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Review

Artifacts in trimethylsilyl derivatization reactions and ways to avoid them

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Abstract

Trimethylsilyl derivatives are routinely employed in gas chromatography to increase the volatility and stability of organic compounds containing active hydrogens. Normally only the desired derivative is formed when organic compounds are derivatized with common silylation reagents. However, some compounds form additional unexpected derivatives or by-products (artifacts). Artifact formation leads to multiple peaks for the same compound or unexpected components in the gas chromatographic analysis of mixtures. This review includes silylation artifacts identified in our laboratory by mass spectrometry during the last 20 years and references to those found in the literature. Also, means of avoiding artifact formation are discussed in detail.

Keywords: Artefacts; Derivatization, GC; Reviews; Trimethylsilyl derivatives; Formalin; Silyl derivatives; Bis-(trimethylsilyl)acetamide; Bis(trimethylsilyl)trifluoroacetamide

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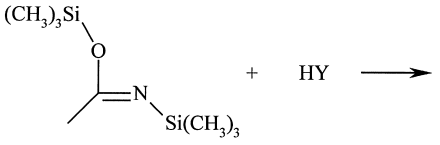
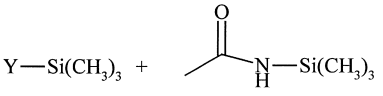
1. Introduction

Trimethylsilyl derivatives are routinely employed [1–5] in gas chromatography (GC) to increase the volatility and stability of organic compounds containing active hydrogens (see Fig. 1). Normally only the desired derivative is formed when organic compounds are derivatized with common silylation reagents such as BSA [*N*,*O*-bis(trimethylsilyl)acetamide] and BSTFA [*N*,*O*-bis(trimethylsilyl)trifluoroacetamide].



BSA



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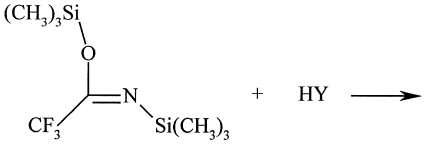
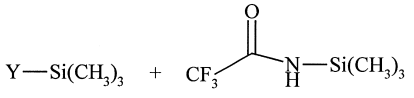


BSTFA



2

However, some functional groups such as aldehydes, amides, carboxylic acids, esters, ketones and phenols under certain conditions form additional unexpected derivatives from silylation reagents and their by-products (e.g., **1** and **2**). We refer to these unexpected derivatives as silylation artifacts. Furthermore, even the derivatization reagent can react with itself, inorganic reagents, other organic reagents, or organic solvents to yield artifacts.

Artifacts are a common problem in analytical chemistry [6] and are noted in a wide variety of chromatography techniques. Artifact is either spelled artifact or artefact and both spellings are acceptable. The former spelling was employed in this article since searches [6] of several common databases showed that scientists prefer to spell the word with an ''i''.

Artifact formation in silylation reactions leads to multiple peaks for the same compound or unexpected components in the gas chromatographic analysis of mixtures. This leads to confusion about the concentration of a component or the number of components present in the sample. For quantitative analyses, the responses for the multiple components can be summed (assuming equal responses) or derivatization conditions changed to avoid artifact formation. This report includes the types of artifacts noted in our laboratory during the last 20 years and references to those found in the literature. Also, means of avoiding artifact formation are discussed.

2. Experimental

2.1. Instrumentation and sample preparation

Gas chromatography–mass spectrometry (GC–MS) data were obtained on Finnigan/MAT 4023, VG/Micromass 70, VG/Micromass Autospec, Hewlett-Packard MSD and Finnigan/MAT TSQ-700 mass spectrometers. The source temperature was set at 250°C on magnetic mass spectrometers and at 150°C on quadrupole mass spectrometers.

All reactions were performed in 2-ml disposable glass vials with crimp tops. Septa were sealed by crimping an aluminum top. Typically 1–5 mg of a sample were dissolved in 0.5 ml of a suitable nonprotic solvent such as *N*,*N*-dimethylformamide (DMF), dimethylsulfoxide (DMSO), tetrahydrofuran (THF), acetonitrile, toluene, pyridine, etc. The sam-

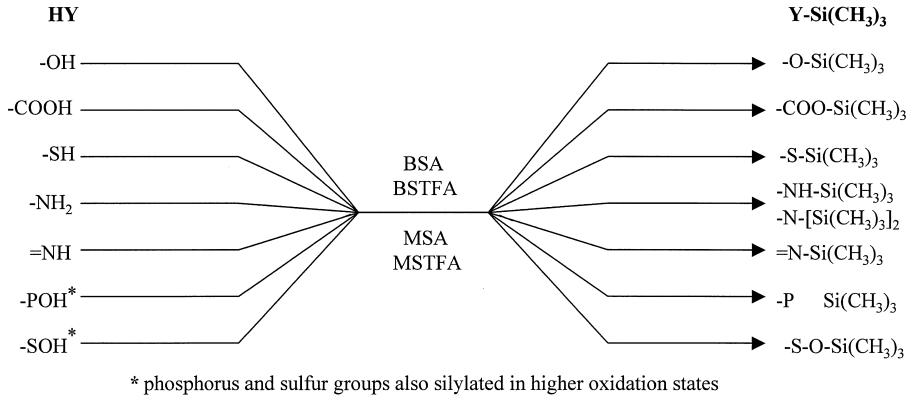


Fig. 1. Functional groups normally derivatized by silylation reagents.

ple was then mixed with 0.5 ml of derivatization reagent (BSTFA, BSA, etc.). Occasionally the sample was dissolved directly in the derivatization reagent and no solvent was employed. Samples were heated for 15–30 min at 50–80°C. Solvent vapors are easily contained in the sealed vials, but caution should be employed when the solvents are heated significantly above their boiling points. Under these conditions, the septum could rupture. Reinforced vials are commercially available for performing reactions at temperatures in excess of 100°C.

Most GC separations were performed on relatively non-polar capillary columns with bonded liquid phases such as DB-17 or DB-5 (J&W, 30 m×0.32 mm I.D., 0.25 μm film thickness). The columns were typically programmed from 50°C to 300°C at 12–15°C/min with a helium backpressure of 12 p.s.i. (1 p.s.i.=6894.76 Pa). Under these conditions a non-retained component traveled through the column at approximately 30 cm/s. Normally 1–3 μl of the solutions were injected in the split mode or 1 μl in the splitless mode with an injection port temperature of 275–310°C.

A wide variety of conditions were employed for sample derivatization depending on the class of compounds and sample matrix. Several references [1–5] discuss factors to consider in optimizing silylation reactions including reaction mechanisms, choice of solvents, choice of reagents, catalysts, temperatures, reaction times, etc. In addition, many distributors of silylation reagents offer excellent technical information.

2.2. Chemical ionization mass spectrometry

Molecular masses were confirmed by ammonia or isobutane chemical ionization (CI) mass spectral data. Ammonia was normally the preferred gas (pressure set to yield a ratio of m/z 18 to 35 of approximately 10:1). Several compounds did not yield molecular mass information with either ammonia or isobutane as the CI gases. These compounds were analyzed with CI gas mixtures of either 3% methylamine in methane or 3% dimethylamine in methane. The pressure of these gases was optimized by setting the ratio of the gas phase monomers (m/z 32 and 46, respectively) to the gas phase dimers (m/z 63 and 91) at approximately 10:1.

2.3. Accurate mass electron impact mass spectral data

Accurate mass electron impact mass spectral data were obtained on a Micromass Autospec GC–MS (magnetic instrument) at about 5000 resolution. Several different ions in **8** ($n=1$) including the molecular ion ($C_6H_{12}NO_2F_3Si$, measured 215.0591, calculated 215.0589, 0.9 ppm error), the molecular ion-methyl ($C_5H_9NO_2F_3Si$, measured 200.0358, calculated 200.0355, 1.5 ppm error), the molecular ion-methyl-formaldehyde ($C_4H_7NOF_3Si$, measured 170.0258, calculated 170.0249, 5.3 ppm error), the molecular ion-methyl-formaldehyde-CF_2 (C_3H_7NOSiF, 120.0288, 120.0281 calculated, 6 ppm error), and the $Si(CH_3)_2F$ ion (measured 77.0212,

calculated 77.0223, 14 ppm error). Accurate mass data were also obtained for **2**, the silylation by-product from BSTFA. Several ions were measured including the molecular ion ($C_5H_{10}NOF_3Si$, 185.0471 measured, 185.0484 calculated, 6.9 ppm error), molecular ion-methyl ($C_4H_7NOF_3Si$, 170.0248 measured, 170.0249 calculated, 0.6 ppm error), molecular ion-methyl-CF_2 (C_3H_7NOSiF, 120.0281 measured, 120.0281 calculated, 0 ppm error), and the $Si(CH_3)_2F$ ion (measured 77.0224, calculated 77.0223, 1.3 ppm error). The measured errors are within the capability of our measurement (control chart data over several years, $n=191$, one standard deviation 5.4 ppm).

2.4. Electron impact mass spectral data

The major ions and relative intensities of the majority of the components (TMS = trimethylsilyl) discussed in the text, but whose mass spectra are not displayed or found in commercial databases, are listed in this section. Full spectra will be sent for consideration as entries in the Wiley Registry of Mass Spectral Data [7] and the NIST/EPA/NIH Mass Spectral Database [8]. The mass spectra are listed with the relative intensities and number of halogens present noted in parentheses.

5a: 73 (30), 147 (100), 189 (80), 213 (40,1Cl), 236 (10,1Cl), 305 (10), 326 (10,1Cl), 380 (20), 415 (30, 1Cl); per-TMS derivative of disilicic acid, CAS No. 20638-18-0: 73 (100), 147 (60), 207 (13), 221 (50), 281 (15), 295 (10), 327 (13), 341 (15), 399 (10), 415 (15), 529 (8), 503 (7), 591 (45); bis-(TMS) derivative of hydrogen peroxide: 59 (6), 117 (5), 119 (4), 133 (60), 163 (100); per-TMS derivative of sulfuric acid: 45 (6), 66 (13), 73 (32), 131 (7), 133 (4), 147 (100), 227 (57); per-TMS derivative of phosphorous acid: 45 (17), 61 (10), 73 (75), 131 (8), 133 (15), 135 (14), 147 (93), 195 (8), 207 (100), 221 (5), 283 (21), 298 (94); bis-TMS derivative of sulfurous acid: 43 (30), 45 (57), 58 (20), 59 (32), 66 (30), 73 (80), 131 (5), 147 (100), 211 (8); bis-TMS derivative of phosphorous acid: 45 (8), 73 (35), 98 (7), 133 (8), 135 (12), 147 (22), 195 (8), 211 (100), 226 (6); **20**: 44 (20), 56 (20), 69 (41), 71 (27), 99 (16), 127 (100); **23**: 42 (7), 61 (100), 172 (8), 185 (62), 233 (26); **24**: 45 (5), 61 (100), 73 (37), 100 (17), 105 (15), 149 (50), 168 (8), 216 (5), 245 (2); BSA artifact with M_r 245: 43 (20), 45 (15), 73 (100), 75 (13), 115 (18), 116 (30), 130 (10), 131 (9) 147 (90), 190 (10), 230 (15), 245 (10); BSA artifact with M_r 275: 56 (38), 73 (100), 113 (18), 114 (12), 117 (18), 131 (8), 147 (40), 172 (5), 260 (15), 275 (5).

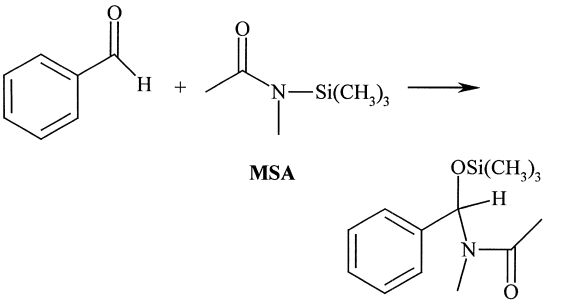
3. Results and discussion

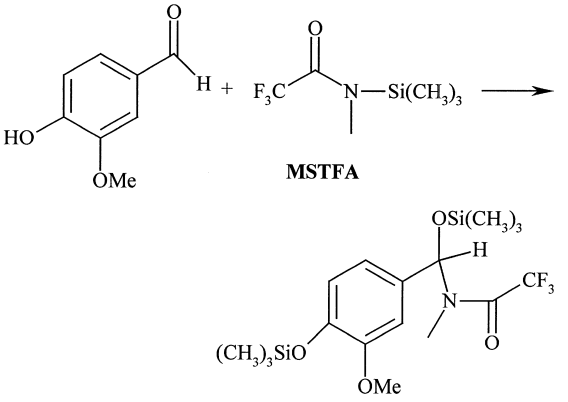
In many of our examples, significant concentrations of silylation artifacts are only noted in the derivatization of reaction mixtures or crude samples, and not in the derivatization of pure samples. Apparently components not present in the pure samples lead to the formation of these artifacts. Many materials are reported [9] to catalyze the silylations of compounds with BSTFA and BSA. Catalysts reported include trimethylchlorosilane (TMCS), trifluoroacetic acid, hydrogen chloride, potassium acetate, piperidine, *O*-methylhydroxylamine hydrochloride, pyridine, oxalic acid and trimethylbromosilane. Thus, it is not surprising that silylation artifacts are noted in reaction mixtures or crude samples since they often contain salts, bases and acids as contaminants.

Attempts were made to isolate several of the artifacts by preparative GC for characterization by proton nuclear magnetic resonance (NMR) analyses. However, the only artifact successfully isolated was an artifact of an amide. This is not surprising since many silylated components tend to decompose when exposed to moisture in the air. Therefore, the majority of the artifacts noted in our laboratory were identified by interpretation of electron impact mass spectra, by confirmation of molecular masses with CI data, and by proposing structures from reasonable reaction mechanisms.

3.1. Aldehyde artifacts

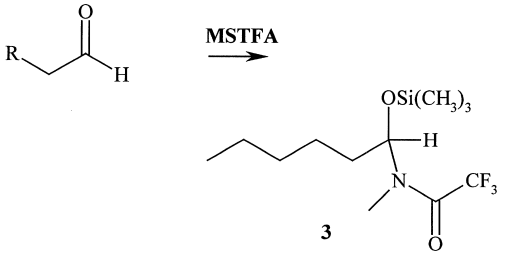
Aldehydes form artifacts in a variety of ways with silylation reagents. Aromatic aldehydes were noted to react with MSA [10–12] or *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) [13] to yield the following types of artifacts:

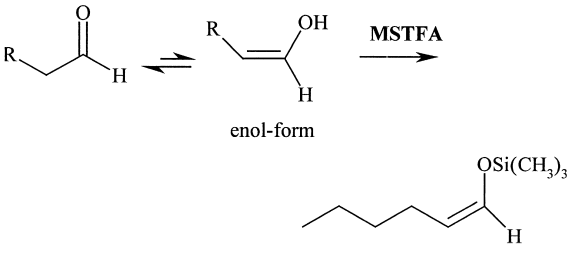




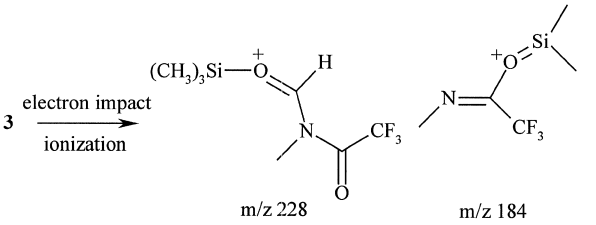
The MSTFA adducts of aromatic aldehydes [13] show characteristic molecular ion-H (M−1) and the molecular ion-$N(CH_3)COCF_3$ (M−126) fragments in their electron impact mass spectra.

Aldehydes with α-hydrogens can react with MSTFA [13] to yield two different types of artifacts:



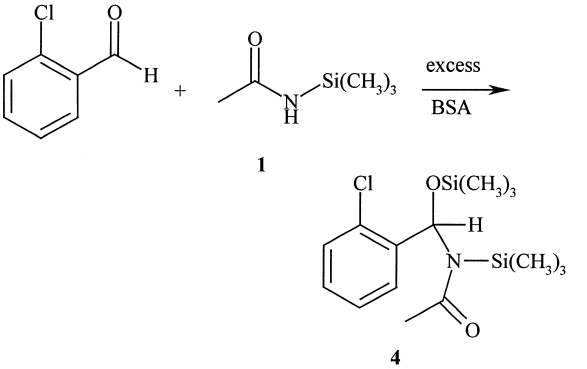


The major ions noted in the electron impact mass spectra [13] for MSTFA adducts of aliphatic aldehydes such as **3** are shown below:

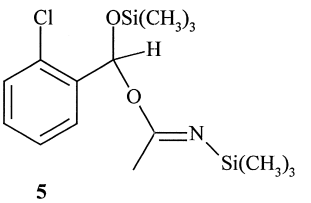


The ion at *m*/*z* 184 is thought to be formed [13] by the neutral loss of hexanal from the molecular ion to yield an odd electron ion for the *O*-TMS form of MSTFA. This intermediate ion then loses a methyl radical to yield the fragment ion proposed for *m*/*z* 184 above.

We have noted the analogous types of artifacts such as **4** with BSA and aromatic aldehydes. Others [13] propose that this type of artifact is formed directly from the silylation product. However, it could just as well be formed from the derivatization by-product, **1**, in the presence of excess BSA.



The electron impact mass spectrum (see Fig. 2) of the artifact formed from 2-chlorobenzaldehyde is most consistent with **4** above. However, the TMS group readily migrates in the electron impact mass spectra of organic compounds [14]. Thus, another likely structure for the artifact could be **5**. Indeed two isomers are sometimes noted in our work for related aldehyde artifacts.



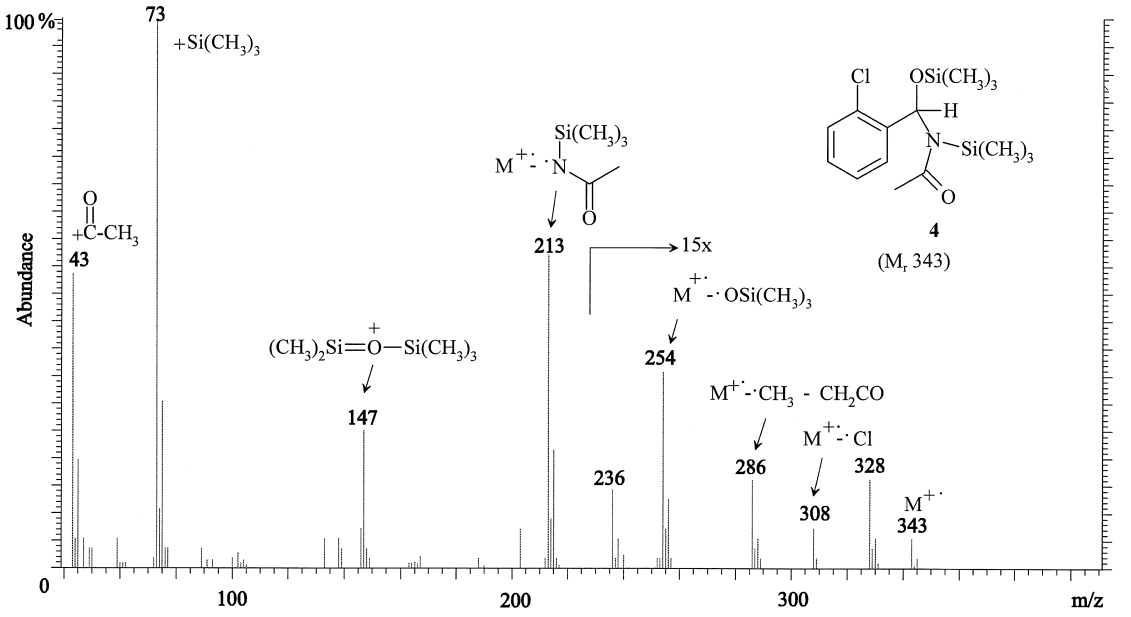
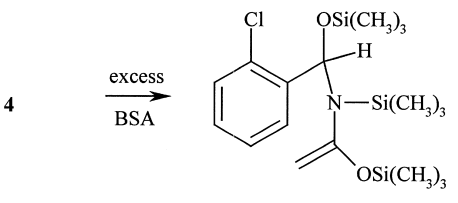


Fig. 2. Electron impact mass spectrum of **4**, an aldehyde artifact formed with BSA.

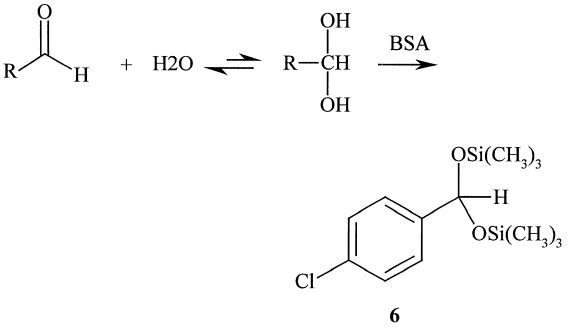
We also have noted smaller concentrations of other artifacts, for example **5a**, formed from further reactions of **4** with BSA:



5a (or isomer)

The concentrations of these BSA artifacts were significantly reduced in this example by substituting BSTFA for BSA. We also plan to evaluate the use of *N*-trimethylsilylimidazole (TMSI) to determine if it might also reduce the concentrations of these types of artifacts.

We have also noted acetal artifacts such as **6**. This type of artifact is likely formed by the reaction of BSA or BSTFA with the *gem*-diol (hydrate) of the aldehyde to form a bis(trimethylsiloxy)acetal. The electron impact mass spectrum of the acetal formed from BSA and 4-chlorobenzaldehyde is shown in Fig. 3.



6

We identified several artifacts (see Fig. 4) in the silylation of a 36% formalin with BSTFA when repeating work performed in Ref. [15]. Formalin solutions are complex mixtures of ‘‘poly-acetals’’ and ‘‘poly-hemiacetals’’ formed by mixing water, methanol and formaldehyde gas. The artifacts in the silylation of formalin solutions are likely formed by the reaction of **7** with one or more of the oligomeric compounds present in the formalin mixture.

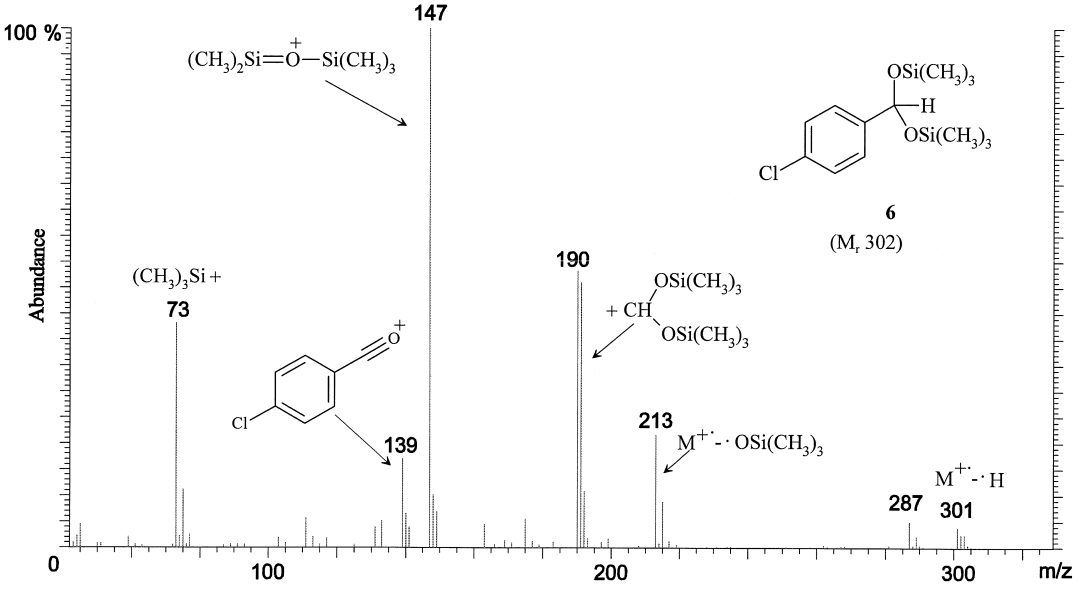


Fig. 3. Electron impact mass spectrum of **6** formed from silylation reagent and *gem*-diol form of aldehyde.

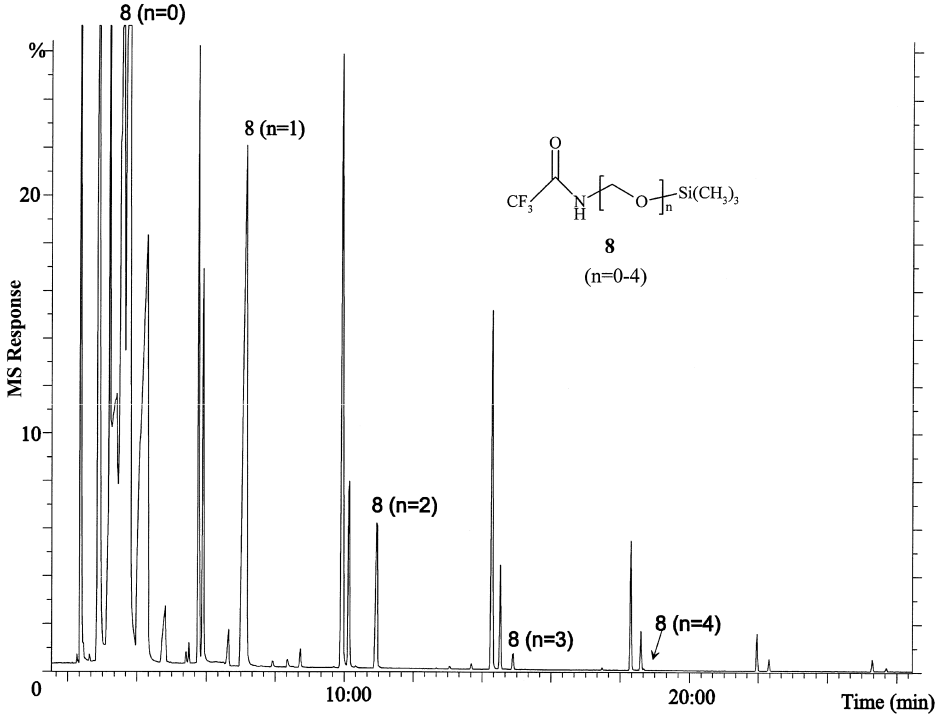
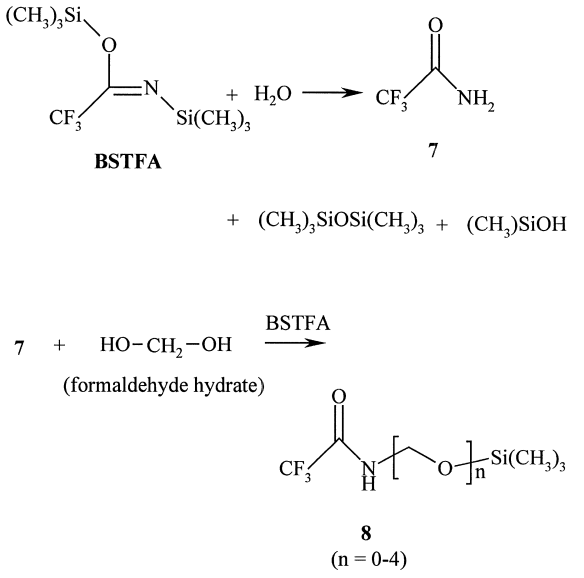


Fig. 4. GC–MS total ion chromatogram of TMS derivatized formalin solution showing retention times of BSTFA artifacts.



The authors of the original study calculated the oligomer distribution of the formalin solution after derivatizing with BSTFA. However, they did not identify or comment on the silylation artifacts present in the analysis. The concentrations and origin of these artifacts should have been considered in the calculation of the oligomer distribution in their original work.

We identified these artifacts by their EI and CI mass spectra. **8** ($n=1$) shows a very unusual ion at m/z 77 in its electron impact mass spectrum in Fig. 5. We have proposed this ion to have a structure of $+Si(CH_3)_2F$. We suspect it is formed by some type of an intramolecular rearrangement since its relative intensity decreases as the distance between the TMS and triflurometyl groups increases. This decrease is shown in the electron impact mass spectra for **8** ($n=1$ and $n=3$) in Fig. 5.

Others have studied a very similar rearrangement [16] that leads to the formation of the $+Si(CH_3)_2F$ ion in the electron impact mass spectrum of trimethylsilyl trifluoroacetate. B/E (constant linked scan of magnetic and electric sector) scan and accurate mass data show that the ion is formed in a multi-step mechanism. A methyl group is initially lost from the molecular ion with subsequent losses of

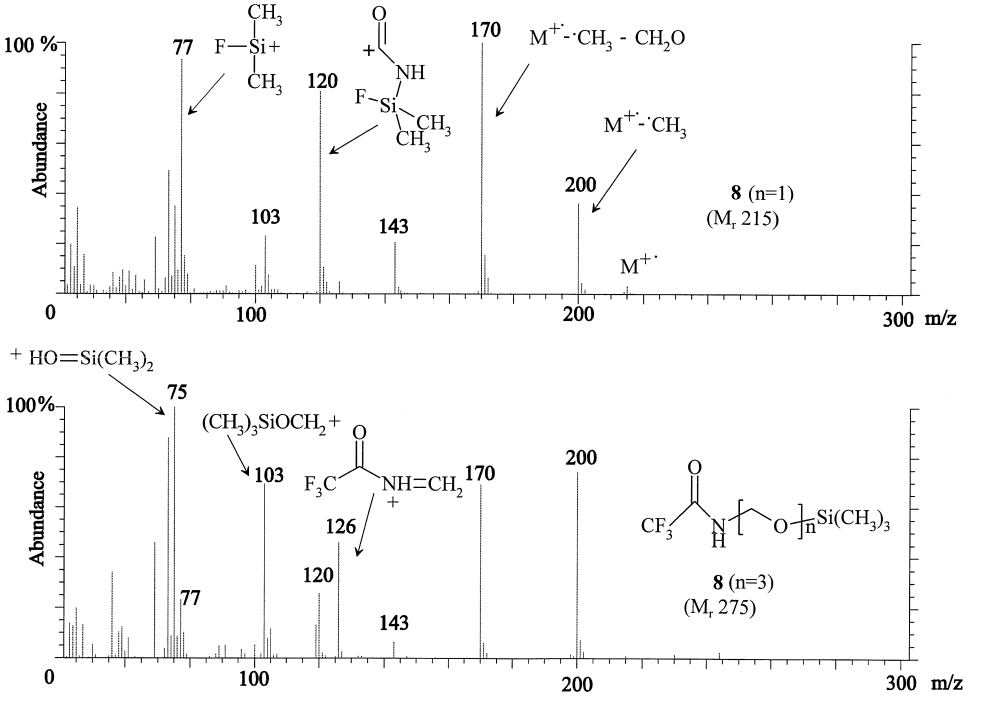
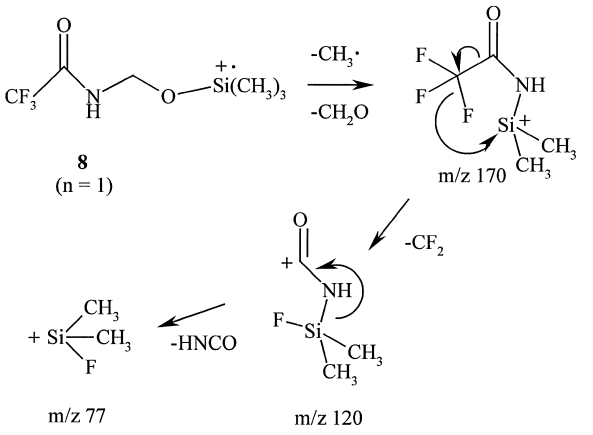


Fig. 5. Electron impact mass spectra of two artifacts, **8** ($n=1$, 3) in formalin solution derivative.

difluorocarbene and carbon dioxide to yield *m*/*z* 77. Furthermore, *m*/*z* 77 is the base peak [17] in the electron impact mass spectrum of **2**, the silylation by-product from BSTFA. We confirmed by accurate mass data that **2** likely fragments by an analogous mechanism as that of trimethylsilyl trifluoroacetate (see Experimental).

Thus our data and literature references support the following fragmentation mechanism for **8** (*n*=1):



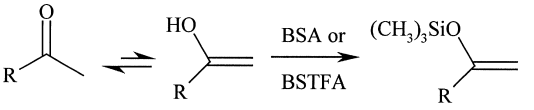
8 (n = 1)

Using a larger excess of the derivatization reagent, BSTFA, might reduce the formation of these artifacts. This would decrease the relative concentration of **7**, and thus form lower concentrations of the observed artifacts. However, diluting the sample with more derivatization reagent would make it difficult to detect many of the low-boiling components. Another possibility is substituting another silylation reagent for BSTFA.

Aldehydes readily form hemiacetals and acetals. Sugars are probably the most well known example of acetals formed during silylation. These acetals are formed by intramolecular attack of an alcohol group on the aldehyde group found in reducing sugars at equilibrium. Attempts to silylate these hemiacetals by silylation often lead to several peaks for each individual sugar [18]. These types of artifacts are best avoided by converting the aldehyde to methoxime or hydroxylimine derivatives before silylation. The only significant problem with forming imine derivatives is that they exist as *syn*- and *anti*-geometric isomers, which are separated by many non-polar GC columns.

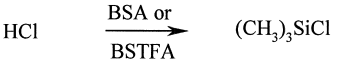
3.2. Ketone artifacts

Ketones form the same type of artifacts noted for aldehydes. Ketones with α-hydrogens react to form artifacts through their enol-form as noted below:



keto-form enol-form

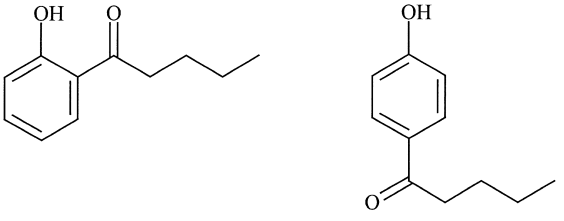
Pure samples of ketones form varying amounts of these types of artifacts depending on variables [19] such as reaction time or the addition of catalysts such as TMCS. Of course the presence of HCl in crude samples would have the same effect since TMCS would be formed in situ.



TMCS

Several schemes are available for avoiding artifacts from ketones that can form enol-trimethylsilyl ethers. They include avoiding acid catalyst when using BSA and TMSIM as derivatization reagents or converting the ketone to a methoxime derivative [19]. The methoxime derivative will still yield multiple peaks on certain stationary GC phases due to the formation of *syn*- and *anti*-geometric isomers. The formation of silylquinoxalinol derivatives from 1,2-diaminobenzene and a silylation reagent avoids the formation of *syn*- and *anti*-geometric isomers for α-keto acids [20].

The electron impact mass spectra of the ketone artifacts formed from **9** and **10** are shown in Fig. 6.



9 **10**

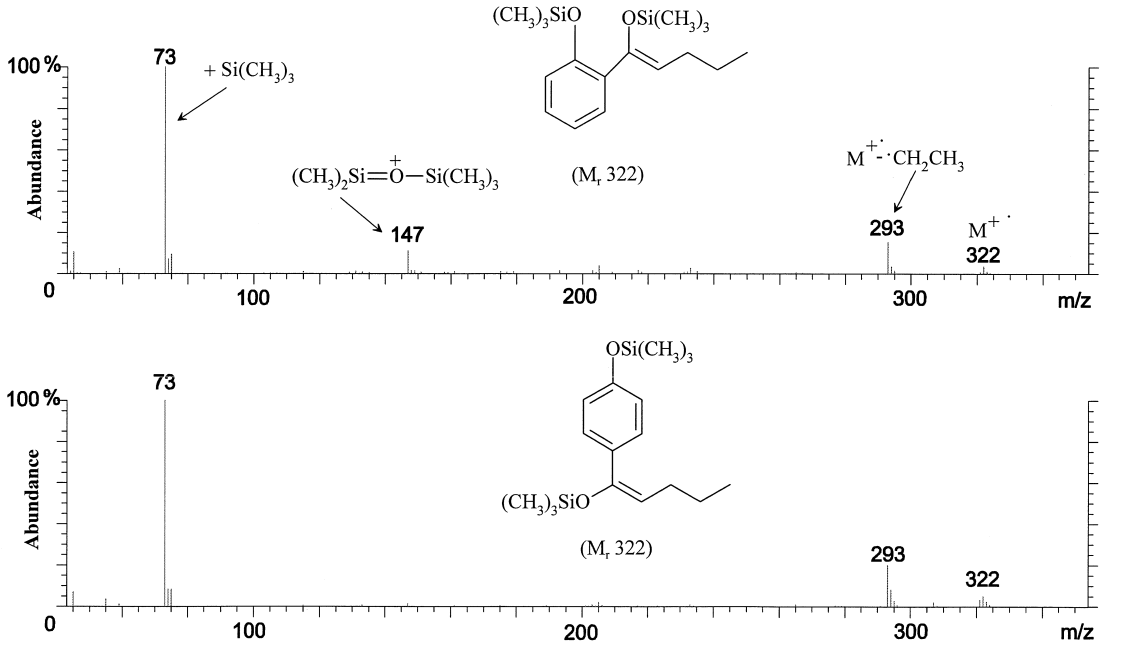
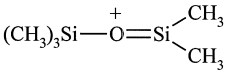


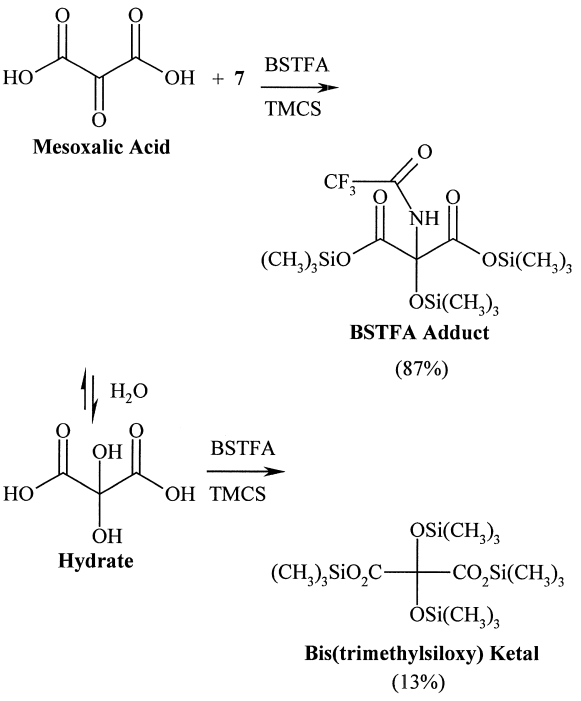
Fig. 6. Electron impact mass spectra of isomeric silylation artifacts **9** (top) and **10** (bottom) showing difference in intensity of m/z 147.

The *ortho*-isomer, **9**, shows a significantly larger ion at m/z 147 formed from an intramolecular rearrangement. This ion is characteristic of two closely spaced TMS groups within a molecule [21]. This ion is much smaller in the *para*-isomer, **10**. Therefore, in some cases this ion can be employed to distinguish isomers.



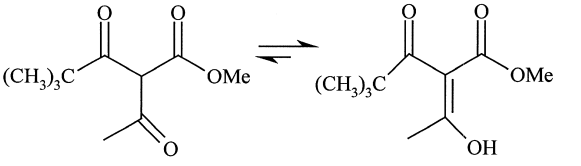
m/z 147

We have not noted the reaction of the reaction by-products (**1**, **2** and **7**) with ketones. This is not surprising since ketones are much less likely to form ketal-like compounds while aldehydes readily form acetal-like compounds. However, others have even noted the by-product of BSFTA, **7**, reacting with highly electron deficient ketones [22,23].

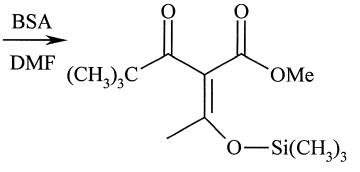


The analogous BSA adduct was noted (2–5%) when BSA (no TMCS added) was employed. The formation of the bis(trimethylsiloxy) ketal is not surprising since mesoxalic acid is isolated in crystalline form as its hydrate. Possibly eliminating the TMCS or increasing the ratio of the silylation reagent would form less of the nitrogen-containing artifact. The latter approach would lead to a smaller concentration of **7**.

The formation of trimethylsilyl enol-ethers can be useful in the analysis of keto-carbonyl compounds such as acetoacetamides, alkyl acetoacetates, α,α-diketo esters, etc. These classes of compounds elute as very broad peaks or two peaks connected by a valley. This poor chromatography is noted on even highly deactivated bonded-phase capillary columns. The broadening of the peak is due to the dynamic equilibrium of the keto- and enol-forms of the diketo compounds. For example, derivatizing of **11** with BSTFA and *N*,*N*-dimethylformamide as the solvent normally yields complete trapping of the enol-form as its trimethylsilyl enol-ether.

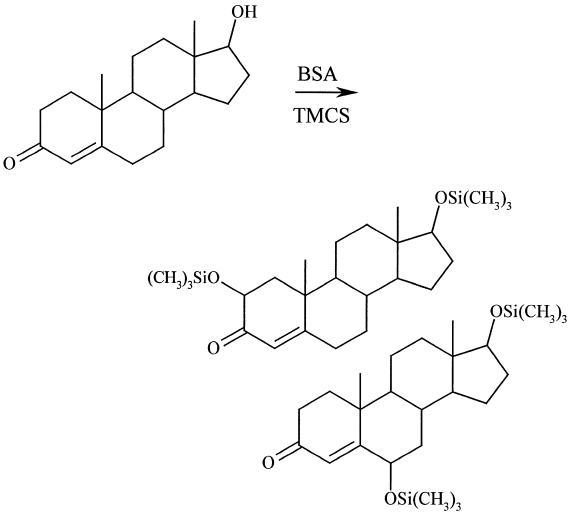


11



The resulting chromatogram yields a very sharp peak that can easily be quantified or two peaks in some cases. Two peaks are sometimes noted because the trimethylsilyl enol-ether can exist in *E*- and *Z*-confirmations.

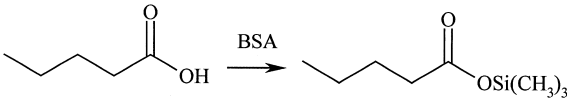
Others have noted the attack of a TMS radical on the enol-trimethylsilyl ether double bond for α,β-ketones such as testosterone [24] that subsequently eliminates a TMS group. The reactions forming the artifacts were presumed to be free radical in nature and catalyzed by ultraviolet light or dibenzoyl peroxide [25].



Free Radical Artifacts

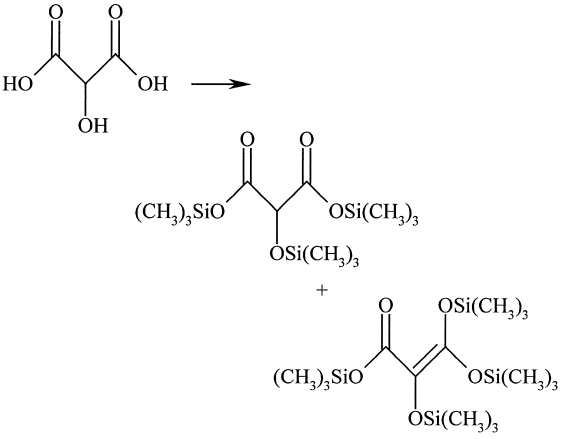
3.3. Carboxylic acid and ester artifacts

Carboxylic acids and alkyl esters tend to form silylation artifacts much less readily than either ketones or aldehydes. Alkyl esters do not normally silylate and carboxylic acids form the expected TMS derivatives.

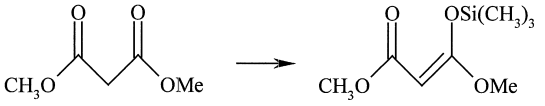


However, malonic acid, α-hydroxymalonic acid, α-methylmalonic acid and alkyl diesters of malonic acid were shown by MS data [26] to form artifacts from the silylation of the enol-form of their ester groups. For example, α-hydroxy malonic acid forms the expected Tris-trimethylsilyl derivative and the

unexpected tetrakis-trimethylsilyl artifact shown below:

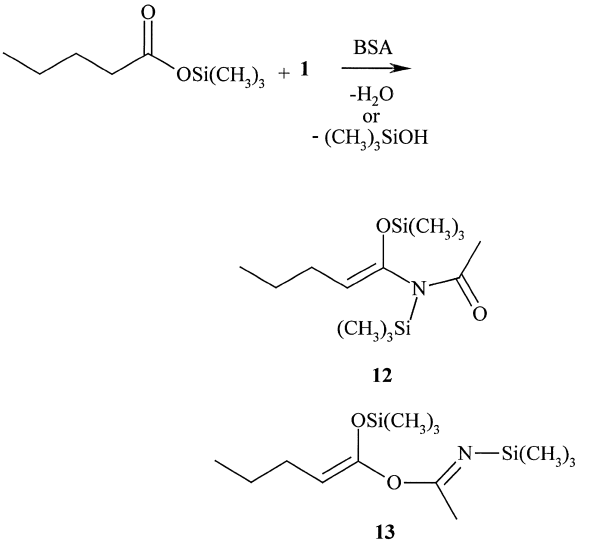


Alkyl esters of malonic acid such as dimethyl malonate form a mono-trimethylsilyl artifact:



These types of artifacts are observed because the enol-forms of the compounds are stabilized as α,β-unsaturated esters.

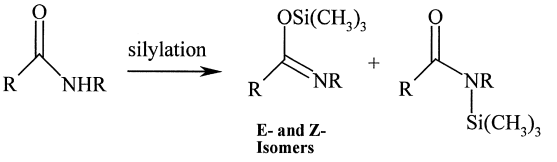
We have noted that carboxylic acids with at least one α-hydrogen occasionally form artifacts. Apparently the derivatization by-product from BSA, **1**, can attack the initially formed trimethylsilyl ester to yield an artifact. The electron impact mass spectrum of an artifact, **12** or **13**, formed from the reaction of BSA and **1** with pentanoic acid is shown in Fig. 7. The electron impact mass spectrum is most consistent with the structure for **13**. The electron impact mass spectrum of **12** would be expected to be more similar to that of **4** shown in Fig. 2. In particular, **12** should show ions at m/z 43 for the presence of an acetyl group and the loss of ketene from either the molecular ion or the molecular ion-methyl group.



The formation of this artifact in this particular sample matrix was avoided by employing BSTFA as the derivatization reagent. Furthermore, this artifact was not noted in the derivatization of the pure sample of pentanoic acid with BSA under the same reaction conditions. Apparently some component or mixture of components present in the crude sample catalyzed the formation of the artifact. Possible ways to avoid these acid artifacts are to select a different silylation (BSTFA, TMSIM, etc.) reagent or to select an all-together different type of derivatization reagent [2–5].

3.4. Amide artifacts

Usually the main problem in the silylation of amides is that they can be detected in three different forms after silylation. The amide usually appears in its underivatized form since it tends to be one of the least reactive groups [27]. However, it can form both *N*-trimethylsilyl and *O*-trimethylsilyl derivatives.



The only amide silylation artifact that we have noted at significant concentrations was formed from

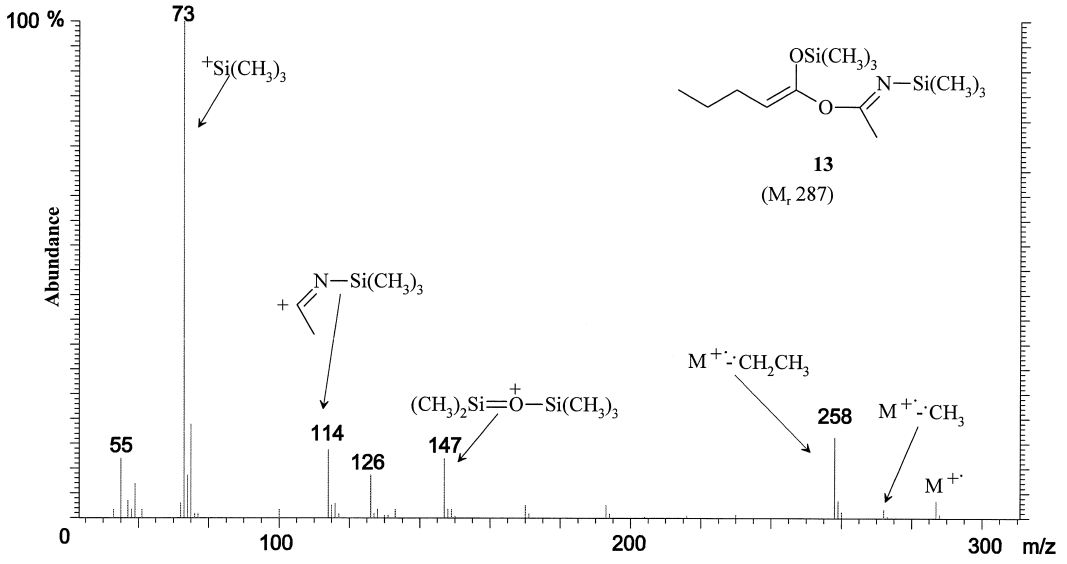
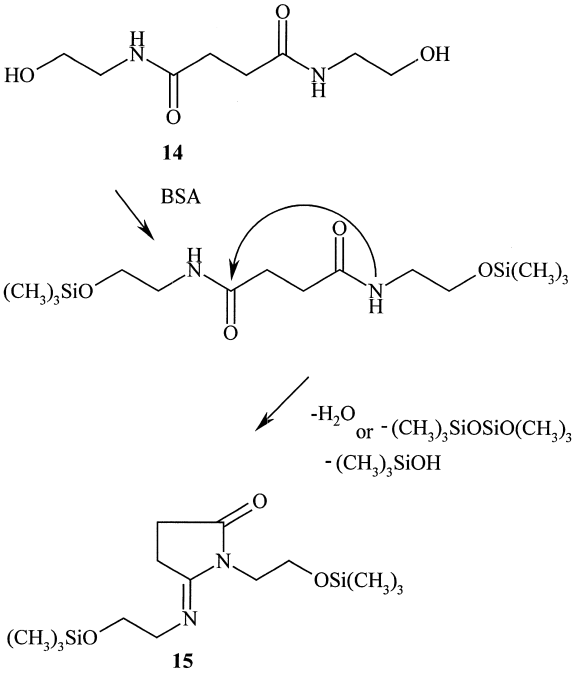


Fig. 7. Electron impact mass spectrum of acid silylation artifact, **13**.

the intramolecular condensation of the bis-(trimethylsilyl)-derivative of **14**.



The structure of this artifact, **15**, was confirmed by proton NMR analysis and could easily be formed in high yields by the reaction of a pure sample of the starting material, **14**, with BSA. The electron impact mass spectrum of the amide artifact is shown in Fig. 8. Normally no ions should be noted whose m/z is greater than that of the molecular ion cluster in an electron impact mass spectrum. However, trimethylsilyl derivatives often yield ions [28,29] corresponding to $M+1$ and $M+73$ ions due to intermolecular transfer of a proton or the trimethylsilyl group via ion molecule reactions. The relative abundance of these ions will increase as the concentration of the analyte present in the electron impact source increases. Analyzing the compound by CI allowed the molecular mass of the compound to be determined since this intermolecular transfer of groups is suppressed in the CI mode.

Decreasing the reaction time and/or temperature of the silylation reaction might significantly reduce this concentration of **15**. The alcohol groups are likely silylated at room temperature or upon exposure to the hot GC injection port in the presence of excess silylation reagent and should require no additional heating. Another approach would be to decrease the silyl donor strength. The relative silyl donor strengths [30] of common commercial reagents is noted below:

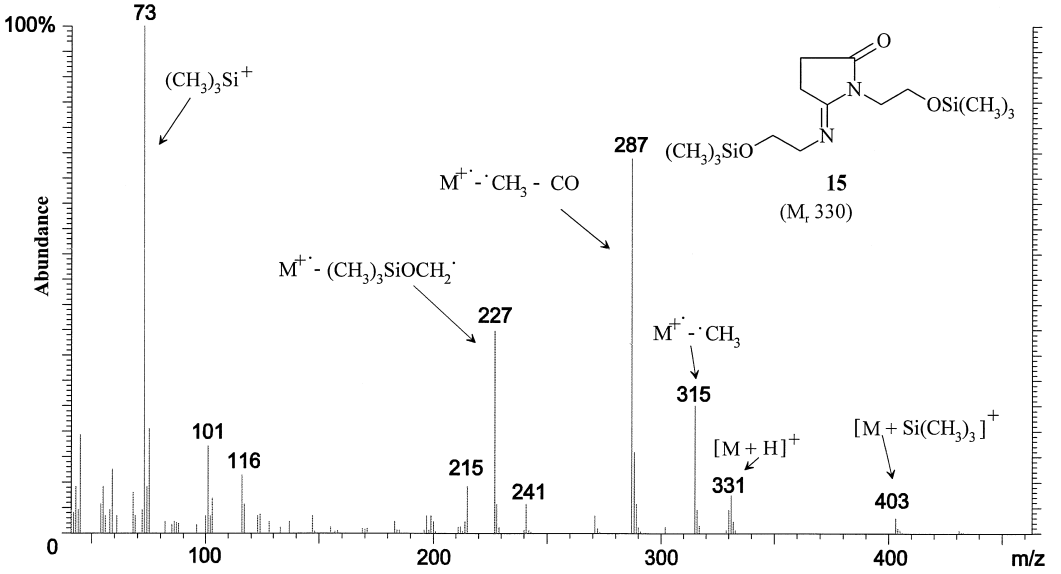


Fig. 8. Electron impact mass spectrum of **15** showing ions formed from intermolecular transfer of proton and trimethylsilyl groups.

TMSI > BSTFA > BSA > MSTFA > TMSDMA
> TMSDEA > MSA
> TMCS (with base catalyst) > HMDS

All the silylation reagents are catalyzed by the addition of 1–10% TMCS.

3.5. Inorganic reagent artifacts

TMS derivatives of inorganic reagents (see Fig. 9) are not truly artifacts since they are expected to undergo derivatization. However, they are often found unexpectedly in crude samples and their presence can catalyze the formation of artifacts

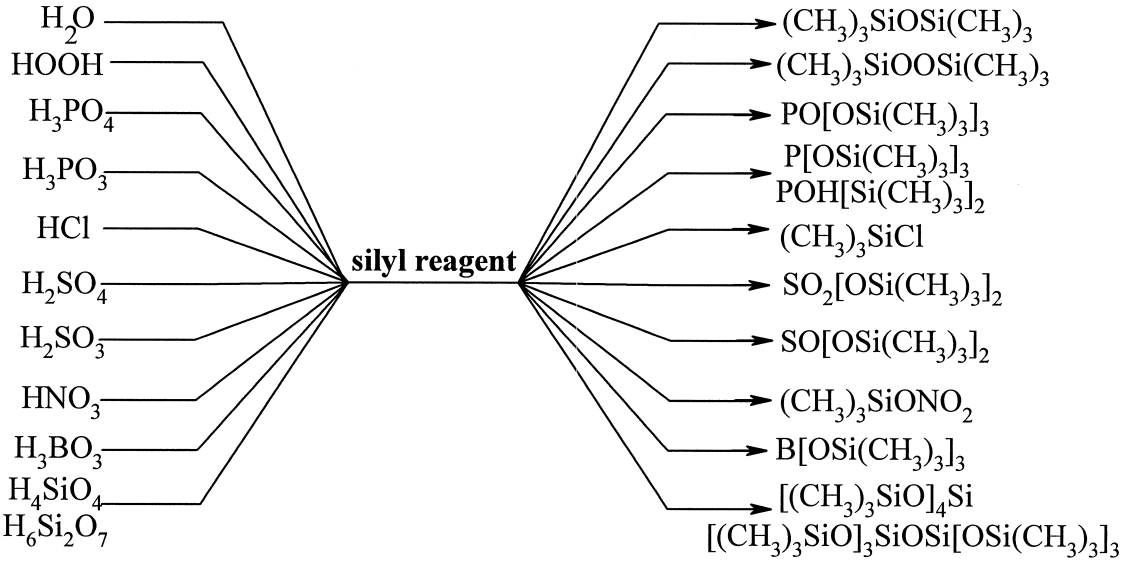


Fig. 9. Trimethylsilyl derivatives of inorganic compounds.

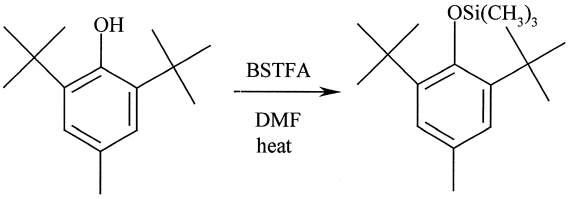
[9,19]. The silylation of samples suspected to contain inorganic acids is very desirable since in their underivatized form they can seriously damage GC columns. The per-trimethylsilylated derivatives of silicic acid and disilicic acid are usually noted [31] when samples are isolated from TLC (thin layer chromatography) plates and then derivatized.

Many of the TMS derivatives of the inorganic compounds shown in Fig. 9 are already present in large commercial electron impact mass spectral databases such as the NIST/EPA/NIH Mass Spectral Database and the Wiley Registry of Mass Spectral Data. However, there were several spectra not present, which were obtained in our laboratory. In addition, several discrepancies between our spectra and those found in the commercial databases were noted. All of our spectra will be donated to these two commercial databases [7,8].

The discrepancies were noted for the per-trimethylsilylated derivatives of hydrogen peroxide, phosphorous acid and sulfuric acid. The spectra sent, which were not in either of the commercial databases, were the per-trimethylsilyl derivative of disilicic acid and sulfurous acid and the bis-trimethylsilyl derivative of phosphorus acid.

3.6. Solvent artifacts

When BSTFA is employed by itself or with other common solvents to derivatize mixtures of hindered phenols such as **16**, a mixture of derivatized, **17**, and underivatized phenol is obtained. However, when BSTFA and DMF are employed for the reaction, **16** is completely derivatized in 20–30 min with heat.



16 **17**

A DMF artifact is always noted at significant concentrations in this derivatization procedure. The artifact was identified by its electron impact mass spectrum (see Fig. 10) as **19**. We have proposed that **19** is formed from reaction intermediate **18**, shown below:

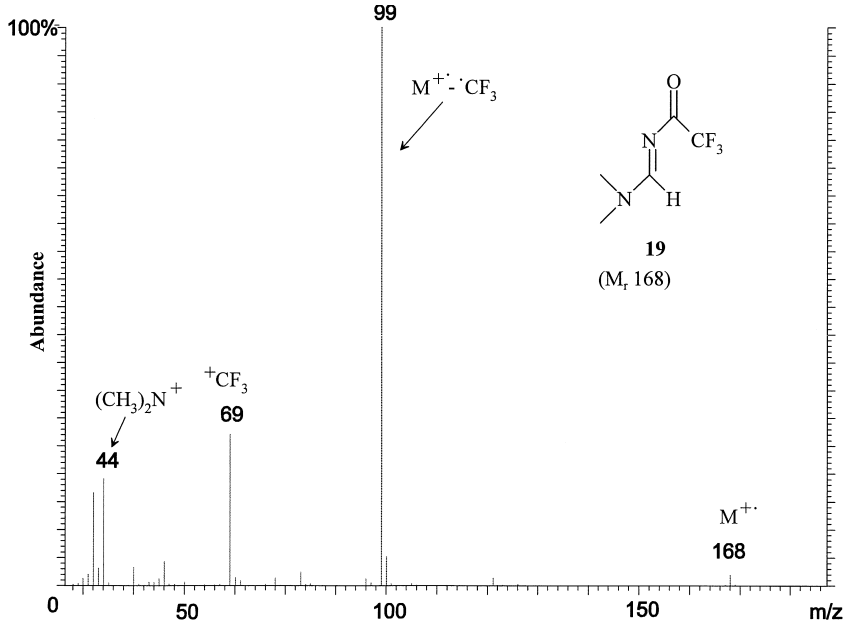
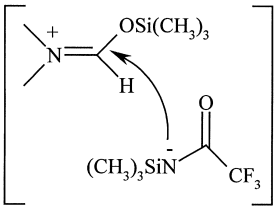
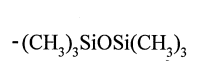
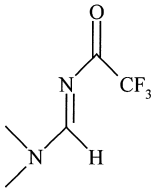


Fig. 10. Electron impact mass spectrum of artifact **19**, commonly noted in reaction mixtures of DMF and BSTFA.



18

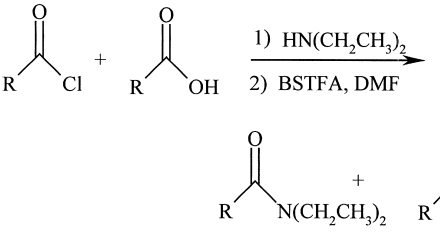




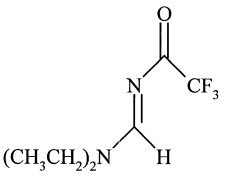
19

Reaction intermediate **18** is similar to the imidoyl chloride cation formed in the Vilsmeier–Haack synthesis [32]. The total ion chromatogram for the GC–MS analysis of **17** showing the relative retention time and concentration of **19** is shown in Fig. 11. It is proposed [33] that the relatively higher dielectric constant of DMF facilitates the solvation of the charge in the transition state of the trialkylphenols leading to the more efficient silylation of hindered phenols with hexamethyldisilazane.

A mixture of **19** and **20** is noted when a two step derivatization procedure is employed to determine the amount of carboxylic acid present in an acid chloride sample. The acid chlorides are first derivatized with diethylamine to convert acid chlorides to amides. The mixture is then treated with BSTFA in DMF to derivatize any free carboxylic acid present.



The diethylamine employed to derivatize the acid chloride is involved in a ‘‘transamidation’’ reaction with either **19** or reaction intermediate **18** to yield **20**.



20

Both **19** and **20** can be avoided by employing

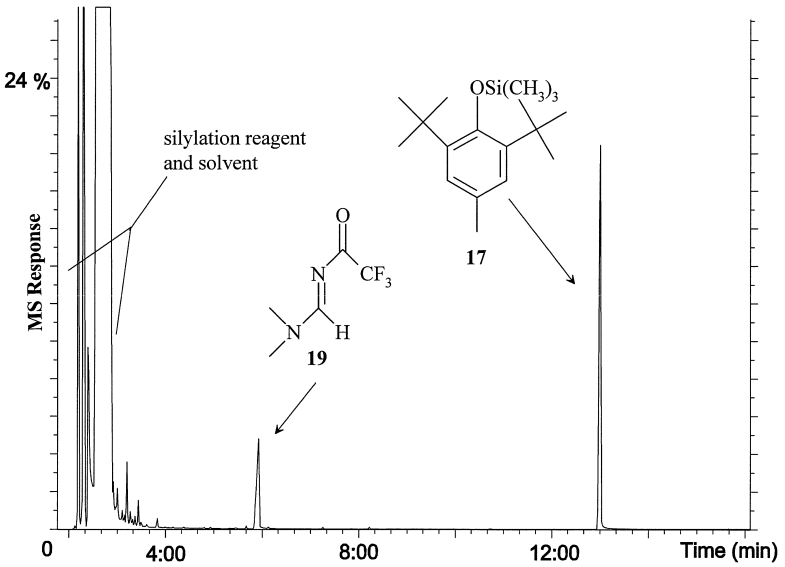
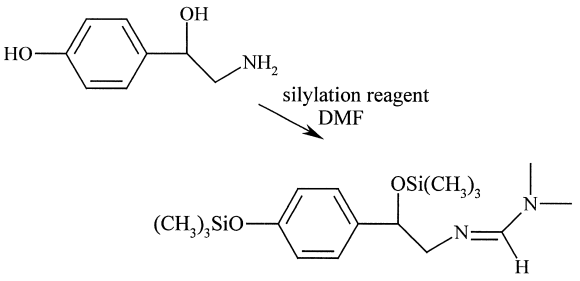


Fig. 11. GC–MS total ion chromatogram of silyl derivative of hindered phenol showing retention time and relative concentration of BSTFA–DMF artifact, **19**.

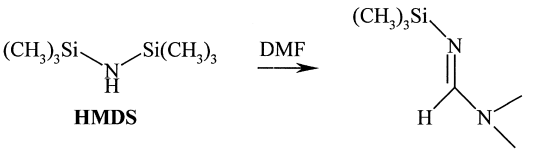
another solvent such as pyridine in the derivatization of acid chlorides. Neither the acid chlorides nor free carboxylic acids need DMF to be converted to their respective derivatives.

Other artifacts formed from DMF and silylation reagents were also noted in the literature. Amines form silylation artifacts with DMF. For example, octopamine was noted [34,35] to form the following type of artifact:

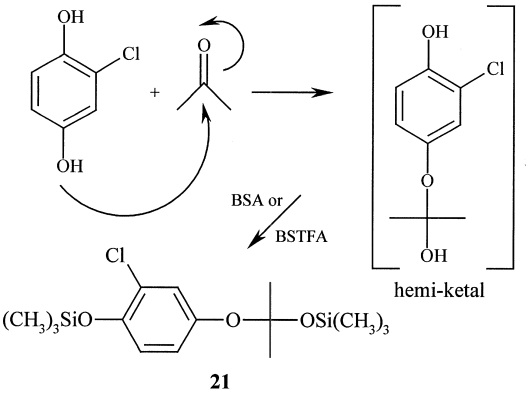


This type of DMF artifact was not formed when the glass surfaces of the reaction tubes were silanized [35].

Hexamethyldisilazane (HMDS) was noted to form an artifact with DMF in the derivatization of tertiary alcohols [34,36]. The artifact was not characterized in their work. A reasonable structure for the artifact would be the structure shown below or a similar amidine:



The use of acetone as a solvent in the derivatization of compounds with either BSA or BSTFA was noted to yield artifacts. For example, in the analysis of a mixture of hydroquinones, several artifacts such as **21** were observed. The electron impact mass spectrum of one of these artifacts formed from 2-chlorohydroquinone is shown in Fig. 12.



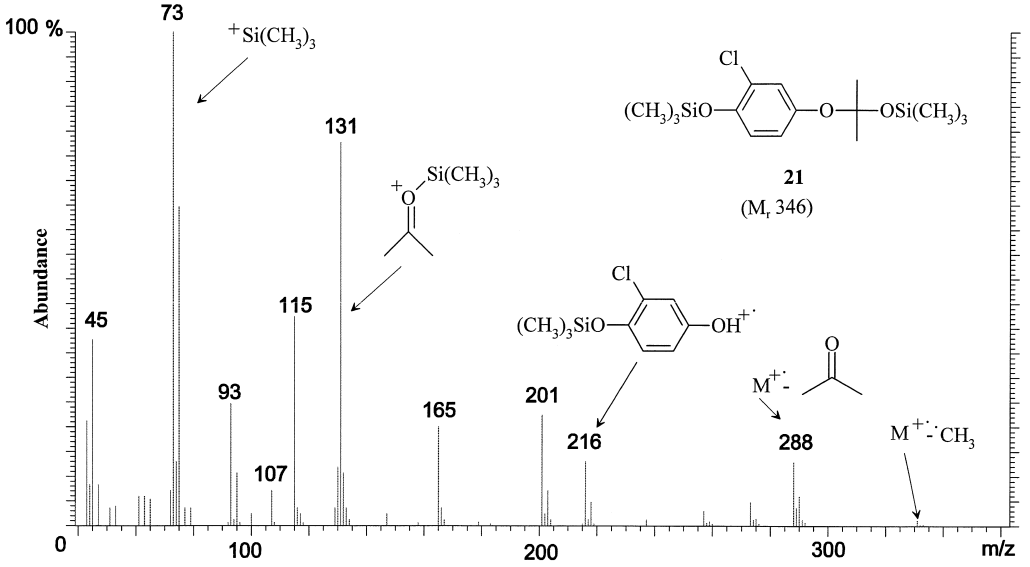
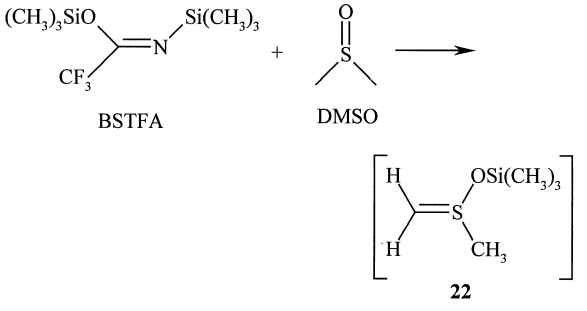


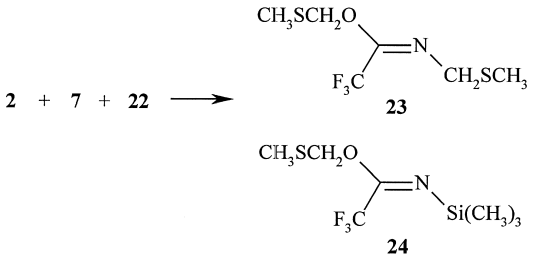
Fig. 12. Electron impact mass spectrum of silylation artifact, **21**, formed from acetone as solvent.

Apparently this artifact is formed by the reaction of acetone with the phenol to give a ''hemi-ketal'' intermediate which is then silylated to yield the observed artifact. These artifacts were not noted when a pure sample of the 2-chlorohydroquinone was derivatized in acetone.

DMSO was noted to give many different artifacts in the preparation of polyester samples for composition analysis [37]. It was proposed that BSTFA reacted with DMSO in a Pummerer reaction [38] to form intermediate **22**.



By-products from the silylation reaction, **2** and **7**, react with **22** to yield **23** and **24** plus many additional artifacts at lower concentrations. GC–IR, GC–MS, CI GC–MS (accurate mass), and deuterium-labeled reagent experiments were used to identify the artifacts.

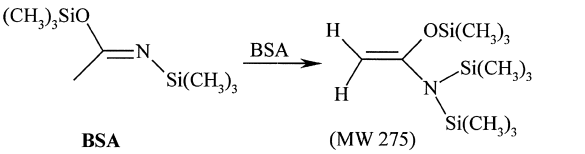


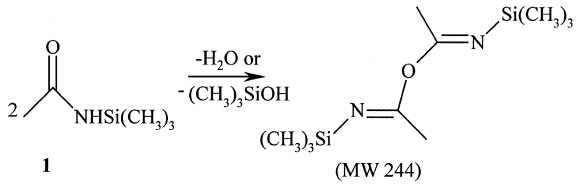
Similar reactions were noted [39] when *N-tert.*-butyldimethylsilyl derivatives of imidazole were heated with DMSO at very high temperatures.

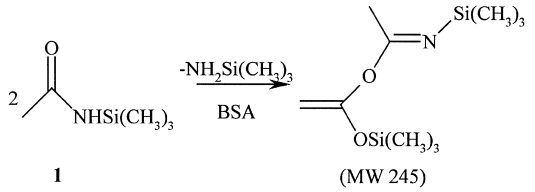
The DMSO artifacts [37] were avoided in the polyester analysis by substituting BSA for BSTFA. BSA is a weaker silyl donor than BSTFA that retarded the formation of the reaction intermediate. Indeed, all detectable artifacts are avoided by substituting BSA for BSTFA. However, BSA and its by-products are higher boiling than BSTFA and its by-products and could obscure lower boiling components of interest. Another possible reagent for this application is MSTFA which has not been tested. MSTFA and its by-products are more volatile than BSA and its by-products.

3.7. Silylation reagent artifacts

Three structures are proposed for artifacts frequently noted at lower concentrations in samples derivatized with BSA. They are proposed to be formed by the reaction of BSA and its by-product, **1**. Analogous by-products are not noted with BSTFA since it and its by-product, **2**, do not contain any active hydrogens and its by-product does not add as readily across active carbonyls.

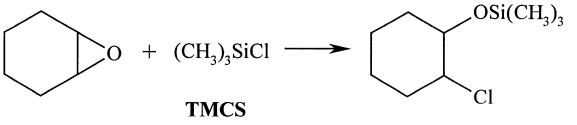






3.8. Other miscellaneous literature artifacts

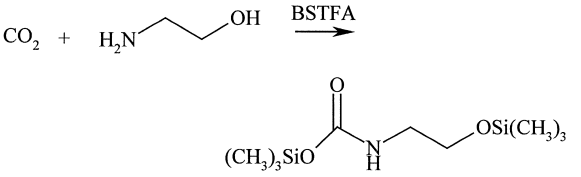
Several other references were found in the literature for miscellaneous silylation artifacts. Epoxides [40] were reported to react with TMCS as follows:



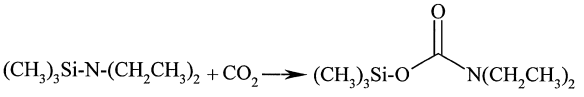
Epoxides could react with by-products such as **1**, **2** and **7** in a similar manner to yield silyl derivatives;

however, no literature references were found for these types of artifacts.

Silylation artifacts can be formed from carbon dioxide dissolved in samples. Amines such as glycine, serine, alanine and ethanolamine [41] were noted to form carboxylates. For example:

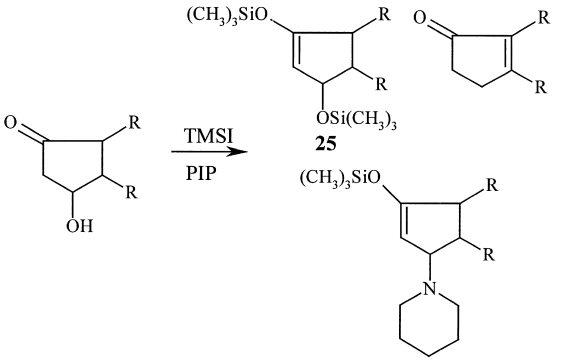


These types of artifacts were avoided by adding dilute hydrochloric acid during the final stages of drying a sample for derivatization. Silylamines add to carbon dioxide to give the following carbamate ester [42–44].



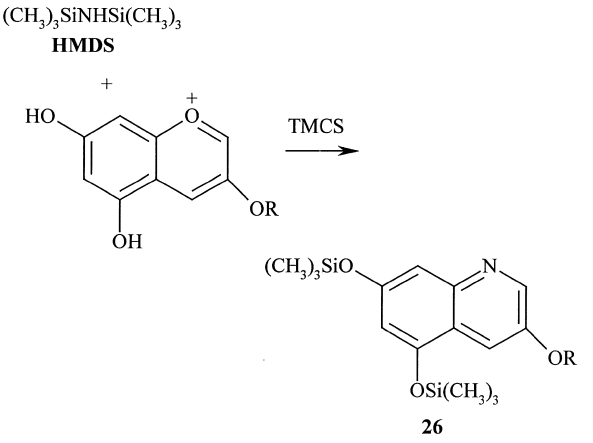
Carbon disulfide [42,45] and sulfur trioxide [42,46] also react with silylamines to give similar products.

Prostaglandins [47] were noted to form three different chromatographic peaks when silylated with trimethylsilyl imidazole (TMSI) containing piperidine (PIP). For example, a prostaglandin having an unstable β-hydroxy ketone ring structure was found to form the following derivatives:



When a mixture of BSTFA and PIP were used for the derivatization, only one peak was noted by GC for **25**. The piperidine-containing artifact might also have been avoided by employing TMSI with a tertiary amine as a catalyst instead of piperidine.

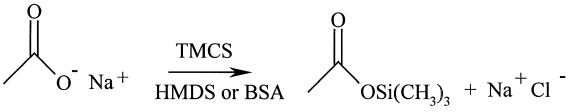
An artifact was noted to form in the of silylation [48] of glycosides with hexamethyldisilazane (HMDS) and TMCS. This artifact was desirable since it formed volatile derivatives of anthocyanin-2-arylbenzopyrilium salts. The compounds are converted into quinoline-like structures such as **26** by reaction of the salts with mono-trimethysilylamine.



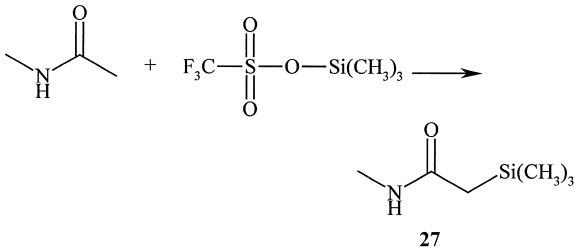
We have noted that THF containing peroxides can lead to artifact formation. Others have also noted oxidations during silylation reactions. Trimethylsilylation of 7-methylpurine nucleosides using BSTFA and TMCS yielded 7-methyl-8-oxo compounds as artifacts [49]. The compounds were shown by $^{18}O_2$ labeling experiments to be formed by an oxidation reaction (dissolved oxygen) during the derivatization reaction. The resulting artifacts were found to be very useful for analyzing 7-methylpurine nucleosides because they were amenable to gas chromatography and exhibited structurally diagnostic mass spectra. Components in hydrolyzed DNA were noted to oxidize [50] during silylation for GC–MS analyses. To prevent the artifactual formation of oxidized bases during the silylation, a preparative high-performance liquid chromatography (HPLC) method was developed to remove the interferences. Molecular oxygen [51] was involved in the dehydrogenation of nucleosides during vigorous trimethylsilylation to yield artifacts. The reaction was accelerated by heat and certain free radical initiators and was inhibited by diethyldithiocarbamate and gal-

vinoxyl free radical. This reaction was found useful as a synthetic approach in nucleoside synthesis. Persilylation of norethynodrel and 5(10)estrene-17β-ol-3-one was noted [52] to aromatize the A-rings of these compounds.

Salts of inorganic or organic acids are normally not derivatized when reacted with BSA or BSTFA alone. However, under certain circumstances they will be detected as their trimethylsilyl ester derivatives. Ammonium salts of many different inorganic acids [53] were readily derivatized. K or Na salts were first converted to ammonium salts by cation exchange. Several Na and K salts of mono-, di- and tribasic organic acids and sodium salts of fatty acids were directly silylated [54] with a mixture of hexamethyldisilazane and trimethylchlorosilane. Na salts of organic acids are converted to trimethylsilyl esters [55] employing mixtures of hydroxylamine hydrochloride–BSA or trimethylchlorosilane–BSA. The conversion of volatile organic acids to sodium salts with subsequent derivatization [55] can be a very useful means of avoiding their loss when samples are concentrated by lyophilization.

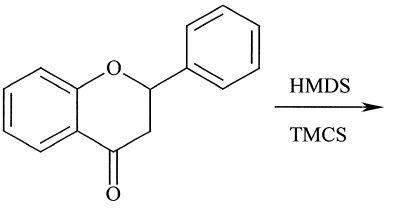


The presence of triflic acid in samples leads to silylation artifacts from C-silylation at the α-position of amides and esters. For example, the trimethylsilyl ester of triflic acid was shown [56] to react with *N*-methylacetamide after aqueous work-up to yield **27**.

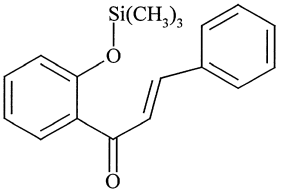


27

Flavanone aglycons were noted to ring open to their corresponding chalcones [57]. This ring opening was confirmed by GC–MS, silylation of standards and UV spectrophotometric data.

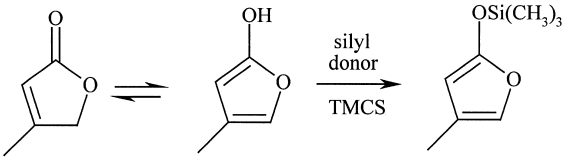


Flavanone Aglycons

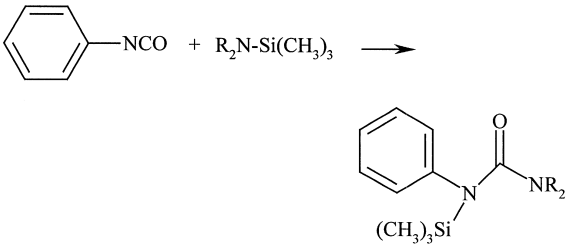


Chalcone

Compounds containing α,β-unsaturated lactone rings are derivatized to form enol-TMS ethers. The conversion is quantitative when TMCS and a powerful silyl donor are employed [58].



Secondary silylamines react with isocyanates to give *N*-silylureas [42,59–61].



The primary silylamines add to the isocyanate to give two different products, which were shown by NMR data to be in equilibrium with each other via silyl-proton exchange [42,61]. Isothiocyanates and silylamines form analogous products [42,61].

4. Summary of ways to avoid or minimize artifact formation

Normally silylation reactions yield the desired

derivative with minimal optimization of reaction conditions. However, many different artifacts can be formed under certain circumstances. In addition, multiple peaks can be noted due to incomplete silylation of compounds. Several excellent references [1–5] discuss factors to consider in optimizing silylation reactions including reaction mechanisms, solvents, derivatization reagents/reagent mixtures, catalysts, temperatures, reaction times, etc. In addition many distributors of silylation reagents offer technical information on the selection and use of silylation reagents.

Several ways to avoid or to minimize artifact formation were noted in our studies and literature references. Ones that we have found generally useful are summarized below:

1. The first step is to characterize all components in the mixture by electron impact GC–MS. In some cases, additional analyses were required by CI GC–MS, accurate mass and isotope labeling to identify unknowns. Silylation conditions can only be efficiently optimized if components are identified and reactions leading to their formation understood. Several references [14,28,29,62–64] discuss the general interpretation of the electron impact mass spectra of silylated compounds. Other references discuss the fragmentation of MSTFA adducts [13] and the TMS derivatives of nucleotides [65].
2. Reaction times should be optimized for the components/functional groups of interest. For many compounds, derivatization reactions are complete upon mixing or injection onto the hot GC injection port when excess derivatization reagent is employed.
3. Select a different silylation reagent. Selecting a weaker silyl donor [30] will often minimize artifacts formed from over-silylation.
4. By-products from silylation reactions form artifacts by adding to analytes or by reacting with themselves or with solvents. Thus selecting another derivatization reagent can avoid or minimize many of these types of artifacts.
5. Select another solvent for the derivatization. Polar solvents increase the rate of silylation, but we have often noted artifacts from their reactions with analytes and the silylation reagents.
6. Derivatization methods developed for pure standards often yield different products than those noted for crude samples containing additional solvents, inorganic acids, inorganic salts, etc. Therefore it is best to develop derivatization procedures for compounds with matrices identical or similar to the targeted process samples.
7. There is a multitude [2–5] of other derivatization reactions for organic compounds. Consider a totally different class of derivatization reagent.
8. Use one class of reagent to derivatize one type of functional group in a sample and a different class of reagents to derivatize another type of functional group. For example, a combination of oxime and silyl reagents is used to derivatize keto-acids.
9. Switch to a GC column which does not require derivatization of the sample or analyze the sample by HPLC without derivatization.

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