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GAS–LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY OF MONO- AND DITHIOLS AS THEIR *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES

DEBORAH C. LANDRUM and THOMAS P. MAWHINNEY*
Departments of Biochemistry and Child Health, University of Missouri-Columbia, 322-A Chemistry Building, Columbia, MO 65211 (U.S.A.)
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SUMMARY

The use of gas–liquid chromatography and mass spectrometry with derivatizing agents that give stable derivatives and consistent fragmentation patterns allows for accurate identification of a variety of compounds. In this study either N-methyl-N-*tert.*-butyldimethylsilyltrifluoroacetamide or N-*tert.*-butyldimethylsilylimidazole were employed to derivatize a range of mono- and dithiols: from ethanethiol to 1-hexadecanethiol and 1,2-ethanedithiol to 1,9-nonanedithiol. When analyzed in this way, the resulting *tert.*-butyldimethylsilyl derivatives of the 24 thiols tested were readily distinguishable. Complete baseline separation of each derivative by capillary gas–liquid chromatography was achieved, and each produced a prominent mass minus 57 [$M^{\ddagger}$ − 57] fragment ion. The *tert.*-butyldimethylsilyl-thioethers were colorless and were stable at room temperature for over 3 months. This method may provide a convenient approach to the analysis of thiol compounds from a wide variety of sources.

INTRODUCTION

Thiols are found in a variety of natural sources and are by-products of many industrial processes[1,2]. In order to study or monitor them, efforts have been made towards developing convenient and sensitive analytical methods for determining low levels of thiol compounds from different sources. Though many methods are available for the detection and determination of thiols[3], gas–liquid chromatography (GLC) has remained an important analytical tool[2–13]. Because of their high volatility and tendency to exidize[3,6], most thiol samples require immediate analysis or derivatization[9]. Owing to these difficulties most analyses have been primarily limited to alkanethiols of six carbons or less.

This investigation reports an analytical method for the analysis of a broad molecular weight spectrum of mono- and dithiols as their *tert.*-butyldimethylsilyl (*t*BDMS) derivatives. Derivatization is accomplished in a single step employing N-methyl-N-*tert.*-butyldimethylsilyltrifluoroacetamide or N-*tert.*-butyldimethylsilylimidazole yielding *t*BDMS-thioethers amenable to packed or capillary GLC analysis and detected by flame ionization and by mass spectral (MS) electron ionization. With

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the exception of 2,3-butanedithiol, each of 24 derivatized mono- and dithiols tested displayed a single peak and, in mixture, were readily resolved by capillary GLC. In addition, these *t*BDMS-thioethers are stable at room temperature for over 3 months. The mass spectra for these derivatized thiols are relatively simple, displaying prominent and unambiguous mass minus 57 [$M^+ - 57$] fragment ions. Because of their different GLC retention times and dominant, high molecular weight, mass spectral fragment ions, employing combined GLC–MS analysis of each *t*BDMS-thioether provides a unique chromatographic–mass spectral fingerprint.

EXPERIMENTAL

Materials

All monothiol and dithiol compounds were obtained from Aldrich (Milwaukee, WI, U.S.A.). N-Methyl-N-*tert.*-butyldimethylsilyltrifluoroacetamide (MTBSTFA), with and without 1% *tert.*-butyldimethylsilyl chloride (*t*BDMS-Cl), was either synthesized in this laboratory[14] or was purchased from Regis (Morton Grove, IL, U.S.A.). N-*tert.*-Butyldimethylsilylimidazole (TBSI) was synthesized in this laboratory, as described below. Tetrahydrofuran (THF), N,N-dimethylformamide (DMF), acetonitrile (ACN), dimethyl sulfoxide (DMSO), ethyl acetate and chloroform were purchased from Aldrich and were redistilled prior to use.

Gas chromatography

Direct capillary GLC analysis was performed with a Varian GLC system, Model 3700 (Varian Assoc., Park Ridge, IL, U.S.A.) equipped with dual flame ionization detectors. The chromatographic column employed was a 30 m × 0.32 mm I.D. fused-silica capillary column, packed 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 290°C. After an initial hold of 1 min at 100°C the column was temperature-programmed at 4°C/min to 250°C. Packed-column GLC was performed using a Perkin-Elmer Sigma 3 instrument equipped with dual flame ionization detectors. The chromatographic column was 6 ft. × 1/8 in. O.D. (1.8 mm I.D.) glass column 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 290°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 of 218°C.

Synthesis of N-tert.-butyldimethylsilylimidazole (TBSI)

To a 2.0-l flask containing 150 ml of dry THF was added 28.79 g of NaH (1.2 mol) under dry nitrogen. The flask was then equipped with an addition funnel, with pressure equalizing line, containing 68.08 g of imidazole (1 mol) dissolved in 500 ml of

dry THF. The contents of the addition flask were slowly added to the stirred THF–NaH suspension while the reaction temperature was kept at 0°C. Hydrogen evolved was vented from the flask via the pressure equalizing line of the addition funnel. Upon completion of the addition the reaction mixture was stirred for 1 h at room temperature. Then 165.8 g of *t*BDMS-Cl (1.1 mol) dissolved in 250 ml of THF were added dropwise under stirring, with the reaction mixture temperature held at 0°C. After this addition, the reaction flask was allowed to come to room temperature and was stirred overnight. Under a blanket of dry nitrogen the solution was filtered and the filtrate was reduced to a constant volume at room temperature at 2666 Pa. TBSI was then distilled at 73°C at 0.75 mmHg (d_4^{23} 0.9363). The yield was 87.4%. Mass spectrum: *m/e* (relative intensity) 182 (M^+, 12), 167 (19), 125 ($M^+ - 57$, 100). Anal. calcd. for $C_9H_{18}SiN_2$: C, 59.34; H, 9.89; N, 15.38; Si, 15.38. Found: C, 59.21; H, 9.77; N, 15.42; Si, 15.29.

Thiol standard solution

Thiols (100 μl each) were kept in a 50-ml amber serum vial sealed with a Teflon-faced silicone septum. Benzyl mercaptan and 1-pentanethiol were included at the same concentration as internal standards. For GLC and GLC–MS analysis, 50-μl aliquots of this stock solution were used.

Derivatization of thiols

In the respective Reactivial, equipped with a small PTFE-coated stir bar and a PTFE-faced silicone septum, 10 μl of DMF were added to the sample to be derivatized. Then 200 μl of MTBSTFA, containing 1% *t*BDMS-Cl, was added with stirring. It was then heated at 70°C for 10 min and cooled to room temperature. The reaction was monitored by GLC at each step and at different time intervals at room temperature. Derivatization with TBSI was performed similarly, with the exception that the sample was heated for 5 min at 70°C. For packed and capillary column GLC analysis, 1.5 and 0.1 μl were injected per analysis, respectively. In other experiments, ACN, DMSO, THF, ethyl acetate, chloroform, or no solvent was substituted for DMF. Additionally, MTBSTFA without *t*BDMS-Cl, as a catalyst, was employed in several studies.

Some low-molecular-weight monothiols coeluted from the GLC column with the derivatizing reagent TBSI and the by-product imidazole. Thiol samples, derivatized with TBSI (and also with MTBSTFA), were therefore dissolved in 1.0 ml of benzene and washed twice against water to destroy unchanged TBSI (or MTBSTFA) and to remove the imidazole before GLC analysis.

RESULTS AND DISCUSSION

Derivatization

With the exception of 2,3-butanedithiol, *tert.*-butyldimethylsilylation of the standard thiol mixture with MTBSTFA containing 1% *t*BDMS-Cl was complete within 60 min at room temperature. Initially heating the sample for 10 min at 70°C had little effect on the reaction time. With the exception of 2,3-butanedithiol, derivatization of the same thiol mixture with TBSI was complete within 30 min at room temperature or within 15 min if initially heated for 5 min at 70°C. Substitution of ACN,

DMSO, THF, ethyl acetate, chloroform, or no solvent, for DMF did not significantly affect the derivatization rate. With either MTBSTFA containing 1% *t*BDMS-Cl, or with TBSI, *tert.*-butyldimethylsilylation of 2,3-butanedithiol was complete in 18 h. This protracted derivatization time probably reflects the large steric hindrance of placing the bulky *tert.*-butyldimethylsilyl function on two vicinal secondary thiols.

Thiols derivatized with MTBSTFA without *t*BDMS-Cl required a minimum of 24 h for complete derivatization. Substitution of ACN, DMSO, THF, ethyl acetate or chloroform for DMF as the thiol solvent did not affect the derivatization rate.

Stability of the tBDMS-thiols as a function of time

The stability of each *t*BDMS-thiol derivative was determined by *tert.*-butyldi-

TABLE I

STABILITY OF THE *tert.*-BUTYLDIMETHYLSILYL THIOL DERIVATIVES

Data expressed as the mean relative weight response (RWR) of six different sample injections of each respective thiol relative to the relative weight response of 1-pentanethiol at 0 h; [RWR = (thiol/1-pentanethiol)]. A 50-μl aliquot of a standard thiol solution containing 5 μmol/ml of each thiol was taken up in 10 μl of DMF and derivatized with 200 μl of MTBSTFA containing 1% *t*BDMS-Cl. The sample was heated at 70°C for 5 min and then allowed to stand at room temperature for 60 min. Aliquots (0.2 μl) were injected on a 30 m × 0.32 mm I.D. capillary column packed with 0.25 μm bonded SPB-1. The helium flow-rate was 5 ml/min, with injector and detector temperatures of 290°C. After an initial hold of 1 min at 100°C the column was temperature-programmed at 4°C/min to 250°C.

Thiol	*Relative weight response*				
	0 h	*6 h*	*12 h*	*18 h*	*24 h*
Monothiols					
Ethanethiol	1.20	1.18	1.16	1.16	1.15
1-Propanethiol	1.21	1.20	1.20	1.18	1.15
2-Propanethiol	1.18	1.14	1.15	1.14	1.11
1-Methyl-1-propanethiol	1.10	1.11	1.10	1.07	1.06
2-Methyl-1-propanethiol	1.06	1.05	1.05	1.03	1.03
2-Methyl-2-propanethiol	1.08	1.10	1.09	1.07	1.08
1-Butanethiol	1.12	1.09	1.10	1.09	1.08
2-Methyl-2-butanethiol	1.05	1.07	1.08	1.08	1.07
3-Methyl-1-butanethiol	1.09	1.05	1.07	1.05	1.05
1-Pentanethiol	1.00*	0.99	1.00	0.99	0.97
1-Hexanethiol	0.95	0.98	0.96	0.97	0.97
Heptyl mercaptan	0.88	0.88	0.88	0.86	0.86
Benzyl mercaptan	1.45	1.44	1.45	1.46	1.45
Nonyl mercaptan	0.78	0.77	0.74	0.75	0.74
1-Decanethiol	0.51	0.55	0.52	0.50	0.50
1-Dodecanethiol	0.39	0.40	0.39	0.38	0.38
1-Hexadecyl mercaptan	0.25	0.25	0.24	0.24	0.24
Dithiols					
1,2-Ethanedithiol	1.97	1.98	1.98	1.96	1.95
2,3-Butanedithiol (I + II)	0.77	0.85	0.91	1.03	1.05
1,3-Propanedithiol	1.84	1.88	1.88	1.85	1.82
1,4-Butanedithiol	1.45	1.39	1.37	1.38	1.36
1.5-Pentanedithiol	1.16	1.13	1.14	1.13	1.13
1,6-Hexanedithiol	0.95	0.94	0.94	0.93	0.91
1,9-Nonanedithiol	0.70	0.67	0.66	0.66	0.65

methylsilylating a thiol standard mixture, in 10 μl of DMF, with 250 μl of MTBSTFA containing 1% *t*BDMS-Cl. It was then heated at 70°C for 5 min and kept at room temperature for 60 min. Then, following an immediate GLC analysis (time 0), aliquots of this standard were injected at 6-h intervals for 48 h (Table I). With the exception of 2,3-butanedithiol, all the monothiols and dithiols demonstrated excellent stability for 24 h. As indicated above, complete derivatization of 2,3-butanedithiol was accomplished in *ca.* 18 h, from which time no significant degradation was noted. Though not shown, injection of derivatized standards after 3 months also showed no significant degradation, and no additional chromatographic peaks were observed with time.

GLC separations

Separation of the *t*BDMS derivatives of a thiol standard mixture on an SPB-1 (bonded) fused-silica capillary GLC column is presented in Fig. 1. With the exception of 2,3-butanedithiol, for which two adjoining peaks were observed, each *t*BDMS-monothioether and $(t\text{BDMS})_2$-dithioether derivative displayed a single sharp symmetrical peak with no significant tailing or indication of any decomposition on the column. In addition, baseline separation was achieved in a single run for all the thiols studied.

Separation of the standard *t*BDMS-thioethers on an SP-2250 packed GLC column (Fig. 2) provided the same general elution pattern as seen on the SPB-1 column, though several compounds produced overlapping peaks and ethanethiol was not resolvable from the solvent front.

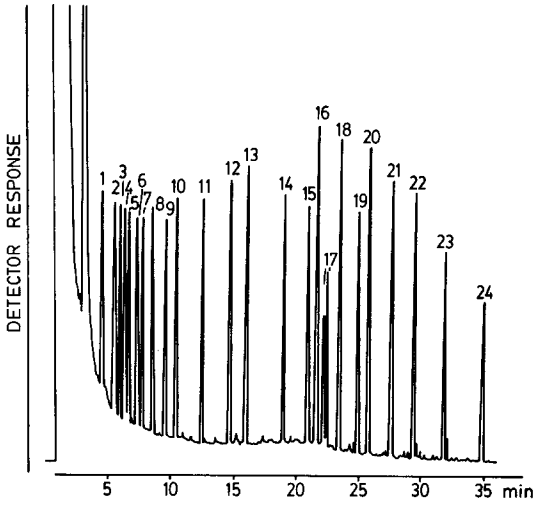

Fig. 1. Gas–liquid chromatogram of the *t*BDMS derivatives of 24 thiols. Separation was performed on a 30 m × 0.32 mm I.D. SPB-1 (bonded) capillary GLC column with a film thickness of 0.25 μm. The injected sample contained 2.5 nmol of each *t*BDMS thiol. After an initial hold of 1 min at 100°C, the column was temperature-programmed at 4°C/min to 250°C. Helium was the carrier gas at a flow-rate of 5 ml/min. Peaks: 1 = ethanethiol; 2 = 2-propanethiol; 3 = 2-methyl-2-propanethiol; 4 = 2-methyl-2-butanethiol; 5 = 1-propanethiol; 6 = 1-methyl-1-propanethiol; 7 = 2-methyl-1-propanethiol; 8 = 1-butanethiol; 9 = 3-methyl-1-butanethiol; 10 = 1-pentanethiol; 11 = 1-hexanethiol; 12 = 1-heptanethiol; 13 = benzyl mercaptan; 14 = 1-nonanethiol; 15 = 1-decanethiol; 16 = 1,2-ethanedithiol; 17 = 2,3-butanedithiol (two peaks); 18 = 1,3-propanedithiol; 19 = 1-dodecanethiol; 20 = 1,4-butanedithiol; 21 = 1,5-pentanedithiol; 22 = 1,6-hexanedithiol; 23 = 1-hexadecanethiol; 24 = 1,9-nonanedithiol.

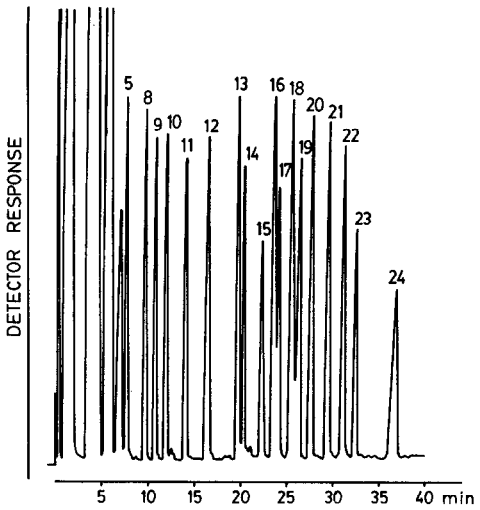

Fig. 2. Gas–liquid chromatogram of the *t*BDMS derivatives of eighteen thiols. Separation was performed on a 3.0% SP-2250 packed glass column (6 ft. × 1/8 in. O.D., 1.8 mm I.D.), employing Supelcoport, 100–120 mesh. The injected sample contained 8 nmol of each *t*BDMS thiol. After an initial hold of 1 min at 60°C the column was temperature-programmed at 4°C/min to 260°C. The nitrogen flow-rate was 18 ml/min, with injector and detector temperatures of 290°C. Peaks as in Fig. 1.

Retention data

Retention times and relative retention times for each *t*BDMS-monothioether and $(t\text{BDMS})_2$-dithioether derivative on an SPB-1 (bonded) capillary column and a 3.0% SP-2250 packed GLC column are given in Table II. As is generally noted with non-polar columns, for each group of thiols, the thiols emerged from the SPB-1 capillary column primarily in the order of the molecular weight of the *t*BDMS-thioether derivatives. This is also true for the results achieved on the 3.0% SP-2250 intermediate polarity column.

Quantitative aspects

The relative weight response (RWR) of the *t*BDMS-thioethers is shown in Table III. As indicated, the relative standard deviation (R.S.D.) for each RWR on the SPB-1 capillary column was less than 1.85% in all cases, demonstrating the excellent precision in the RWR of each *t*BDMS-thioether derivative. On the 3.0% SP-2250 packed column the R.S.D. for each thioether was less than 3.0%.

A linear response curve in the range 0.6–50 nmole was obtained for each mono- and dithiol standard, using flame ionization detection.

Mass spectrometry

Each synthetic *t*BDMS-thioether was subjected to GLC–MS analysis. Tables IV and V show the mass spectral results for the mone- and dithiols, respectively.

All *t*BDMS-monothioethers produced the same general fragmentation, yielding mass spectra that were dominated by a single unique $[M^{+} - 57]$ fragment ion in the high-molecular-weight region and also by a prominent common fragment ion at m/z

TABLE II

RETENTION TIMES (t_R) AND RELATIVE RETENTION TIMES (RRT) OF THIOLS AS THEIR *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES BY CAPILLARY AND PACKED COLUMN GLC ANALYSIS

The SPB-1 column was a 0.25 μm bonded phase, 30 m × 0.32 mm I.D. capillary. The helium flow-rate was 5 ml/min, with injector and detector temperatures of 290°C. After an initial hold of 1 min at 100°C the column was temperature-programmed at a rate of 4°C/min to 250°C. The 3.0% SP-2250 packed GLC column was a 6 ft. × 1/8 in. O.D. (1.8 mm I.D.) glass column, employing Supelcoport, 100–120 mesh, as the support. The nitrogen flow-rate was 18 ml/min, with injector and detector temperatures of 290°C. After an initial hold of 1 min at 60°C the column was temperature-programmed at 4°C/min to 260°C. The retention time is expressed in minutes; the relative retention time is relative to the indicated internal standard, marked with an asterisk.

Thiol	*Capillary column SPB-1 (bonded)*		*Packed column 3.0% SP-2250*	
	t_R	*RRT*	t_R	*RRT*
Monothiols				
Ethanethiol	3.93	0.27	Not seen	Not seen
1-Propanethiol	5.33	0.36	5.63	0.31
2-Propanethiol	4.53	0.30	4.33	0.24
1-Methyl-1-propanethiol	5.93	0.40	4.55	0.25
2-Methyl-1-propanethiol	6.18	0.42	5.98	0.33
2-Methyl-2-propanethiol	4.61	0.31	4.06	0.34
1-Butanethiol	6.58	0.45	6.95	0.38
2-Methyl-2-butanethiol	4.99	0.34	4.52	0.25
3-Methyl-1-butanethiol	7.71	0.52	7.88	0.44
1-Pentanethiol	8.62	0.58	9.00	0.50
1-Hexanethiol	10.88	0.74	11.30	0.63
Heptyl mercaptan	13.34	0.90	13.72	0.76
Benzyl mercaptan	14.77	1.00*	17.95	1.00*
Nonyl mercaptan	17.94	1.22	18.42	1.03
1-Decanefhiol	20.19	1.37	20.62	1.15
1-Dodecanethiol	24.41	1.65	24.58	1.37
1-Hexadecyl mercaptan	31.91	2.16	32.20	1.79
Dithiols				
1,2-Ethanedithiol	20.83	1.41	22.21	1.24
2,3-Butanedithiol (I)	21.61	1.46	22.46	1.25
2,3-Butanedithiol (II)	22.10	1.50	Not seen	Not seen
1,3-Propanedithiol	22.84	1.55	24.32	1.35
1,4-Butanedithiol	25.33	1.72	26.35	1.47
1,5-Pentanedithiol	27.34	1.85	28.62	1.59
1,6-Hexanedithiol	29.28	1.98	30.69	1.71
1,9-Nonanedithiol	35.31	2.39	36.15	2.01

91. Notably, since sulfur has natural isotopes (% isotopic abundance) of $^{32}_{16}S$ (95), $^{33}_{16}S$ (0.76) and $^{34}_{16}S$ (4.2) and silicon of $^{28}_{14}S$ (92.2), $^{29}_{14}S$ (4.7) and $^{30}_{14}Si$ (3.1), all fragment ions possessing sulfur, silicon or both displayed readily distinguishable fragment ions 1 and 2 atomic mass units (amu) above the most abundant isotope. As is typical of *t*BDMS derivatives, the $[M^{\ddagger} - 57]$ fragment ion, *i.e.*, $R\text{–}S^{+}=Si(CH_3)_2$, results from the elimination of $^{\bullet}C(CH_3)_3$ from the molecule. This fragment ion is so intense it serves

TABLE III

RELATIVE WEIGHT RESPONSE (RWR) AND RELATIVE STANDARD DEVIATION (R.S.D.) OF THE *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES OF MONO- AND DITHIOLS

Data expressed as the RWR and R.S.D. (%) of six different sample injections of each respective thiol relative to 1-pentanethiol on the SPB-1 capillary and SP-2250 packed columns. Program and derivatization procedure as described in Tables I and II.

Thiol	*SPB-1 (bonded) (n = 6)*		*SP-2250 (packed column) (n = 6)*	
	RWR	*R.S.D. (%)*	*RWR*	*R.S.D. (%)*
Monothiols				
Ethanethiol	1.20	1.32	Not seen	Not seen
1-Propanethiol	1.21	1.59	1.08	1.67
2-Propanethiol	1.18	1.37	0.92	2.31
1-Methyl-1-propanethiol	1.10	1.71	0.89	2.89
2-Methyl-1-propanethiol	1.06	1.56	0.89	2.79
2-Methyl-2-propanethiol	1.08	1.82	0.85	2.67
1-Butanethiol	1.12	1.66	0.80	2.89
2-Methyl-2-butanethiol	1.05	1.49	0.81	2.66
3-Methyl-1-butanethiol	1.09	1.63	0.79	2.38
1-Pentanethiol	(1.00)		(1.00)	
1-Hexanethiol	0.95	1.71	0.78	2.41
Heptyl mercaptan	0.88	1.80	0.74	2.35
Benzyl mercaptan	1.45	0.96	1.19	1.15
Nonyl mercaptan	0.78	1.80	0.59	2.16
1-Decanethiol	0.51	1.71	0.44	2.30
1-Dodecanethiol	0.39	1.68	0.21	2.09
1-Hexadecyl mercaptan	0.25	1.66	0.11	2.11
Dithiols				
1,2-Ethanedithiol	1.97	1.48	1.63	1.81
2,3-Butanedithiol (I + II)	0.77	1.51	0.61	2.84
1,3-Propanedithiol	1.84	1.48	1.57	1.75
1,4-Butanedithiol	1.45	1.43	1.22	1.77
1,5-Pentanedithiol	1.16	1.42	0.94	1.70
1,6-Hexanedithiol	0.95	1.51	0.78	1.82
1,9-Nonanedithiol	0.70	1.53	0.41	1.80

as the base fragment ion in all straight-chained primary thiol mass spectra.

Continued fragmentation of the $R\text{–}S^+=Si(CH_3)_2$ ion through hydrogen rearrangement and adjacent σ-bond dissociation of the respective alkyl chain (R) from the sulfur heteroatom, *i.e.*, $[M^{\cdot+} - {}^{\cdot}C(CH_3)_3 - C_nH_{2n}]$, results in the formation of $HS^+=Si(CH_3)_2$, *m/z* 91 (dimethylsilanethiol). This fragment ion, though prominent in all *t*BDMS-monothioether mass spectra, serves as the base fragment ion for all branched and secondary thiols. The ratio of the relative intensities of *m/z* 91 to $[M^{\cdot+} - 57]$ increases with the increased stability of the leaving alkyl group R′ in the order tertiary > secondary > primary, respectively (*e.g.* 2-methyl-2-propanethiol > 1-methyl-1-propanethiol > 1-propanethiol). For benzyl mercaptan the intense *m/z* 91 fragment ion also probably reflects a contribution by the tropylium ion ($C_7H_7^+$, *m/z* 91). For all *t*BDMS-monothioethers, the molecular weight difference between $[M^{\cdot+} - 57]$ and *m/z* 91 divided by 14 (*i.e.*, CH_2) yields the number of carbons in R.

TABLE IV

INTERPRETATION AND RELATIVE INTENSITIES OF THE IMPORTANT MAJOR FRAGMENT IONS IN THE MASS SPECTRA OF THE *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES OF MONOTHIOLS

Data expressed as *m/z* (% relative intensity) where the intensities of the fragment ions of each monothiol are given relative to the indicated base fragment ion, *i.e. m/z* (100), observed in the respective mass spectrum. Weak fragment ions *m/z* 71, 85 and 99, though not in the Table, were noted in most mass spectra.

Monothiols	M^+	*M – 15*	*M – 57*	*M – 149*	*Other*					
Ethanethiol	176(8.2)	161(1.2)	119(100)	27(11)		91(35)	73(12)	57(10)	43(8)	29(21)
1-Propanethiol	190(9.5)	175(0.6)	133(100)	41(70)	105(4)	91(61)	73(25)	57(6)	43(10)	29(20)
2-Propanethiol	190(6.6)	175(0.8)	133(70)	41(19)		91(100)	73(15)	57(8)	43(15)	29(11)
1-Methyl-1-propanethiol	204(4.8)	189(0.7)	147(50)	55(2.5)	105(2)	91(100)	73(16)	57(14)	43(4)	29(20)
2-Methyl-1-propanethiol	204(5.8)	189(1.9)	147(76)	55(3.6)	105(2)	91(100)	73(24)	57(20)	43(10)	29(18)
2-Methyl-2-propanethiol	204(2.0)	189(2.7)	147(27)		105(2)	91(100)	73(9.5)	57(29)	43(8)	29(25)
1-Butanethiol	204(2.6)	189(1.3)	147(100)	55(30)	105(3)	91(30)	73(24)	57(20)	43(50)	29(22)
3-Methyl-1-butanethiol	218(1.7)	203(1.9)	161(91)	69(28)	105(98)	91(100)	73(47)	57(9)	43(18)	29(8)
2-Methyl-2-butanethiol	218(1.6)		161(22)			91(100)	73(10)	57(5)	43(19)	29(10)
1-Pentanethiol	218(8.7)	203(1.1)	161(100)	69(26)	105(10)	91(98)	73(45)	57(20)	43(56)	29(40)
1-hexanethiol	232(5.3)	217(0.9)	175(100)	83(14)	105(6)	91(98)	73(37)	57(15)	43(41)	29(24)
Benzyl mercaptan	238(0.5)	223(0.2)	181(43)			91(100)	73(6.8)	57(10)	43(2)	29(8)
1-Heptanethiol	246(4.4)	231(0.8)	189(100)	97(8.6)	105(4)	91(69)	73(21)	57(22)	43(18)	29(18)
1-Nonanethiol	274(2.4)	259(0.6)	217(100)		105(4)	91(71)	73(31)	57(24)	43(25)	29(28)
1-Decanethiol	288(2.0)	273(0.7)	231(100)	139(2.0)	105(6)	91(78)	73(38)	57(22)	43(26)	29(29)
1-Dodecanethiol	316(1.6)	301(0.6)	259(100)		105(5)	91(75)	73(38)	57(27)	43(25)	29(17)
1-hexadecanethiol	372(0.6)	357(0.5)	315(100)		105(4)	91(75)	73(57)	57(35)	43(48)	29(28)

TABLE V

INTERPRETATION AND RELATIVE INTENSITIES OF THE IMPORTANT MAJOR FRAGMENT IONS IN THE MASS SPECTRA OF THE *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES OF DITHIOLS

Data expressed as m/z (% relative intensity) where the intensities of the fragment ions of each (tBDMS)$_2$-dithioether are given relative to the indicated base fragment ion, *i.e.* m/z (100), observed in the respective mass spectrum. Fragment ions displayed as [m/z] were calculated but not observed.

Dithiols	M^+	$M-15$	$M-57$	$M-187$	*Other*					
1,2-Ethanedithiol	[322]	307(3)	265(39)	135(17)	115(37)	91(15)	73(100)	57(22)	41(22)	29(18)
1,3-Propanedithiol	336(2)	321(4)	279(53)	149(50)	115(39)	91(16)	73(100)	57(17)	41(17)	29(9)
1,4-Butanedithiol	[350]	335(5)	293(28)	163(25)	115(16)	91(14)	73(100)	57(11)	41(13)	29(11)
2,3-Butanedithiol (I)	[350]	[335]	293(13)	163(12)	115(13)	91(13)	73(100)	57(9)	41(11)	29(9)
2,3-Butanedithiol (II)	[350]	[335]	293(12)	163(12)	115(11)	91(14)	73(100)	57(10)	41(10)	29(12)
1,5-Pentanedithiol	[364]	349(8)	307(55)	177(29)	115(17)	91(17)	73(100)	57(6)	41(8)	29(5)
1,6-Hexanedithiol	[378]	363(8)	321(26)	191(9.1)	115(15)	91(18)	73(100)	57(6)	41(14)	29(9)
1,9-Nonanedithiol	[420]	405(9)	363(37)	233(16)	115(15)	91(25)	73(100)	57(7)	41(12)	29(8)

In addition to the [$M^{\ddagger} - 57$] fragment ion, each derivative displayed a molecular ion ($M^{\ddagger}$) of low intensity, and a very weak [$M^{\ddagger} - 15$] fragment ion corresponding to the loss of $\cdot CH_3$ from the derivative. Also noted is the typical fragment ion series of $[C_nH_{2n+1}]^+$ (*i.e.*, *m/z* 29, 43, 57, 71, 85, 99), which result from σ-bond dissociation within the saturated alkyl side-chains and which show the typical higher relative intensities around C_3 or C_4. Use of this series of fragment ions as the sole indicator of the alkyl side-chain size is made difficult by the contribution of the fragment ions of *m/z* 99, 57, 41 and 29, which result from fragmentation of the *t*BDMS or $C(CH_3)_3$ group with the proposed structures of $^+Si(CH_3)_2C(=CH_2)(CH_3)$, $^+C(CH_3)_3$, $^+CH_2CH=CH_2$ and $^+CH_2CH_3$, respectively. Fragment ions *m/z* 73 ($CH_2=CHS^+=CH_2$) and 105 ($CH_2=S^+-SiH(CH_3)_2$) are also seen. Fragment ions [$M^{\ddagger} - 149$] correspond to the loss of both $\cdot Si(CH_3)_2C(CH_3)_3$ and H_2S.

Table V shows the major fragment ions and abundances in the mass spectra of $(t\text{BDMS})_2$-dithioethers. Like the *t*BDMS-monothioethers derivatives, each dithiol displayed a prominent fragment ion at [$M^{\ddagger} - 57$]. Also each [$M^{\ddagger} - 57$] exhibited the associated fragment ions 1 and 2 amu above the most abundant isotope ion, indicating the presence of two silicon and two sulfur atoms and their natural isotopic abundances. In contrast to the *t*BDMS-monothioether derivatives, each $(t\text{BDMS})_2$-dithioether mass spectrum showed weak [$M^{\ddagger} - 15$], no molecular ion [$M^{\ddagger}$] and the base fragment ion of *m/z* 73. Fragment ion *m/z* 91 is still present in each spectrum but with an abundance of 25% or less. Though a very weak ion in the mass spectra of monothiols, *m/z* 115 ($^+Si(CH_3)_2C(CH_3)_3$) is seen in $(t\text{BDMS})_2$-diethioether mass spectra with abundances greater than 15%. Another important fragment ion common to all $(t\text{BDMS})_2$-dithioether mass spctra is [$M^{\ddagger} - 187$], which corresponds to the combined losses of *t*BDMS, $C(CH_3)_3$ and CH_3, *i.e.* [$M^{\ddagger} - 115 - 57 - 15$]. Fragment ions *m/z* 29, 41 and 57 are also seen and are as described above. No significant fragment ions related to the alkyl chain itself are seen to provide an indication of its size.

Mass spectral examination of the two contiguous capillary GLC peaks produced by 2,3-butanedithiol (Table V) demonstrated that the resulting fragment ions of each, and their relative intensities, were identical. Each 2,3-butanedithiol capillary GLC peaks exhibited the same important [$M^{\ddagger} - 57$] and [$M^{\ddagger} - 187$] fragment ions (*i.e.*, *m/z* 293 and 163, respectively) indicating that both 2,3-butanedithiol peaks were completely *tert.*-butyldimethylsilylated species, and that the two GLC peaks represented resolved stereoisomers of the derivatized compound.

CONCLUSION

A method is described in which mono- and dithiols are derivatized to their respective *t*BDMS derivatives and analyzed by GLC and GLC–MS. The derivatives are readily made and have excellent capillary and packed-column characteristics. In addition, each *t*BDMS-thioether shows a unique, unambiguous [$M^{\ddagger} - 57$] fragment ion when analyzed by MS, which allows for thiol identification.

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REFERENCES

1 L. B. Ryland and M. W. Tamele, in J. H. Karchmer (Editor), *The Analytical Chemistry of Sulfur and its Compounds*, Part I, Wiley-Interscience, New York, London, Sydney, Toronto, 1970.
2 P. C. Jocelyn, *Biochemistry of the SH Group*, Academic Press, London, New York, 1972, p. 1.
3 A. Fontana and C. Toniolo, in S. Patai (Editor), *Chemistry of the Thiol Group*, Part 1, Wiley, London, New York, Sydney, Toronto, 1974, p. 271.
4 M. R. F. Ashworth, in R. Belcher and D. M. W. Anderson (Editors), *The Analysis of Organic Materials*, Vol. 2, Academic Press, London, New York, San Francisco, CA, 1976, p. 239.
5 P. Ronkainen, J. Denslow and O. Leppanen, *J. Chromatogr. Sci.*, 11 (1973) 384.
6 J. W. Gramshaw and A. Hussain, *J. Chromatogr.*, 157 (1978) 267.
7 B. Zygmunt and R. Staszewski, *J. Chromatogr.*, 119 (1976) 599.
8 J. Kangas, *J. Chromatogr.*, 346 (1985) 405.
9 A. Przyjazny, W. Janicki, W. Chrzanowski and R. Staszewski, *J. Chromatogr.*, 292 (1984) 199.
10 B. Zygmunt and A. Przyjazny, *J. Chromatogr.*, 294 (1984) 117.
11 T. A. Misharina, D. N. Grigor'eva, R. L. Golovnya and A. F. Aerov, *Zh. Anal. Khim.*, 42 (1987) 929.
12 L. Huber and H. Obbens, *J. Chromatogr.*, 349 (1985) 465.
13 C. D. Pearson, *J. Chromatogr. Sci.*, 14 (1976) 154.
14 T. P. Mawhinney and M. A. Madson, *J. Org. Chem.*, 47 (1982) 3336.