SEPARATION AND ANALYSIS OF SULFATE, PHOSPHATE AND OTHER OXYANIONS AS THEIR *tert.*-BUTYLDIMETHYLSILYL DERIVATIVES BY GAS–LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY

THOMAS P. MAWHINNEY

University of Missouri, Department of Biochemistry, 322-A Chemistry Building, Columbia, MO 65211 (U.S.A.)

(Received September 27th, 1982)

SUMMARY

The *tert.*-butyldimethylsilyl derivatives of the oxyanions carbonate, sulfite, sulfate, selenite, selenate, molybdenate, borate, phosphite, phosphate, vanadate, arsenite, arsenate and pyrophosphate were prepared by reacting the free acid and the ammonium salt form of the oxyanions with N-methyl-N-(*tert.*-butyldimethylsilyl)trifluoroacetamide in N,N-dimethylformamide. All derivatives were amenable to gas chromatographic analysis and, except for molybdenate, were stable for over a week at room temperature and over six months at 4°C. Each *tert.*-butyldimethylsilylated oxyanion produced an easily interpretable mass spectrum dominated by a unique $M - 57$ fragment ion.

INTRODUCTION

Relative to the analysis of organic compounds, application of gas chromatography (GC) to the separation of inorganic oxyanions has received little attention. Hashizume and Sasaki[1], in their study of nucleotides, first reported the GC detection of the trimethylsilyl (TMS) derivative of orthophosphate which was later shown to be $(TMS)_3PO_4$ by combined gas chromatography–mass spectrometry (GC–MS) analysis[2]. Similar studies of nucleotides and nucleosides demonstrated the same volatile derivative of phosphate[3–6]. However these investigations, including those of sugar phosphates[7], phosphorylated pesticides[8] and alkyl phosphates, were focused primarily on the GC analysis of the naturally occurring and synthetic organophosphates and not of the inorganic oxyanions itself.

Separation of several silicates as their TMS derivatives by GC was first reported by Lentz[9]. Wu *et al.*[10] similarly trimethylsilylated five different silicates employing trimethylchlorosilane and studied the resulting derivatives by GC–MS. Though many different silyl esters of inorganic oxyanions have been reported, few used inorganic oxyanions directly or had procedures amenable to routine GC analysis[11–13]. A major advancement in the derivatization, separation and analysis of inorganic oxyanions by GC and GC–MS was presented by Butts[14] and by Butts and Rainey[15]

respectively. In these studies arsenate, arsenite, borate, carbonate, oxalate, phosphate, phosphite, sulfate and vanadate were converted into their respective TMS derivatives with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). The ammonium salts of the oxyanions were employed and N,N-dimethylformamide (DMF) was used as the reaction solvent to facilitate both the solubilization and the derivatization of the salts. Trimethylsilylated samples were then injected directly into a gas chromatograph and the different oxyanions were separated on a GC column of intermediate polarity.

With the exception of phosphate and oxalate, all TMS derivatives of oxyanions demonstrate immediate and progressive degradation in the derivatizing cocktail at room temperature. Trimethylsilylated sulfate degradation is the most pronounced. Increasing the amount of BSTFA has little effect upon the rate of degradation. In addition, TMS-oxyanions readily display thermal decomposition at temperatures above 50°C that is not reversible. Decomposition of the TMS-oxyanions also occurs on the GC column during chromatography producing highly polar products that do not elute from the column. Furthermore, attempts to trimethylsilylate sulfite results in the oxidation of sulfite to sulfate during analysis. Lastly, all TMS-oxyanions exhibit one or more volatile secondary products that can be shown by GC.

In the present investigation we report on the preparation and separation of the *tert.*-butyldimethylsilyl (*t*BDMS) derivatives of the above mentioned oxyanions and also of selenate, selenite and pyrophosphate employing N-methyl-N-(*tert.*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) as the silyl donor. As the *t*BDMS derivative, sulfite can be analyzed without detectable oxidation to sulfate. Furthermore, each *t*BDMS-oxyanion displays a single, sharp and symmetrical chromatographic peak by GC, demonstrates excellent on-column characteristics, is stable at room temperature for over one week and over 6 months at 4°C and each yields a characteristic mass spectrum dominated by a unique molecular weight minus 57 base peak fragment ion.

MATERIALS AND METHODS

Reagents

N-methyl-N-(*tert.*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) was either synthesized in our laboratory[16] or purchased from Regis Chemical Company (Morton Grove, IL, U.S.A.). Analytical-reagent grade DMF, pyridine, acetonitrile, tetrahydrofuran and dimethylsulfoxide (DMSO) were obtained from Aldrich Chemical Company (Milwaukee, WI, U.S.A.) and were dried and redistilled before use.

Derivatization procedure

The *t*BDMS derivatives were prepared by placing 50–100 μg of the respective oxyanion in the free acid form into a 1.0-ml Teflon-faced silicone-rubber septum-capped reaction vial followed by the sequential addition of 50 μl of DMF and 150 μl of MTBSTFA. Upon mixing, aliquots (0.5–2.0 μl) from the vials can be immediately injected into the gas chromatograph for analysis. Alternatively, the equivalent of the oxyanion in the ammonium salt form, in lieu of the free acid, was placed into a reaction vial equipped with a small magnetic stir bar. To this vial was then added 100 μl of DMF and 200 μl of MTBSTFA. Reactions were then allowed to stir overnight at

room temperature or until the complete dissolution of the salts. Aliquots were then subjected to GC analysis.

Gas chromatography

A Perkin-Elmer Sigma III gas chromatograph with dual flame ionization detectors equipped with a 3 % SE-30 on Chromosorb W HP, 100–120 mesh, 6 ft. × 1/8 in. I.D. nickel column and a 3 % SP-2250 on Supelcoport, 100–120 mesh, 6 ft. × 1/8 in. I.D. nickel column, was employed. Identical GC conditions were used for both columns. The injector temperature was 280°C and the *t*BDMS-oxyanions were injected at an oven temperature of 120°C. After an initial hold time of 2 min at 120°C the oven temperature was programmed at 6°C/min to 250°C. Nitrogen was used as the carrier gas at a flow-rate of 20 ml/min. A Hewlett-Packard 2300 integrator was used for peak-area measurements.

Stability of derivatives

To determine the stability of the *t*BDMS-oxyanion derivatives 100 μg of each oxyanion in its ammonium salt form were individually *tert.*-butyldimethylsilylated under dry nitrogen in DMF with MTBSTFA, as described above, and stored at room temperature. A parallel series of derivatives were prepared and stored at 4°C after 1 h at room temperature. Phenyl ether and *n*-butyl phenyl ether were employed as internal standards. Aliquot were then taken from each *t*BDMS-oxyanion and analyzed by GC to determine the progress of the reaction and the long-term stability of each *t*BDMS-oxyanion.

Gas chromatography–mass spectrometry

For mass spectral studies the above GC columns were placed in an H and F gas chromatograph interfaced with a CEC model 21-110C mass spectrometer. Mass spectra were recorded at 70 eV with an ionization current of 50 μA, a source temperature of 260°C and a transfer temperature of 240°C.

RESULTS AND DISCUSSION

Derivatization

Formation of the *t*BDMS derivatives of the free acid forms of sulfate, selenite, selenate, borate, phosphite, phosphate, vanadate, arsenite, arsenate and pyrophosphate in DMF with MTBSTFA were complete upon mixing (< 1.0 min). Substitution of acetonitrile, tetrahydrofuran or DMSO for DMF in the above reaction had no noticeable effect upon the derivatization rate. In contrast, substitution of pyridine for DMF resulted in the formation of the pyridinium salts of the oxyanions which, in turn, did not react to completion with MTBSTFA even after 3 days reaction time at room temperature. All attempts to form the *t*BDMS derivative of inorganic oxyanions as their sodium or potassium salts failed, presumably due to the apparent lack of solubility of these salts in the derivatizing reagent. When the ammonium salts of the above inorganic oxyanions and those of sulfite, carbonate and molybdenate were employed, total derivatization of the oxyanions was coincident with the complete dissolution of the salts in the derivatizing reagent consisting of DMF and MTBSTFA. Samples up to 100 μg of the ammonium salt form of the oxyanion were
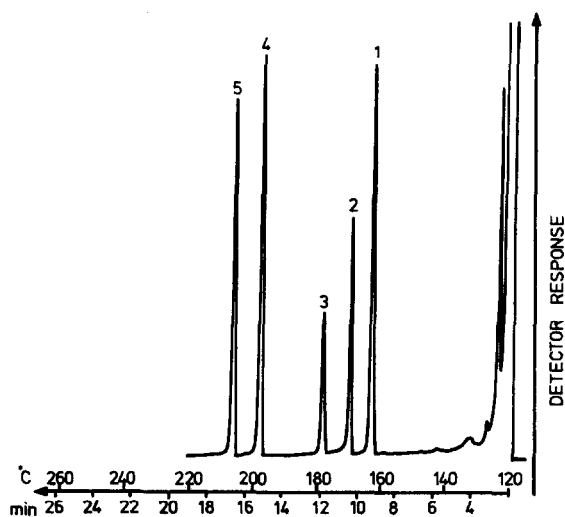
Fig. 1. Gas–liquid chromatogram of a *tert.*-butyldimethylsilylated oxyanion sample separated on a 3 % SP-2250 GC column and containing 50 ng each of borate (1), sulfate (2), arsenite (3), phosphate (4) and arsenate (5). For chromatographic conditions, see Materials and methods.

completely derivatized in less than 1 h. Larger quantities of the ammonium salts or larger ammonium salt crystals were generally derivatized completely if allowed to stir overnight at room temperature. Substitution of acetonitrile, DMSO, tetrahydrofuran or pyridine for DMF as the solvent in the derivatization of the oxyanions in their ammonium salt form resulted in very poor yields of the desired *t*BDMS product.
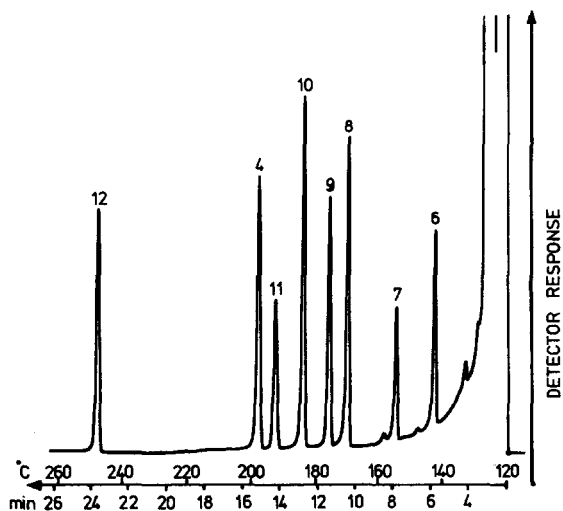

Fig. 2. Gas–liquid chromatogram of a *tert.*-butyldimethylsilylated oxyanion sample separated on a 3 % SP-2250 GC column and containing 50 ng each of carbonate (6), sulfite (7), selenite (8), phosphite (9), selenate (10), vanadate (11), phosphate (4) and pyrophosphate (12). For chromatographic conditions, see Materials and methods.

GC separations

The GC separation of the *t*BDMS derivatives of borate, sulfate, arsenite, phosphate and arsenate is shown in Fig. 1. Employing the same 3% SP-2250 GC column and the same column conditions as in Fig. 1, the *t*BDMS derivatives of carbonate, sulfite, selenite, phosphite, selenate, vanadate, phosphate and pyrophosphate are separated in a single GC run (Fig. 2). As shown, each *t*BDMS-oxyanion derivative displayed a very sharp single chromatographic peak and exhibited no peak tailing. Similar results were obtained when the GC separations were performed on the very non-polar SE-30 GC column. Retention data, expressed in terms of methylene unit values, for each *t*BDMS-oxyanion on SE-30 and SP-2250 are given in Table I. Generally it can be stated that the observed retention time on SP-2250 for each *t*BDMS-oxyanion appears to be directly related to both the molecular weight of the derivative and on the presence or absence of one or more polar double-bonded oxygens to the central hetero-atom. Pyrophosphate, which has the largest derivative molecular weight (MW = 635) of the oxyanions studied and which also possesses two double-bonded oxygens, is observed to have the longest retention time. Carbonate, which has the smallest derivatized molecular weight (MW = 290) and possesses one double-bonded oxygen, has the shortest retention time. Where a specific element possesses more than one oxo acid form the derivatized oxyanion form that has the larger number of oxygens also has the longer retention time. In this case sulfate, selenate, phosphate and arsenate have longer retention times than sulfite, selenite, phosphite and arsenite, respectively. Lastly, *t*BDMS-oxyanions that do not possess free double-bonded oxygens, *i.e.* do not form *t*BDMS inorganic esters, have retention times that

TABLE I

GAS CHROMATOGRAPHIC RETENTION DATA FOR THE OXYANIONS AS THEIR *tert.-*BUTYLDIMETHYLSILYL DERIVATIVES

Anion	Anion derivatized	Methylene unit values	
		*SE-30**	*SP-2250***
Carbonate	(*t*BDMS)$_2$CO$_3$	12.98	14.00
Sulfite	(*t*BDMS)$_2$SO$_3$	14.63	15.14
Sulfate	(*t*BDMS)$_2$SO$_4$	15.44	16.60
Selenite	(*t*BDMS)$_2$SeO$_3$	15.75	16.60
Selenate	(*t*BDMS)$_2$SeO$_4$	16.48	17.85
Molybdenate	(*t*BDMS)$_2$MoO$_4$	17.18	18.00
Borate	(*t*BDMS)$_3$BO$_3$	16.80	16.14
Phosphite	(*t*BDMS)$_3$PO$_3$	17.60	17.17
Phosphate	(*t*BDMS)$_3$PO$_4$	19.06	19.38
Vanadate	(*t*BDMS)$_3$VO$_4$	19.08	18.95
Arsenite	(*t*BDMS)$_3$AsO$_3$	18.00	17.42
Arsenate	(*t*BDMS)$_3$AsO$_4$	19.49	20.32
Pyrophosphate	(*t*BDMS)$_4$P$_2$O$_7$	24.18	25.10

* 3% SE-30 on Chromosorb W HP, 100–120 mesh. Column: nickel, 6 ft. × 1/8 in. I.D. Program: temperature was increased from 120°C to 250°C at a rate of 6°C/min following an initial 2-min hold at 120°C. Nitrogen was used as the carrier gas at a flow-rate of 20 ml/min.

** 3% SP-2250 on Supelcoport, 100–120 mesh. Column: nickel, 6 ft. × 1/8 in. I.D. Program: same as above.

TABLE II

RELATIVE INTENSITIES OF PROMINENT FRAGMENT IONS IN THE MASS SPECTRA OF THE tert.-BUTYLDIMETHYLSILYLATED DERIVATIVES OF OXYANIONS

The intensities of fragment ions of each derivatized oxyanion are given relative to their respective [M − 57] fragment ion. In those cases where a specific oxyanion produced more than one [M − 57] fragment ion due to the central element having more than one stable isotope in high natural abundance, the intensities of fragment ions are given relative to the [M − 57] fragment ion of the stable isotope with the greatest natural abundance. All molecular ions (M^+) had relative intensities of less than 2%.

Anion	Anion derivatized	m/e (relative intensity, %)							$M - 57$	$M - 15$	M^+
Carbonate	$(tBDMS)_2CO_3$	73(56)	75(34)	115(11)	147(44)	189(15)			233(100)	275(8)	290
Sulfite	$(tBDMS)_2SO_3$	73(43)	75(27)	115(6)	147(31)	189(10)			253(100)	295(8)	310
Sulfate	$(tBDMS)_2SO_4$	73(37)	75(19)	115(15)	147(32)	189(18)			269(100)	311(11)	326
Selenite	$(tBDMS)_2SeO_3$	73(29)	75(30)	115(14)	147(28)	189(7)	297(16)* 299(56)*	298(18)* 303(27)*	301(100)*	343(10)	358
Selenate	$(tBDMS)_2SeO_4$	73(43)	75(40)	115(7)	147(39)	189(14)	313(20)* 315(50)*	314(16)* 319(23)*	317(100)*	359(6)	374
Molybdenate	$(tBDMS)_2MoO_4$	73(30)	75(23)	115(11)	147(18)	189(11)	329(65)* 332(68)* 334(43)*	331(41)* 333(71)* 337(42)*	335(100)*	377(8)	392
Borate	$(tBDMS)_3BO_3$	73(37)	75(26)	115(9)	147(21)	189(22)		347(26)*	348(100)*	390(16)	405
Phosphite	$(tBDMS)_3PO_3$	73(27)	75(19)	115(10)	147(29)	189(16)			367(100)	409(11)	424
Phosphate	$(tBDMS)_3PO_4$	73(33)	75(29)	115(13)	147(33)	189(9)			383(100)	425(7)	440
Vanadate	$(tBDMS)_3VO_4$	73(36)	75(34)	115(5)	147(23)	189(7)			403(100)	445(9)	460
Arsenite	$(tBDMS)_3AsO_3$	73(27)	75(26)	115(8)	147(30)	189(17)			411(100)	453(7)	468
Arsenate	$(tBDMS)_3AsO_4$	73(41)	75(33)	115(13)	147(29)	189(20)			427(100)	469(5)	484
Pyrophosphate	$(tBDMS)_4P_2O_7$	73(32)	75(27)	115(4)	147(34)	189(14)			578(100)	620(3)	635

* Multiple [M − 57] fragment ions for selenite, selenate, molybdenate and borate resulting from the multiple stable isotopes that exist for selenium, molybdenum and boron in nature.

are shorter than those anticipated if their molecular weights alone are considered relative to the other tBDMS-oxyanions. The tri-O-tBDMS derivatives of borate, phosphite and arsenite are examples of this type.

Quantitative aspects

We have found, as others have indicated[15], that flame ionization detectors are very sensitive to derivatized inorganic oxyanions. Borate, phosphite, phosphate, vanadate, arsenite and arsenate were detectable at 50 pmole and, taking into account the total peak-area of each oxyanion, the response curves for each were similar and linear over the wide range of 50 pmole to 40 nmole. In contrast to these derivatized oxyanions which possess three tBDMS functions, carbonate, sulfite, sulfate, selenite and selenate, which are smaller in derivatized molecular weight and possess only two tBDMS groups, were not detectable at 50 pmole but were at 150 pmole and each produced a linear response curve in the range of 150 pmole to 40 nmole.

Derivative stability

All oxyanions studied were tested for their stability when kept under dry nitrogen at room temperature and when stored at 4°C. Except for molybdenate, all tBDMS-oxyanions displayed no detectable degradation after 24 h when kept at room temperature and exhibited less than 2% degradation after a week under the same conditions. At 4°C these same derivatives failed to show any significant degradation after 6 months. In contrast to these findings, the di-O-tBDMS derivative of molybdenate demonstrated rapid decomposition after 8 h ($\approx 20\%$) at both room temperature and 4°C.

During GC analysis no decomposition of the tBDMS-oxyanions could be demonstrated. The lack of peak tailing and the consistency in the ratio of peak area to oxyanion concentration indicate that these derivatives do not undergo decomposition on the GC column. In addition, after 500 repeated injections of an oxyanion standard (1 μg of each named oxyanion per μl, 1 μl injected each time) no trace of acidic decomposition products could be found on the removed GC column packing.

Mass spectrometry

All synthesized tBDMS-inorganic oxyanions were each subjected to combined GC–MS analysis. The results are presented in Table II. The tBDMS-oxyanions containing carbon, sulfur, phosphorus, vanadium or arsenic as the central hetero-atom yielded mass spectrums dominated by a singular unique [M − 57] fragment ion. 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[17]. In contrast, tBDMS-oxyanions containing selenium, molybdenum or boron yielded mass spectrums exhibiting more than one large [M − 57] fragment ion reflecting the fact that these specific elements possess more than one stable isotope in relatively high natural abundance. Selenium has six stable isotopes ($i.e.$, $_{34}$Se^{74}, $_{34}$Se^{76}, $_{34}$Se^{77}, $_{34}$Se^{78}, $_{34}$Se^{80}, $_{34}$Se^{82}), molybdenum possesses seven stable isotopes ($i.e.$, $_{42}$Mo^{92}, $_{42}$Mo^{94}, $_{42}$Mo^{95}, $_{42}$Mo^{96}, $_{42}$Mo^{97}, $_{42}$Mo^{98}, $_{42}$Mo^{100}) and boron exists in two stable isotopic forms ($i.e.$ $_5$B^{10}, $_5$B^{11}). Each of these stable isotopes are seen with the exception of selenium-74 which has a very low percent natural abundance. It should be noted that sulfur and vanadium also possess more than one stable isotope but that these are not observed

for two reasons. The first is that the stable isotopes sulfur-32 and vanadium-51 account for over 95 % and 98 % of their total element, respectively; thus the very low natural abundance of the related stable isotopes makes them difficult to see. Secondly, in the absence of high-resolution MS, recognition of the fragment ions possessing these central stable isotopes is complicated by overlap of fragment ions having similar m/e that possess the low natural abundant stable isotopes of silicon, carbon and oxygen that are also present in the tBDMS derivatives.

All tBDMS-oxyanions displayed very weak molecular ions (M^+, $<2\%$ relative intensity). [M $-$ 15] fragment ions, resulting from the loss of a methyl function from the molecule, were also noted for each derivative in low relative intensity. Characteristic of each mass spectrum are the fragment ions at m/e 73, 75, 115, 147 and 189 which are interpreted to be $(CH_3)_3Si^+$, $HO^+=Si(CH_3)_2$, $[(CH_3)_3C](CH_3)_2Si^+$, $(CH_3)_3Si-O^+=Si(CH_3)_2$ and $[(CH_3)_3C](CH_3)_2Si-O^+=Si(CH_3)_2$, respectively.

It is evident that the isotopic ratio of boron, of molybdenum and of selenium is not present in any major fragment ion with a m/e less than M $-$ 57 in the mass spectrum of $(t$BDMS$)_3BO_3$, of $(t$BDMS$)_2MoO_4$ and of $(t$BDMS$)_2SeO_3$ and $(t$BDMS$)_2SeO_4$, respectively. This suggests that these central hetero-atoms are lost early in the fragmentation pattern and that rearrangement of the tBDMS groups predominates in the lower m/e region of the mass spectrum. The similarity in the mass spectrum profiles of the other tBDMS-inorganic oxyanions also suggests that these derivatives may behave analogously. In studies on the TMS derivatives of some of these oxyanions, similar results are reported[15].

In conclusion, the *tert.*-butyldimethylsilylation of inorganic oxyanions, in either their free acid or ammonium salt form, with MTBSTFA proceeds smoothly at room temperature producing tBDMS-oxyanions that are amenable to both GC and GC–MS.

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