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STUDY OF REARRANGEMENT REACTIONS OCCURRING DURING GAS CHROMATOGRAPHY OF *tert.*-BUTYLDIMETHYLSILYL ETHER DERIVATIVES OF URIDINE

MICHAEL A. QUILLIAM*, KELVIN K. OGILVIE**, KRISHAN L. SADANA*** and JOHN B. WESTMORE*

Department of Chemistry, University of Manitoba, Winnipeg, Manitoba R3T 2N2 (Canada)

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SUMMARY

The gas chromatography of partial O-*tert.*-butyldimethylsilyl (TBDMS) derivatives of uridine is complicated by the occurrence of a 2′ ↔ 3′ rearrangement of the TBDMS group when uridine derivatives having a 2′- or 3′-O-TBDMS group and an underivatized OH group in the 3′- or 2′-position are injected into a gas chromatograph. When a 5′-O-TBDMS group is also present these rearrangements are accompanied by a thermal decomposition thought to involve elimination of methane. Further derivatization by acetylation or trimethylsilylation (but not trifluoroacetylation), both of which prevent the rearrangement and decomposition reactions, together with gas chromatography–mass spectrometry selected ion recording techniques, is suggested as an analytical procedure for the synthetically useful partial TBDMS derivatives of ribonucleosides.

INTRODUCTION

In recent years there has been increasing use of bulky silyl groups for the protection of hydroxyl functions in a variety of compounds, both from the point of view of organic synthesis and for analytical methods based on chromatography and/or mass spectrometry (MS). Perhaps the most widely used of these groups has been the *tert.*-butyldimethylsilyl (TBDMS) group, which produces ether derivatives which retain most of the desirable features of the trimethylsilyl (TMS) ethers for gas chromatography (GC), but are much more stable to hydrolytic conditions and are preferable for the various types of liquid chromatography, and for combined techniques such as high-performance liquid chromatography (HPLC)–MS.

* Present address: Department of Chemistry, McMaster University, Hamilton, Ontario L8S 4M1, Canada.

** Present address: Department of Chemistry, McGill University, Montreal, Quebec H3C 3G1, Canada.

*** Present address: Department of Microbiology, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada.

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During studies on the synthesis of oligonucleotides[1–7] it was necessary to find methods for producing, in high yield, selectively protected nucleoside monomers. Protection of hydroxyls as "sterically crowded" trialkylsilyl ethers presented an attractive solution to the synthetic problems. To aid in optimizing reaction conditions for preparation of the protected monomers methods have been developed for rapid, sensitive and precise analysis of reaction products. The problems involved, and recommended solutions, in the quantitative analysis of the deoxynucleoside thymidine by GC have been reported previously[8]. In this paper we describe studies on the analysis of partial O-TBDMS ether derivatives of the ribonucleoside uridine by GC. These analyses present difficulties of a different kind, the resolution of which forms an interesting analytical problem.

EXPERIMENTAL

Reagents

tert.-Butylmethyldichlorosilane (tBuMeSiCl$_2$) was prepared by reacting equimolar amounts of *tert.*-butyllithium (Alpha Products, Ventron, Danvers, MA, U.S.A.) under nitrogen and methyltrichlorosilane (PCR, Gainesville, FL, U.S.A.) dissolved in pentane. After filtration, and evaporation of pentane, the white solid product was used without further purification. (Mass spectrum: m/z 170/172/174, M^+; m/z 155/157/157, $(M - CH_3)^+$; m/z 135/137, $(M - Cl)^+$; m/z 113/115/117, $(M - t\text{Bu})^+$.) Tri-Sil-Z (1.2 *M* trimethylsilylimidazole in pyridine) and trifluoroacetylimidazole (TFAIm) were purchased from Pierce (Rockford, IL, U.S.A.). Reagent-grade acetic anhydride was distilled from phthalic anhydride and stored in the dark. Reagent-grade pyridine was distilled from *p*-toluenesulfonylchloride, redistilled from calcium hydride, and stored over Linde molecular sieve 3A. Reagent grade N,N-dimethylformamide (DMF) was refluxed over calcium hydride, followed by distillation and storage 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. Triethylamine was distilled from calcium hydride and stored over molecular sieve. *n*-Alkanes were purchased from Applied Science Labs. (State College, PA, U.S.A.).

Nucleoside derivatives

Uridine was purchased from Sigma (St. Louis, MO, U.S.A.). Full details of the preparation, isolation and characterization of TBDMS ether derivatives of ribonucleosides have been reported previously[4,6,7]. In brief, partial TBDMS derivatives were prepared by reacting uridine with the silylating reagent (TBDMSCl, 1 *M*, and imidazole, 2 *M*, in DMF) in slightly more than the stoichiometric amount and isolating and purifying the products. Excess silylating reagent was used to prepare Tris-O-TBDMS-U. When necessary, a 5'-O-TBDMS group could be removed selectively with 80% acetic acid. All products were characterized by elemental analysis, and, in the case of isomeric compounds by chemical conversion to known compounds. For example, acetylation of 2',3'-bis-O-TBDMS-U followed by desilylation with tetra-*n*-butylammonium fluoride in THF yields 5'-O-acetyl-U.

Acetylation of partial O-TBDMS derivatives of uridine was accomplished with acetic anhydride–pyridine (1:2, v/v), and trifluoroacetylation with 1.5 *M*

TFAIm in pyridine. Reactions were complete within 30 min at room temperature as confirmed by thin-layer chromatography (TLC) and/or GC analysis (prior to GC analysis, excess reagent and side products were evaporated *in vacuo*, and the residue was dissolved in dichloromethane for injection into the gas chromatograph). Trimethylsilylations were complete within 10 min using Tri-Sil-Z. Under the foregoing conditions, derivatization of the nucleobase moiety was insignificant, as verified by GC and MS. Such derivatization, accomplished under more vigorous conditions, was readily detected by these techniques.

5'-O-TBDMS-2',3'-*tert.*-butylmethylsilyldioxy-U was prepared by reacting 5'-O-TBDMS-U with *tert.*-BuMeSiCl$_2$ (1 *M* in THF–triethylamine, 5:1, v/v) for 1 h at room temperature, and used for GC and MS.

Chromatography

TLC was performed on strips of Eastman Chromagram sheets (No. 6060, silica gel with fluorescent indicator) with a solvent migration distance of 10 cm using diethyl ether (Fisher) as development solvent.

GC was performed on a Hewlett-Packard Model 5711A gas chromatograph equipped with a dual flame-ionization detector. The off column injectors and the detectors had glass inserts and were at 250 and 300°C, respectively. Column A (glass, 1 m × 2 mm I.D. × 6 mm O.D.) and B (glass, 3.75 m × 2.4 mm I.D. × 6 mm O.D.) were packed with 10% OV-1 on 80–100 mesh Gas-Chrom Q (Applied Science Labs.). Nitrogen carrier gas flow-rates were 30 ml/min and the column oven was operated isothermally. Column A was fitted with an effluent splitter between the detector and collector in the ratio 1:4, so that eluted material could be trapped in glass capillaries which could then be fitted into the solid probe of the mass spectrometer. The combined GC–MS technique was not used for the results described here because a Biemann-Watson glass frit separator installed in our GC–MS instrument at the time of these experiments proved unsuitable for any type of nucleoside derivative. Our recent experiments have demonstrated that analysis can be performed by GC–MS with a glass jet separator interface. Kováts' isothermal retention indices[9] were determined by mathematical interpolation of a plot of log of adjusted retention time *vs.* carbon number for *n*-alkanes at 230°C, and have a precision of ± 5 units.

Mass spectrometry

A Finnigan Model 1015 quadrupole mass spectrometer operating at an electron energy of 70 eV was used to record mass spectra. Samples were introduced through the solid probe inlet. The instrument was carefully tuned for unit resolution and optimum sensitivity at high mass. The spectrum shown in Fig. 4 has been corrected for quadrupole mass filter discrimination[10] as described previously[11].

RESULTS AND DISCUSSION

Gas chromatography of TBDMS ether derivatives

The GC behavior of mono-TBDMS ether derivatives of uridine on OV-1 columns is shown in Fig. 1 (column A). Injection of pure 5'-O-TBDMS-U yielded a single GC peak. Injection of pure 2'-O-TBDMS-U or 3'-O-TBDMS-U yielded essentially identical chromatograms with two peaks in each. The GC behavior of

bis-TBDMS ether derivatives is illustrated in Fig. 2. Injection of 2',3'bis-O-TBDMS-U yielded a single GC peak. Injection of pure 2',5-bis-O-TBDMS-U or 3',5'-bis-O-TBDMS-U gave different chromatograms which contained three peaks with the same retention times. When the longer column B was used, with a consequently higher column temperature, the peaks became broader and less well resolved in each case. It was established, by the evidence given below, that these effects are due to a thermally induced intramolecular rearrangement of a silyl group between the 2' and 3' positions, together with, for 2',5'-bis-O-TBDMS-U and 3',5'-bis-O-TBDMS-U, partial decomposition to 5'-O-TBDMS-2',3'-*tert.*-butylmethylsilyldioxy-uridine (presumably by elimination of methane). Fig. 3 illustrates the reactions proposed for the bis-TBDMS derivatives. The rearrangements occur primarily in the

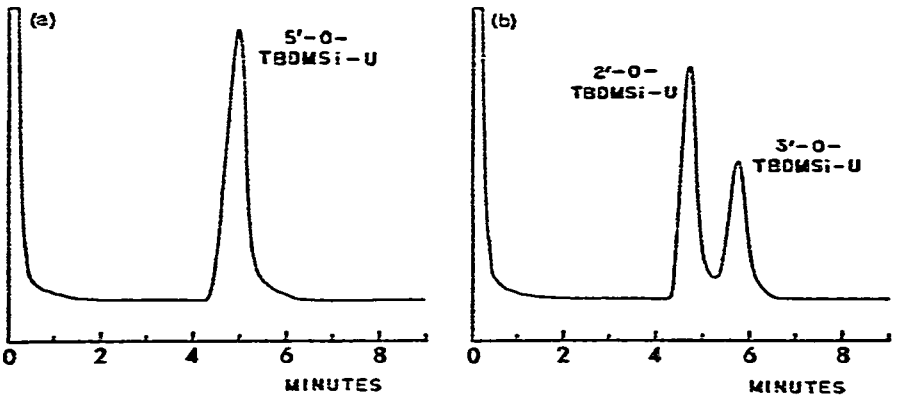

Fig. 1. Gas chromatograms of mono-TBDMS derivatives of uridine: (a) injection of pure 5'-O-TBDMS-U; (b) injection of pure 2'-O-TBDMS-U or pure 3'-O-TBDMS-U (in each an injector port rearrangement gave an additional peak corresponding to the other isomer, resulting in essentially identical chromatograms). Conditions: column A, 10% OV-1, 1 m × 2 mm I.D. × 6 mm O.D.; temperature, 230°C; flow-rate 30 ml/min nitrogen carrier gas.

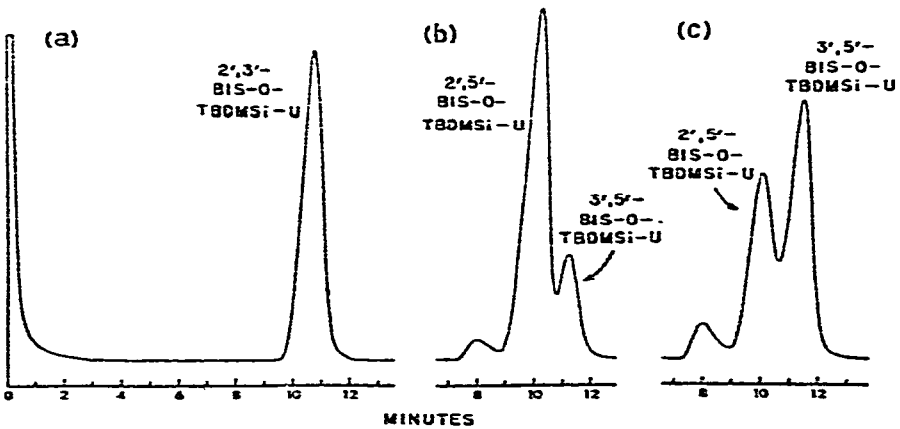

Fig. 2. Gas chromatograms of bis-O-TBDMS-uridine derivatives: (a) injection of pure 2',3'-bis-O-TBDMS-U; (b) injection of pure 2',5'-bis-O-TBDMS-U; (c) injection of pure 3',5'-bis-O-TBDMS-U. Conditions as for Fig. 1.

hot injector port (250°C), but are also induced during elution by higher column temperatures. The latter results in a broadening and fusing of chromatographic peaks. It is interesting to note that the degree of rearrangement is different for the two isomers. Probably, steric repulsion between 5′- and 3′-O-TBDMS groups would mean that 3′,5′-bis-O-TBDMS-U would be formed less readily than 2′,5′-bis-O-TBDMS-U. The slow 2′ ↔ 3′ rearrangement of silyl groups has been observed on silica gel TLC plates, in DMF (especially when imidazole is present), and in wet pyridine, all at room temperature[6].

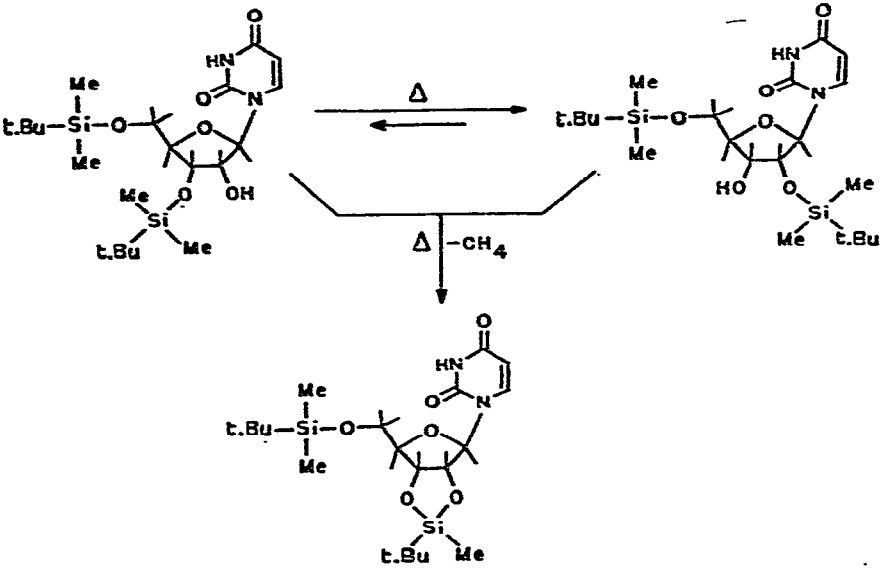

Fig. 3. Rearrangement and partial decomposition of 2′,5′- and 3′,5′-bis-O-TBDMS uridines in the gas chromatograph.

Several pieces of evidence support the foregoing conclusions:

(1) GC eluates corresponding to individual peaks were trapped in glass capillaries, dissolved in dichloromethane, and reinjected into the GC under conditions identical to those used to obtain the original chromatograms. Both the first and second eluates (Fig. 1b) from injection of 2′- or 3′-O-TBDMS-U, when reinjected, gave chromatograms identical to Fig. 1b. The reinjection of the first eluate (Fig. 2b or 2c) resulting from the original injection of 2′,5′- or 3′,5′-bis-O-TBDMS-U gave a single peak at the same retention time as the first peak of Fig. 2b or 2c. Reinjection of the second and third eluates (Figs. 2b and 2c) gave chromatograms identical to those in Figs. 2b and 2c, respectively. These results confirm that rearrangement is actually occurring rather than that the starting compounds were impure. Under similar GC conditions rearrangement of 5′-O-TBDMS-U or 2′,3′-bis-O-TBDMS-U was not detected. Reinjection of the single eluates resulting from injection of these compounds (Figs. 1a and 2a) gave chromatograms identical to Fig. 1a and 2a, respectively.

(2) Collected GC eluates corresponding to individual peaks were studied by TLC. The first eluate from injection of 2′-O-TBDMS-U (Fig. 1b) had the same

R_F value as authentic 2'-O-TBDMS-U, while the R_F value of the second eluate corresponded to authentic 3'-O-TBDMS-U. The second GC eluate from injection of 2',5'- or 3',5'-bis-O-TBDMS-U (Fig. 2b or 2c) had an R_F value corresponding to authentic 2',5'-bis-O-TBDMS-U, while the third GC eluate corresponded to authentic 3',5'-bis-O-TBDMS-U.

(3) Mass spectra of all the possible TBDMS ether derivatives of uridine are characteristically different[12]. Mass spectra of collected GC eluates, when compared with authentic derivatives, confirm the assignments given in (2) above, as well as confirming the non-rearrangement of 5'-O-TBDMS-U (Fig. 1a) and 2',3'-bis-O-TBDMS-U (Fig. 2a).

(4) The mass spectrum of the first eluate from injection of 2',5'- or 3',5'-bis-O-TBDMS-U corresponded to a compound with molecular weight 16 less than the injected compounds, and with mass spectral features (Fig. 4) consistent with 5'-O-TBDMS-2',3'-*tert.*-butylmethylsilyldioxy-uridine. This was confirmed independently since its mass spectrum was identical to that of an authentic sample synthesized by reacting 5'-O-TBDMS-U with tBuMeSiCl$_2$.

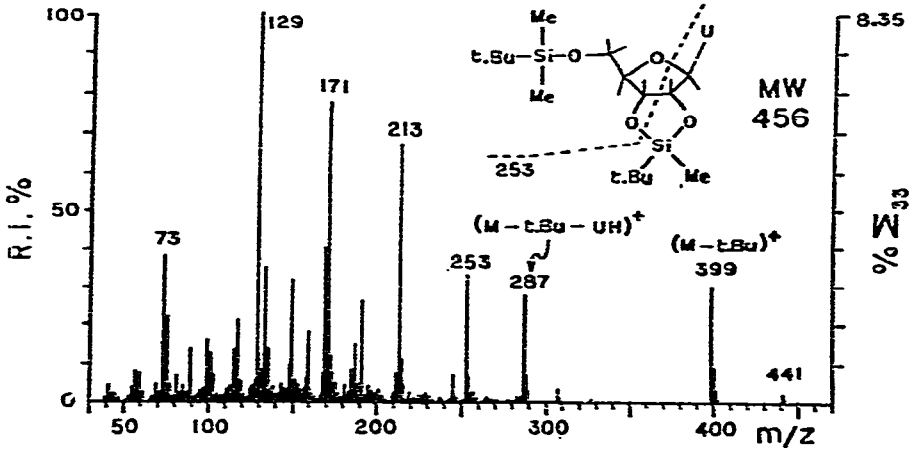

Fig. 4. Mass spectrum of 5'-O-TBDMS-2',3'-*tert.*-butylmethylsilyldioxy-uridine (same as the mass spectrum of the first eluate in the chromatograms of Figs. 2b and c). Me = Methyl; t.Bu = *tert.*-butyl.

(5) Trapped GC eluates corresponding to individual GC peaks were subjected to micro-scale acetylations. The mass spectra and TLC R_F values of the products of the acetylations were compared with those from authentic samples and confirm the assignments given above.

Prevention of thermal rearrangements

The decomposition and rearrangements just described are undesirable for the gas phase analysis of partial O-TBDMS derivatives of ribonucleosides. Since variations of instrumental conditions did not produce a solution to this problem, the formation of fully protected derivatives by acetylation, trifluoroacetylation, and trimethylsilylation was investigated.

Acetylation, using acetic anhydride, gave, in quantitative yield (without acetylation of the nucleobase), mixed acetyl–TBDMS derivatives. These derivatives were

TABLE I

KOVÁTS' ISOTHERMAL RETENTION INDICES[a] FOR DERIVATIVES OF O-TBDMS URIDINES, ON OV-1 AT 230°C

Substrate	*Derivative*		
	bis-O-acetyl	*bis-O-TFA*	*bis-O-TMS*
2'-O-TBDMS-U	2689	2272	2669
3'-O-TBDMS-U	2718	decomp.	2660
5'-O-TBDMS-U	2706	decomp.	2682
	mono-O-acetyl	*mono-O-TFA*	*mono-O-TMS*
2',3'-bis-O-TBDMS-U	2905	2688	2879
2',5'-bis-O-TBDMS-U	2905	2690	2899
3',5'-bis-O-TBDMS-U	2919	decomp.	2891

stable during isolation by TLC, had characteristic mass spectra, and gave single symmetrical GC peaks. By methods described above, it was established that no detectable decomposition or rearrangement occurred in the gas chromatograph at temperatures up to 280°C. Table I reports the retention indices for the various derivatives examined. Some of the isomers can be separated as acetyl derivatives by using high efficiency columns.

Unexpectedly, trifluoroacetylation was found to be an unsuitable method. Mixed trifluoroacetyl–TBDMS derivatives of uridine with a 2'-O-trifluoroacetyl group decomposed during GC analysis although derivatives of the other isomers behaved well.

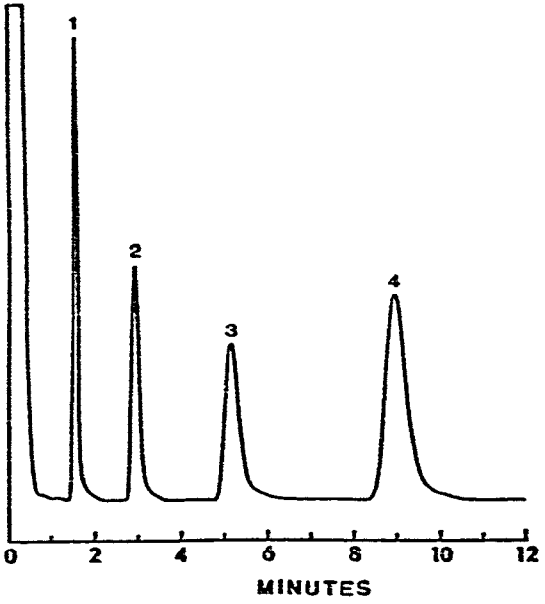

Fig. 5. Gas chromatogram of a mixture of TMS–TBDMS derivatives of uridine: Peak 1 = tris-O-TMS-U; peak 2 = bis-O-TMS-mono-O-TBDMS uridines (3 isomers); peak 3 = mono-O-TMS-bis-O-TBDMS uridines (3 isomers); peak 4 = tris-O-TBDMS-U. Conditions as for Fig. 1 except temperature = 250°C).

Trimethylsilylation of the partial O-TBDMS derivatives of uridine produced mixed TMS–TBDMS derivatives which, individually, had excellent GC properties. Unfortunately, separation of isomeric derivatives requires very efficient columns. Fig. 5 shows a chromatogram of a trimethylsilylated mixture of uridine and its mono-, bis- and tris-TBDMS derivatives. Analysis of such a mixture requires either a much more efficient column, or GC–MS using selected ion recording techniques. These derivatives are well suited to this latter method since all derivatives have characteristic mass spectra[12].

One potential concern in the above derivatization methods is the possibility of 2′ ↔ 3′ rearrangement of the TBDMS group during further derivatization. However, it was readily established by TLC, GC and MS methods that such rearrangements did not occur during acetylation and trimethylsilylation of partial TBDMS derivatives of uridine.

CONCLUSIONS

Provided that suitable instrumentation is available, preparation of acetyl or TMS derivatives should provide a method for the analysis of synthetically useful partial TBDMS derivatives of ribonucleosides. Only the TBDMS derivatives of uridine have been examined in detail, but their behavior should be representative for this entire class of compound.

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