES OF UNSATURATED MONOCARBOXYLIC ACIDS

The intensities of fragment ions of each derivatized carboxylate are given relative to the indicated base fragment ion, *i.e.*, *m/z* (100), observed in the respective mass spectrum. M^+ were recognized for each derivative and each had relative intensities that were less than 1.0%. Fragment ion interpretations are presented in Table III.

Unsaturated monocarboxylic acid	*m/z (relative intensity, %)*								
	M^+	$M - 15$	$M - 57$						
Myristoleic	340 (0.1)	325 (1.8)	283 (100)	131 (7.3)	129 (13)	117 (2.5)	115 (2.9)	75 (56)	73 (18)
Palmitoleic	368 (0.1)	353 (1.5)	311 (100)	131 (7.7)	129 (11)	117 (2.1)	115 (2.4)	75 (45)	73 (14)
Oleic	396 (0.1)	381 (1.4)	339 (100)	131 (2.2)	129 (10)	117 (2.2)	115 (1.4)	75 (36)	73 (11)
Linoleic	394 (0.1)	379 (2.9)	337 (100)	131 (10)	129 (23)	117 (4.6)	115 (4.8)	75 (64)	73 (34)
Linolenic	392 (0.1)	377 (1.3)	335 (100)	131 (3.0)	129 (11)	117 (1.7)	115 (1.7)	75 (35)	73 (13)
Arachidonic	418 (1.3)	403 (1.4)	361 (87)	131 (12)	129 (20)	117 (15)	115 (5.8)	75 (100)	73 (55)
Erucic	452 (0.1)	437 (1.2)	395 (100)	131 (7.1)	129 (13)	117 (3.4)	115 (2.7)	75 (54)	73 (20)
Nervonic	480 (0.1)	465 (1.2)	423 (100)	131 (7.6)	129 (13)	117 (5.1)	115 (3.1)	75 (60)	73 (25)
Petroselinic	396 (0.1)	381 (2.0)	339 (100)	131 (5.9)	129 (16)	117 (7.5)	115 (3.3)	75 (57)	73 (25)

increase in a second chromatographic peak. The mass spectrum and identification of this new peak for malonate is discussed below.

Quantitative aspects

The relative weight response (RWR) of the tBDMS-carboxylic acids with respect to the internal standard, *i.e.*, hexadecane, is shown in Table I. Though not reported, the standard relative deviation for each RWR was less than 2% in all cases demonstrating the excellent precision in the relative weight response of each carboxylic acid.

Employing carboxylic acid standards, a linear response curve in the range of 0.5–100 nmol was obtained for each mono-, di- and tricarboxylate using flame ionization as the GLC detector. The unsaturated monocarboxylates, because of the decrease in RWR with increased degree of unsaturation, varied in their lower range limit for linear response from 0.75 nmol for myristoleic acid to 1.6 nmol for nervonic acid.

Mass spectrometry

Each synthesized tBDMS-carboxylic acids was subjected to combined GLC–MS analysis. Tables III and IV show the mass spectral results for the saturated and unsaturated monocarboxylates, respectively. Both groups of tBDMS-carboxylates produced the same general fragmentation. All saturated and unsaturated tBDMS-carboxylic acids yielded mass spectrums that were dominated by a singular unique [M − 57] fragment ion in the high-molecular-weight mass spectral region. As is typical of tBDMS derivatives, this fragment ion results from the elimination of one *tert.*-butyl function [*i.e.*, M − $-C(CH_3)_3$] from the molecule. This fragment ion is so intense that it serves as the base fragment ion in many of the mass spectra. In those mass spectra where it is not the base ion the [M − 57] fragment ion had relative intensities above 85% and the fragment ion dimethylsilanol (*m/z* 75) served as the base ion. In addition to the [M − 57] fragment ion, each derivative displayed both a weak molecular ion (M^+, $<1.0\%$) and a low relative intensity [M − 15] fragment ion which is produced by the loss of a methyl group ($-CH_3$) from the derivative. Also typical of these derivatives is fragment ion *m/z* 129 interpreted to be $CH_2=CH-CO-O^+=Si(CH_3)_2$. All other major fragment ions in the mass spectra (*i.e.*, *m/z* 131, 117, 115, 75 and 73) do not possess the parent molecule and are derived from the tBDMS function (Tables III and IV).

Table V presents the relative intensities and interpretations of the major fragment-ions in the mass spectra of the di- and tricarboxylates as their tBDMS derivative. In general, most of these derivatives displayed the fragment ions *m/z* 117, 115, 75 and 73 noted above for the saturated and unsaturated monocarboxylates. In addition, for many derivatives, fragment ions *m/z* 189 [$(TBDMS)O-Si(CH_3)_2$], 147 [*m/z* 246 − $(TBDMS)_2O$ − 99] and 133 [$(CH_3)_2HSi-O^+=Si(CH_3)_2$] are present and represent fragment ions derived from the di-*tert.*-butyldimethylsilyl ether, though this ion (*m/z* 246) itself is not observed. As might be expected, fragment ion *m/z* 129 [*i.e.*, $CH_2=CH-CO-O^+=Si(CH_3)_2$] is observed, with increasing relative intensities, for only those carboxylates possessing two or more methylene carbons within the alkanedioic acid structure (*i.e.*, succinate to dodecanedioicate, Table V).

In contrast to the monocarboxylates (Tables III and IV), the aliphatic alkane-

dioic and alkenedioic acids (*i.e.*, oxalate to dodecanedioate, Table V), and the tricarboxylates (*i.e.*, citrate and aconitate, Table V), showed considerable variation in the relative intensities of the prominent [M − 57] fragment ion ranging from 10% for citric acid to 100% for fumarate. Also, with the exception of fumarate, *tert.*-butyloxide (*m/z* 73) served as the base ion. The loss of both (TBDMS)O and $Si(CH_3)_2$ from malate, tartrate, aconitate and citrate account for the [M − 189] fragment ions *m/z* 287, 417, 327 and 459, respectively.

As indicated above, malonate displays one chromatographic peak upon derivatization (malonate I, Table V). With a molecular ion of *m/z* 332 and an [M − 57]

TABLE V

INTERPRETATION AND RELATIVE INTENSITIES OF THE MAJOR FRAGMENT-IONS IN THE MASS SPECTRA OF THE *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES OF DI- AND TRICARBOXYLIC ACIDS

The fragment ion intensities are given relative to the indicated base fragment ion, *i.e.*, *m/z* (100), observed in the respective mass spectrum. M^+ were recognized for each derivative and each had relative intensities that were less than 1.0%. Fragment ion interpretations of M − 15, M − 57, *m/z* 131, 129, 117, 115, 75 and 73 are presented in Table III. Interpretations: *m/z* 133, $[(CH_3)_2HSi{-}O^+{=}Si(CH_3)_2]$; *m/z* 293, (TBDMS-O) $[(CH_3)_2Si{-}O]C{=}(C_6H_4)^+$; *m/z* 279, M − 115; *m/z* 263, (TBDMS)OOC–(C_6H_4)–$C{=}O^+$; *m/z* 223, $(C_5H_4)^+$–COO(TBDMS); *m/z* 178, (C_6H_4)–$COO^+{=}Si(CH_3)_2$; *m/z* 163, $(C_6H_4){=}CH{-}O^+{=}Si(CH_3)_2$; *m/z* 140, (C_3H_2)–$COO^+{=}Si(CH_3)_2$; *m/z* 105, (C_6H_4)–$C{=}O^+$–H; *m/z* 104, $^+(C_6H_4){=}C{=}O$; *m/z* 77, HOOC–CH_2–$C{\equiv}O$.

Carboxylic acid	*m/z (relative intensity, %)*					
	M^+	*M − 15*	*M − 57*			
Dicarboxylates						
Oxalic	(318)	303 (0.2)	261 (50)			189 (3.0)
Malonic I	(332)	317 (0.6)	275 (47)		143 (10)	189 (7.0)
Malonic II	(446)	431 (3.8)	389 (66)	315 (13)	143 (15)	189 (2.0)
Maleic	(344)	329 (0.7)	287 (24)			189 (0.3)
Fumaric	(344)	329 (2.2)	287 (100)			
Malic	(476)	461 (1.7)	419 (39)	287 (25)		189 (6.6)
Tartaric	(606)	591 (1.3)	549 (33)	417 (2.1)		189 (2.4)
Succinic	(346)	331 (1.2)	289 (26)			189 (1.0)
Glutaric	(360)	345 (1.5)	303 (35)			189 (1.9)
Adipic	(374)	359 (1.6)	317 (34)			
Pimelic	(388)	373 (1.7)	331 (37)			
Suberic	(402)	387 (1.4)	345 (33)			
Azelaic	(416)	401 (2.0)	359 (52)			
Sebacic	(430)	415 (0.3)	373 (62)			
Dodecanedioic	(458)	443 (1.5)	401 (56)			
Phthalic	(394)	379 (2.5)	337 (100)	293 (2.4)	263 (11)	178 (18) 177 (0.8)
Isophthalic	(394)	379 (2.4)	337 (100)	279 (2.3)	263 (10)	178 (25) 177 (3.1)
Terephthalic	(394)	379 (3.0)	337 (100)	223 (4.0)	263 (3.4)	247 (3.0)
Tricarboxylates						
Aconitic	(516)	501 (0.9)	459 (34)	327 (7.7)		77 (29)
Citric	(648)	633 (0.2)	591 (10)	459 (13)	357 (3.9)	77 (22)

ion of m/z 275, this derivative is interpreted to be malonate with two TBDMS functions. However, a second peak for malonate (malonate II, Table V) slowly begins to show. Malonate II yielded a molecular ion of m/z 446 and an [M − 57] fragment ion of 389 which is consistent with a malonate molecule possessing three TBDMS functions and having the parent molecular structure (TBDMS)OOC–CH=C(O–TBDMS)$_2$. Apparently, one of the protons on the methylene carbon of malonate I is sufficiently acidic to be removed allowing a third TBDMS function to be added forming malonate II. The presence of this acidic methylene proton is also evident for malonate I by the existence of fragment ions m/z 143 [O=C=CH–COOSi(CH$_3$)$_2$]

147 (31)	133 (4.3)		117 (2.9)	115 (0.2)	75 (8.9)	73 (100)
147 (22)	133 (8.4)		117 (2.4)	115 (6.3)	75 (72)	73 (100)
147 (40)	133 (10)		99 (13)	77 (16)	75 (63)	73 (100)
147 (12)	133 (5.1)		117 (1.5)	115 (4.2)	75 (19)	73 (100)
147 (7.2)	133 (8.1)		117 (2.2)	115 (3.4)	75 (35)	73 (81)
147 (29)	133 (12)			115 (23)	75 (24)	73 (100)
147 (17)	133 (4.3)			115 (12)	75 (10)	73 (100)
147 (25)	133 (4.5)	129 (3.4)	117 (1.7)	115 (4.5)	75 (25)	73 (100)
147 (20)	133 (4.7)	129 (5.3)	117 (1.6)	115 (4.4)	75 (31)	73 (100)
147 (7.8)	133 (3.1)	129 (6.4)	117 (1.6)	115 (4.6)	75 (45)	73 (100)
147 (6.5)	133 (3.0)	129 (8.1)	117 (1.8)	115 (4.8)	75 (52)	73 (100)
147 (3.8)	133 (2.2)	129 (12)	117 (2.1)	115 (4.8)	75 (57)	73 (100)
147 (3.9)	133 (2.5)	129 (14)	117 (3.2)	115 (5.8)	75 (60)	73 (100)
147 (2.2)	133 (1.4)	129 (11)	117 (2.3)	115 (4.3)	75 (57)	73 (100)
147 (3.0)	133 (1.8)	129 (17)	117 (3.5)	115 (5.5)	75 (69)	73 (100)
140 (41)	133 (1.9)			104 (15)	75 (5.5)	73 (4.8)
		135 (3.8)	134 (3.1)			
140 (41)	133 (5.3)			104 (9.2)	75 (21)	73 (72)
163 (2.0)		135 (9.5)	134 (5.7)	119 (5.4)		
147 (66)	133 (7.0)			104 (8.0)	75 (22)	73 (90)
163 (11)	140 (6.3)	135 (3.7)	134 (2.1)	119 (5.8)	105 (19)	
147 (11)	133 (3.0)		117 (0.8)	115 (3.6)	75 (41)	73 (100)
147 (16)	133 (2.5)		117 (1.4)	115 (3.7)	75 (22)	73 (100)

and *m/z* 99 [$HC{\equiv}C{-}O^{+}{=}Si(CH_3)_2$] in its mass spectrum. Malonate II similarly displays the fragment ion *m/z* 143.

The aromatic dicarboxylates, phthalic, isophthalic and terephthalic acids present very different mass spectra than the aliphatic dicarboxylates. This is due to the fragmentation of the benzyl ring. In each case the [M − 57] fragment ion was very intense and served as the base ion. Also, each demonstrated a weak molecular ion and an [M − 15] fragment ion. Though each derivative has the same molecular weight, the presence, absence or variation in the relative intensities of prominent fragment ions at *m/z* 263, 178, 163, 147, 140, 105 and 104 provides a different mass spectral fingerprint for each compound. Fragment ion interpretations for these and other fragments observed for the phthalates are presented in Table V.

The tricarboxylates aconitate and citrate yield a fragmentation pattern not unlike the aliphatic dicarboxylates. In addition to the [M − 57], [M − 15] fragment ions, and weak molecular ions, both tricarboxylates displayed fragments at *m/z* 147, 133, 117, 115, 75 and 73. Both also show [M − 189] fragments at *m/z* 327 and 459, respectively, which represents the loss of (TBDMS)O–$Si(CH_3)_2$. Citrate also shows a fragment ion at 357 indicating the loss of TBDMS, (TBDMS)OH and CO_2. Lastly, the mass spectra of both aconitate and citrate, as well as malonate II, possess a prominent fragment ion at *m/z* 77 representing the fragment $^{+}O{\equiv}C{-}CH_2{-}COOH$ or $HO^{+}{=}C{=}CH{-}COOH$.

In conclusion, a method is described in which carboxylic acids are derivatized to their respective tBDMS derivative and analyzed by GLC–MS. The derivatives are readily made and have excellent packed and capillary column characteristics. In addition, each tBDMS-carboxylic acid shows a unique, unambiguous [M − 57] fragment ion when analyzed by MS which allows for easy carboxylate identification.

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