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INJECTOR PORT REACTIONS IN GAS CHROMATOGRAPHY

SOURCES OF ERROR IN THE QUANTITATIVE ANALYSIS OF ALKYLSILYL DERIVATIVES OF NUCLEOSIDES

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SUMMARY

The direct monitoring, by gas chromatography, of rates of silylation of various substrates is complicated by accelerated reactions occurring in the hot injector port of the gas chromatograph, *e.g.* further silylation by excess reagent. Monitoring the hydrolysis of silyl derivatives is also prevented since desilylation by hydrolysis media occurs in the injector port. Techniques have been developed to avoid these injector port reactions and include: (a) deactivation of silylating reagent by reaction with excess methanol, (b) deactivation of substrate by further derivatization with an active reagent, and (c) removal of hydrolysis media *in vacuo*. The methods are illustrated by studies on the deoxynucleoside thymidine but should be capable of extension to a wide variety of suitable substrates.

INTRODUCTION

Trimethylsilylation is a well-known method of obtaining volatile derivatives for gas phase analysis of compounds that exhibit hydrogen-bonding tendencies[1]. There have been only limited applications of the method to synthetic chemistry for the protection of, for example, hydroxyl groups, due to the very labile nature of the trimethylsilyl (TMS) group[2]. Recently, there has been an increasing use of more sterically crowded alkylsilyl groups in organic synthesis for the protection of hydroxyl functions in a variety of compounds[3–8]. Such derivatives are vastly more stable than TMS derivatives to both acid and base conditions. They have also been found to be amenable to gas phase analysis[9,10].

We have been investigating methods of monitoring the progress of silylation and desilylation reactions for the purpose of optimizing reaction conditions and yields, as well as to study mechanistic aspects of the reactions. The advantages of gas chromatography (GC), namely, small sample size and rapid, quantitative analysis, are well suited to this project. However, reactions in the injector port can cause serious errors in the analysis. We wish to report here our observations and some solutions that we have found to be valuable.

Most of our attention has been directed towards the synthesis of nucleosides protected in the 3′ or 5′ positions by alkylsilyl groups so that oligonucleotide synthesis can be achieved by creating 3′–5′ phosphate linkages. The synthetic approach, illustrated by the deoxynucleoside thymidine (dT), is outlined in Fig. 1. Reaction 1, with certain conditions, silyl donor and solvents, will give a nearly selective synthesis of the 5′-O-silyl derivative b. The 3′,5′-di-O-silyl derivative c is available when an excess of reagent and vigorous conditions are used (reaction 2). The 3′-O-silyl derivative d is obtained by reaction 3, in which the 5′-O-silyl group is selectively hydrolyzed under acid conditions. Conveniently, the side products are all separable by thin-layer chromatography.

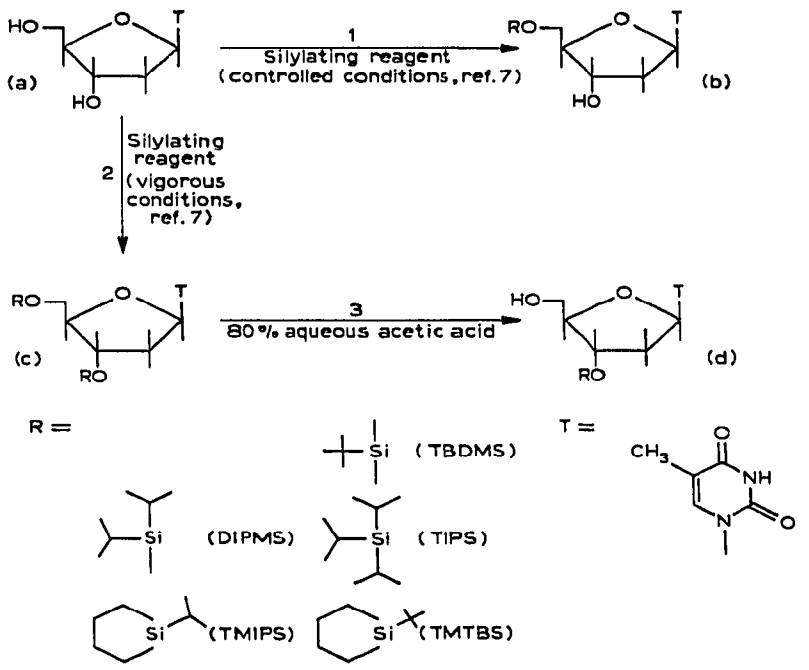

Fig. 1. Synthetic routes to alkylsilyl derivatives of deoxynucleosides.

Since we consider that the silyl derivatives (in particular those listed in Fig. 1) will be of major significance in the nucleoside and nucleotide field, it is important to develop good techniques for their analysis and characterization. The derivatives b, c and d are separable by GC and are easily identified by mass spectrometry (MS)[9,11]. We have developed GC analyses which overcome the interfering injector port reactions to monitor reactions 1 and 3 for various combinations of nucleoside and alkylsilyl reagents.

EXPERIMENTAL

Reagents and chemicals

tert.-Butyldimethylsilyl chloride, m.p. 121–125°, was prepared according to the method of Sommer and Taylor[12]; it is also available commercially from Willow

Brook Labs., Waukesha, Wisc., U.S.A. Other alkylsilyl chlorides were prepared by reacting an alkyllithium with the appropriate alkylchlorosilane. Methods will be given when their properties and reactions are reported in greater detail. *tert.*-Butyldimethylsilylimidazole was prepared by refluxing *tert.*-butyldimethylsilyl chloride (7.5 g, 0.05 mole) with imidazole (3.4 g, 0.05 mole) in triethylamine (TEA) (150 ml) under nitrogen for 3 h. The precipitate of TEA·HCl was filtered off under nitrogen and the filtrate was fractionally distilled to give a clear, viscous liquid, b.p. 150–155° at 77 mm Hg. GC analysis showed the purity to be ~95%. The reagents bis(trimethylsilyl)trifluoroacetamide (BSTFA), trimethylsilylimidazole (TMSIm), Tri-Sil-Z (a 1.2 *M* solution of TMSIm in pyridine), acetylimidazole (AcIm) and trifluoroacetylimidazole (TFAIm) were obtained from Pierce (Rockford, Ill., U.S.A.). Reagent grade acetic anhydride (AcAnh) was distilled from phthalic anhydride and stored in the dark. Trifluoroacetic anhydride (TFAA) was used without further purification. The *tert.*-butyldimethylsilyl derivatives of thymidine were prepared as previously described[6]. Thymidine was purchased from Sigma (St. Louis, Mo., U.S.A.). Reagent grade pyridine was distilled from *p*-toluenesulfonyl chloride, redistilled from calcium hydride, and stored over Linde molecular sieve. Reagent grade tetrahydrofuran (THF) eluted through an activated alumina column was refluxed with powdered $LiAlH_4$, then distilled from $LiAlH_4$ and stored over molecular sieve. TEA was distilled from calcium hydride and stored over molecular sieve.

Procedures

Reactions were performed in small, dry, PTFE-lined septum-capped vials (typically, 3-ml screw-top vials from Pierce) containing small PTFE-covered magnetic stirring bars (approx. 2 mm × 8 mm). A typical study of silylation rate was performed by weighing into the vial approximately 50 μmoles of substrate, adding in the required amount of solvent (usually pyridine, to about 0.1 *M* in substrate) containing internal standard (*e.g.*, pyrene or triphenylene) by syringe, sealing the vial, and then dissolving the substrate by stirring. If required, the vial could then be snugly fitted into a controlled-temperature aluminum block for rate studies at temperatures other than ambient. The appropriate quantity of reagent was then added (amount as determined by the molar proportion of reagent to substrate desired). Timing was started when the reagent had mixed in. The reactions were then monitored at timed intervals by either Method 1 or 2 as appropriate.

Method 1. A 10-μl aliquot of the reaction solution was withdrawn from the vial by syringe. This was added to 20 μl of methanol in a small dry culture tube (50 × 6 mm O.D.) and allowed to stand for 5 min. Volatiles were then removed on a vacuum rack. The residue was dissolved in 20 μl of pyridine or THF (which gives less tailing in the chromatogram) and then 0.5- to 2.0-μl portions were subjected to GC analysis.

Method 2. A 10-μl aliquot of the reaction solution was withdrawn from the vial with a syringe and placed in a dry culture tube fitted with a rubber 5-mm NMR sample tube septum (Kontes Glass Company). To this were added 20 μl of one of BSTFA, TMSIm, TFAIm, TFAA, AcIm or AcAnh reagents. The mixture was then mixed by vibration and allowed to stand for 10 min or longer. Except for the solutions containing acyl anhydrides, 0.5- to 2.0-μl portions were analyzed directly by GC. When anhydrides were present, the volatiles were removed *in vacuo*, the residue was

dissolved in 20 μl of pyridine or THF, and then analyzed by GC. In our studies we observed complete derivatization within 10 min. Incomplete derivatization could be detected from the chromatograms.

Hydrolyses of silyl derivatives by acetic acid on a small scale were studied in a similar way except that the internal standard was not added until a later stage because of its limited solubility in the hydrolysis media. (The internal standard is still required because it serves to prevent a major source of error, *i.e.* variability of injection volume.) Reactions were set up by weighing accurately into a vial about 100 μmoles of silyl derivative followed by addition of 80 % aqueous acetic acid to the appropriate concentration (typically 0.05 *M*). Reactions were usually performed at room temperature, but other temperatures could be used. Stirring and timing were then started. The reactions were monitored at timed intervals by Method 3a or b.

Method 3. An accurately measured 25-μl aliquot of the reaction solution was withdrawn with a syringe (or a micropipette could be used) and placed in a small, dry culture tube. Volatiles were removed on a vacuum rack. The residue was dissolved in 25 μl of pyridine containing the internal standard and the solution was treated in one of two ways: (a) 0.5- to 2.0-μl portions were subjected to GC analysis or (b) 25-μl portions of one of BSTFA, TMSIm, TFAIm, TFAA, AcIm or AcAnh reagents were added and the solution was allowed to stand for 10 min. Except for the solutions containing the acyl anhydrides, portions of the solution were analyzed directly by GC. When anhydrides were present, the volatiles were removed *in vacuo*, the residue dissolved in 25 μl of pyridine or THF, and then portions were subjected to GC analysis.

Analysis and calibration

Areas of GC peaks were measured by triangulation and results were related through the internal standard. Relative molar concentrations were calculated by use of molar response factors. These were obtained from a response curve derived by injecting different volumes of one solution containing the compound of interest and the internal standard of known concentrations. Full details will be given in a later publication. In all injections the "solvent wash" technique was used, *i.e.* all the sample was flushed into the chromatograph by a plug of a suitable solvent, separated in the syringe from the sample solution by an air gap.

The co-injection experiments were performed by drawing into the syringe the required volumes of the solutions under test, ensuring that there was an air gap between the plugs of the two solutions. In this way no reaction could occur until the sample had reached the injector port. In the case of studies of hydrolysis in the injector port, 1.0 μl of 80% aqueous acetic acid was co-injected with varying amounts of silylated nucleoside to imitate 1.0-μl portions of the hydrolysis medium in the desilylation experiments.

Instrumental

Gas chromatography was performed on two different instruments. The first was a Hewlett-Packard Model 5711A gas chromatograph equipped with a dual flame ionization detector (FID). For the nucleoside work, we used a 12.3-ft. $\times$ 4-mm-O.D. $\times$ 2.4-mm-I.D. glass column packed with 10% OV-1 on 80–100 mesh Gas-Chrom Q. The column oven was operated isothermally at 280°, with a nitrogen carrier gas flow-rate of 30 ml/min. The off-column injector had a glass liner and was at 250°.

A second column was modified with an effluent splitter between the FID and a collector in the ratio 1:4, so that eluted material could be trapped out in glass capillaries which could then be fitted into the solid probe of the mass spectrometer.

The second gas chromatograph was a Varian Model 1700 with an FID interfaced (Biemann-Watson separator) to a mass spectrometer. The column effluent was split between the FID and the mass spectrometer in the ratio 1:4. Much of the investigative work was performed on the Varian instrument, while quantitative studies were performed on the Hewlett-Packard instrument.

For mass spectrometry, a Finnigan Model 1015 quadrupole instrument was used.

RESULTS AND DISCUSSION

Silylation reactions

Normally, trimethylsilylation reactions are rapid and the compound under investigation is arranged to be completely derivatized before GC analysis is performed. The reaction solution containing the derivatized compound and excess silyl donor is simply injected into the gas chromatograph and the analysis is straightforward. With sterically crowded alkylsilyl groups, derivatization may take several hours. In attempting to determine the rates of derivatization by GC we have found that an accelerated reaction between underivatized substrates and excess silyl donor can occur in the hot injector port, thus leading to errors in quantitation. This effect is not totally unexpected, since injector port trimethylsilylation of substrates has been reported as a fast method of analysis[13,14]. The effect was proven by a "co-injection" of separate solutions of reagent and substrate, being careful to avoid any mixing of solutions until they had entered the injector port. The extent of derivatization was erratic but was in the order of 50 mole % conversion to silylated products. The amounts of 3'-, 5'- and 3',5'-di-O-*tert.*-butyldimethylsilyl-thymidine formed during the injector port reaction were approximately 1:10:1 mole ratio, respectively. We also investigated the possibility of a "ghost effect" occurring in the injector port. The thymidine that was injected in earlier analyses could remain in the injector port and then be derivatized by the excess reagent in successive injections. To test this, we made a series of injections of thymidine alone, followed by silylating reagent alone. There was no production of silylated thymidine derivatives, indicating that either the thymidine is decomposed in the injector port or is slightly volatile and passes through the GC column but gives a very low FID response.

This problem can be removed by chemical methods directed towards deactivating either (i) the excess silyl donor or (ii) the substrates. The method of choice will depend upon the system under investigation and the kind of information required.

(i) Deactivation of excess silyl donor. With this approach, a large excess of methanol was used to quench the excess reagent. An aliquot of the reaction mixture was mixed into a larger volume of methanol and allowed to react for a short time. The methanol reacts with the reagent much faster than does the nucleoside. Direct analysis of this mixture could not be made, however, as it was found that in the injector port hydrolysis of the silylated nucleoside by the excess methanol occurred to a small extent. This was confirmed by co-injection of silylated nucleoside and methanol.

Thus, to obtain a reliable analysis, it was first necessary to remove excess methanol and other volatiles *in vacuo*, redissolve the residue in a suitable solvent (such as pyridine or THF), and then analyze by GC, relating all areas of peaks to that of an internal standard present in the original reaction mixture. Nucleoside derivatives have a very low vapor pressure at room temperature, which allows the use of vacuum to remove volatiles. This method is obviously not suited to very volatile derivatives. In the study of reaction 1 (Fig. 1), this procedure allows a precise analysis for b, c and d. The non-volatile thymidine (a) does not chromatograph.

(ii) Deactivation of substrates. The substrates were deactivated by treatment with a reagent which reacts rapidly with the unreacted substrate hydroxyls to give derivatives which were amenable to GC analysis and prevent the injector port silylation effect. Methods explored for the nucleoside problem included trimethylsilylation, acetylation and trifluoroacetylation. Table I, which gives the retention data for some of the derivatives that we have studied [R = *tert.*-butyldimethylsilyl (TBDMS)] shows that the TMS and acetyl derivatives of b and d do not separate readily on the OV-1 column used. The use of more efficient columns with different stationary phases was not explored in detail though initial experiments were not promising. Trifluoroacetyl derivatives of b and d are readily separable and, interestingly, the retention times are shorter and reversed with respect to the original compounds.

TABLE I

GC DATA FOR THYMIDINE DERIVATIVES

Column: 10% OV-1 on 80–100 Gas-Chrom Q, 12.3-ft. × 4-mm-O.D. × 2.4-mm I.D., glass; column temperature, 280° isothermal; nitrogen carrier gas flow-rate, 30 ml/min.

Compound	*Relative retention time*	$MU_{OV\text{-}1}$ *index*
3′,5′-diAc-dT	1.103	24.65
3′,5′-diTFA-dT	0.411	20.41
3′,5′-diTMS-dT	1.000*	24.17
3′,5′-diTBDMS-dT	2.648	28.38
3′-TBDMS-dT	1.675	26.38
5′-TBDMS-dT	1.791	26.66
3′-TBDMS-5′-TMS-dT	1.743	26.55
5′-TBDMS-3′-TMS-dT	1.743	26.55
3′-TBDMS-5′-Ac-dT	1.861	26.83
5′-TBDMS-3′-Ac-dT	1.865	26.84
3′-TBDMS-5′-TFA-dT	1.091	24.55
5′-TBDMS-3′-TFA-dT	1.012	24.22

* Retention time = 4.16 min.

The necessary requirements for the reagent which deactivates the substrate are that it reacts rapidly to stop the original silylation reaction, gives a reliable 100% derivatization, and gives a single derivative that is suitable for quantitative GC analysis (*i.e.*, linear detector response with no significant decomposition on column). The reagents that we have studied are: BSTFA, TMSIm, TFAA, TFAIm, AcAnh,

and AcIm. In the procedure used, an aliquot of the reaction solution to be monitored was treated with one of these reagents, as described in Experimental. Then the sample was analysed directly, except in the case of the acyl anhydride reagents, for which the GC column is activated by injection of such reagents and their acidic side products. In this case, the reagents and volatiles must be removed *in vacuo* after derivatization is complete and the residue dissolved in a suitable solvent.

For trimethylsilylations the preferred reagent may depend upon the substrate. Both TMSIm and BSTFA react very rapidly with hydroxyls, but the latter reacts also with amines and enols though more slowly and erratically. With thymidine, BSTFA gives a mixture of two derivatives (di- and tri-TMS-dT), depending upon the extent of trimethylsilylation of the base functions. These are incompletely separated by GC, a shoulder (due to tri-TMS-dT) appearing on the trailing edge of the main GC peak. This can lead to problems in quantitation since it is desirable to have a single derivative rather than a mixture. On the other hand, TMSIm gives a single derivative (3′,5′-di-O-TMS-dT) and a well-shaped GC peak. (The identities of the peaks were verified by trapping the eluates and subjecting them to MS analysis.) Similar problems have been previously noted in the silylation of thymidine[15,16].

Trifluoroacetylations are more conveniently performed with TFAIm than with TFAA, since with the former reagent the reaction solution can be directly injected into the GC while, with the latter, the excess anhydride and trifluoroacetic acid must be removed first.

For acetylations, we have found that AcAnh is the only suitable reagent. AcIm reacts too slowly (usually requires heating for 100% reaction) and would therefore not stop the original reaction fast enough. However, in the case of AcAnh, the excess reagent and acetic acid by-product must be removed before analysis.

Overall, for most applications, trimethylsilylation by TMSIm is preferable since it has all the requirements cited above. However, in particular cases such as our studies with nucleosides, trifluoracetylation can be more useful since it allows for analysis of a, b, c, and d, while trimethylsilylation gives only a, (b + d), and c (see Table I).

We have also been studying a series of model substrates to investigate the rates and selectivity of silylation with sterically crowded silyl groups under a variety of reaction conditions. These substrates include representatives of primary, secondary, and tertiary alcohols, primary and secondary amines, and carboxylic acids. For these simple compounds, trimethylsilylation with BSTFA was found to be the fastest and most efficient procedure. This is a very useful approach for the study of such compounds, which are much more volatile than nucleoside derivatives and which would be lost during removal of methanol in procedure i.

Desilylation reactions

A related problem arises in the monitoring of the hydrolysis of b, c and d by 80% aqueous acetic acid. (Such hydrolysis studies are of interest because if the relative stabilities of the various possible alkylsilyl derivatives are known then the versatility of the synthetic procedures can be improved.) Desilylation of these compounds by excess acetic acid occurs in the hot injector port. There is also activation of the column by the acid, interfering with precise quantitative work. These results are not unexpected since it is well known that acidic solutions should not be injected onto columns

being used for analysis of TMS derivatives, and that the hydrolysis reaction is accelerated at higher temperatures.

The effect is substantiated in Fig. 2, which gives results of another "co-injection" experiment designed to closely resemble conditions for direct analysis of the hydrolysis reactions. Compound b (5'-O-*tert.*-butyldimethylsilyl-thymidine) gives a linear response curve with only slight losses in the chromatograph. Co-injection of a fixed amount of acetic acid with variable amounts of b gives erratic results and a response curve that shows that decomposition is occurring in the injector port and/or on the column.

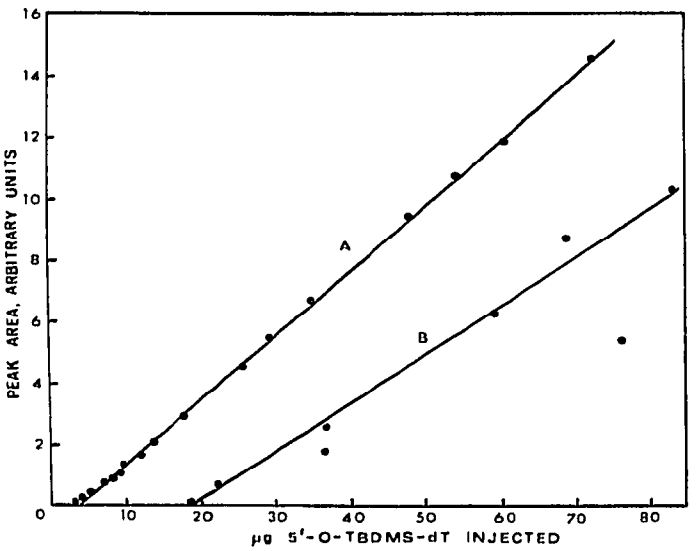

Fig. 2. The effect of the presence of acetic acid upon quantitation of silylated nucleosides by GC. (A) Injection of 5'-O-*tert.*-butyldimethylsilyl-thymidine (b) alone. (B) Co-injection of 5'-O-*tert.*-butyldimethylsilyl-thymidine with 1.0 μl of 80% aqueous acetic acid.

Thus, to obtain an accurate analysis, it was necessary to remove, *in vacuo*, the acetic acid and water from an aliquot of the reaction mixture. The residue could be treated in one of two ways: (a) dissolution in a measured amount of pyridine containing an internal standard, followed by GC for b, c and d or (b) dissolution and reaction in a solvent containing either TMSIm or TFAIm in excess, followed by GC analysis to give a determination for a, b, c and d.

Studies on the formation and hydrolysis of alkylsilyl derivatives of thymidine

The methods described in the previous sections were successfully applied to the determination of the rates of silylation and hydrolysis of alkylsilyl derivatives of deoxynucleosides. Some of the results obtained for *tert.*-butyldimethylsilyl derivatives of thymidine are presented in this section.

Initially, it was necessary to verify the linearity of the GC detector response to the quantity of nucleoside derivative injected and to determine the relative molar response factors. A typical response curve is given by line A, Fig. 2. Although a small

quantity of material appears to be lost between injection and detection, the response is linear and the determinations are reproducible. Reliable analyses can be obtained by using this as a calibration curve. Relative molar response factors are determined from the slopes of the different response curves.

The reaction of thymidine with the reagent *tert.*-butyldimethylsilylimidazole in pyridine solvent was monitored by Method 1 (Experimental), the results being shown in Fig. 3a. Significant concentrations of 3'-O-*tert.*-butyldimethylsilyl-thymidine are never obtained (< 1% by GC analysis) because this can readily react further to give the disilyl compound. In the later stages of the reaction the concentration of c grows at the expense of b (Fig. 1). If a full-scale reaction is being monitored, it can be stopped and worked up at any stage. The maximum analytical yield of b by GC analysis was 92 mole % compared with 91 mole % isolated yield on work-up of the preparative-scale reaction.

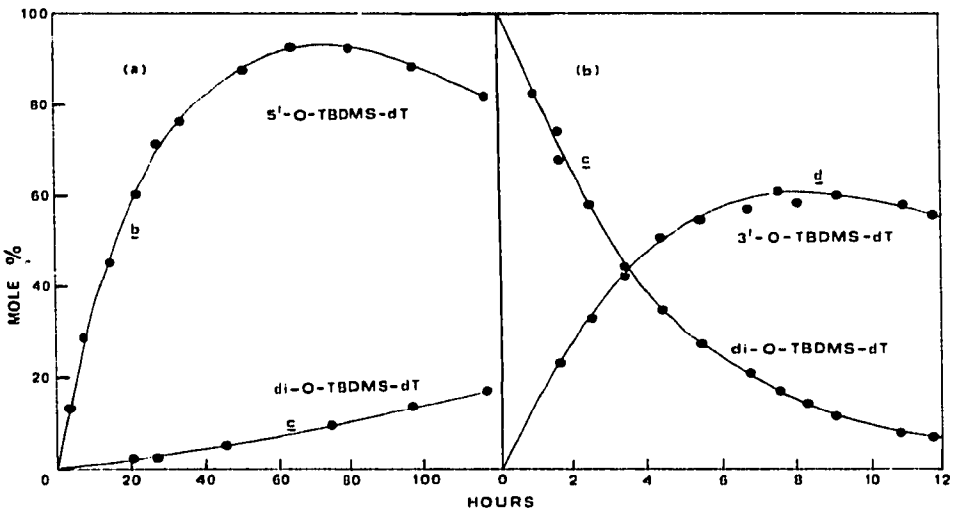

Fig. 3. (a) Reaction of thymidine (32 μmoles, 0.145 *M*) with *tert.*-butyldimethylsilylimidazole (190 μmoles, 0.863 *M*) in pyridine containing internal standard (pyrene, 7 μmoles) at total volume of 220 μl, at room temperature (22°). (b) Hydrolysis of 3',5'-di-O-*tert.*-butyldimethylsilylimidazole-thymidine (0.10 *M*) in 80% aqueous acetic acid at room temperature (22°).

Upon hydrolysis by 80% aqueous acetic acid the 5'-O-silyl group is removed much more readily than the 3'-O-silyl group and Fig. 3b, in which the analytical results were obtained by Method 3a (Experimental), shows that d can readily be obtained from c in good yield (< 5% of b and < 1% of 5'-O-acetyl-3'-O-*tert.*-butyldimethylsilyl-thymidine were also obtained, as determined by gas-phase analysis).

These analytical techniques are also applicable to deoxyadenosine and its silyl derivatives since the analogues of b, c, and d are also amenable to gas-phase analysis[9].

Evaluation of the analytical methods

The relative merits of the analytical procedures for minimizing injector port effects in the study of silylation reactions are summarized in Table II. Some of the

TABLE II

RELATIVE MERITS OF THE EXPERIMENTAL PROCEDURES

*Procedure**	*Advantages*	*Disadvantages*
Method 1: Deactivation of silyl reagent by methanol and evaporation of volatiles.	No concern over 100% derivatization at second step as in Method 2.	(a) Cannot determine underivatized non-volatile substrates. (b) Cannot determine highly volatile substrates and derivatives. (c) Evaporation procedure is inconvenient.
Method 2: Deactivation of substrate by further derivatization by:		
BSTFA, TMSIm, TFAIm, TFAA, AcIm, AcAnh	Can determine substrate.	May not get 100% derivatization at second step.
BSTFA, TMSIm, TFAIm, AcIm	No loss of volatile compounds.	
TFAA, AcAnh		Reagent and acidic side products must be removed; inconvenient and does not allow determination of volatile substrates and derivatives.
TFAIm, TFAA	Separation of 3′- and 5′-O-TBDMS-dT (specific problem for thymidine).	
BSTFA, TMSIm, AcIm, AcAnh		Does not separate 3′- and 5′-O-TBDMS-dT (specific problem for thymidine).
AcIM		Derivatization is slow.

* See Experimental for details.

comments are generally applicable while others relate to specific problems. An additional advantage of the techniques used in this study is that during the monitoring of a reaction, a particular sampling at a certain time can be re-analyzed by GC; there is sufficient sample taken from the reaction and quenched that at least ten analyses could be performed. This will allow determination of the precision of GC analysis of each point. The problem that sometimes arises when a chromatogram is unsatisfactory (*e.g.*, poor injection; too little or too much sample injected; incorrect attenuation setting) is also avoided.

One of the disadvantages (though not a serious one) is that larger samplings are required at each timed analysis. Thus, the reaction has to be kept on a slightly larger scale than would be allowed by direct analysis (if that were possible).

The relative merits of Methods 1 and 2 apply also to Methods 3a and 3b, respectively, for the study of hydrolysis reactions Method 3 is unsuitable for the determination of very volatile compounds that would be lost *in vacuo*.

CONCLUSIONS

Our methods can be generalized to the study of many reactions by GC, in which the presence of an excess reagent or solvent, which will react at an accelerated rate with the compound being analyzed during flash evaporation in the hot injector port of a GC, can lead to serious errors in quantitation.

Undoubtedly, problems similar to ours will arise, and hopefully the techniques we have described here will be of some value. Analysts should examine their analytical methods, possibly by using the co-injection technique we have described.

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REFERENCES

1 A. E. Pierce, *Silylation of Organic Compounds*, Pierce Chemical Company, Rockford, Ill., 1968.
2 A. E. Pierce, *Silylation of Organic Compounds*, Pierce Chemical Company, Rockford, Ill., 1968, Ch. 14, pp. 446–452.
3 G. Stork and P. F. Hudrlik, *J. Amer. Chem. Soc.*, 90 (1968) 4462.
4 E. J. Corey and R. K. Varma, *J. Amer. Chem. Soc.*, 93 (1971) 7319.
5 E. J. Corey and A. Venkateswarlu, *J. Amer. Chem. Soc.*, 94 (1972) 6190.
6 K. K. Ogilvie, *Can. J. Chem.*, 51 (1973) 3799.
7 K. K. Ogilvie, E. A. Thompson, M. A. Quilliam and J. B. Westmore, *Tetrahedron Lett.*, (1974) 2865.
8 K. K. Ogilvie, K. L. Sadana, E. A. Thompson, M. A. Quilliam and J. B. Westmore, *Tetrahedron Lett.*, (1974) 2861.
9 M. A. Quilliam, K. K. Ogilvie and J. B. Westmore, *Biomed. Mass Spectrom.*, 1 (1974) 78.
10 J. T. Watson and B. J. Sweetman, *Org. Mass Spectrom.*, 9 (1974) 39.
11 M. A. Quilliam, K. K. Ogilvie and J. B. Westmore, unpublished results.
12 L. H. Sommer and L. J. Taylor, *J. Amer. Chem. Soc.*, 76 (1954) 1030.
13 G. G. Esposito, *Anal. Chem.*, 40 (1968) 1902.
14 P. M. Wiese and R. H. Hanson, *Anal. Chem.*, 44 (1972) 2393.
15 C. W. Gehrke, D. L. Stalling and C. D. Ruyle, *Biochem. Biophys. Res. Commun.*, 28 (1967) 869.
16 C. W. Gehrke and C. D. Ruyle, *J. Chromatogr.*, 38 (1968) 473.