GAS CHROMATOGRAPHIC RETENTION DATA FOR SILYL AND ACYL DERIVATIVES OF NUCLEOSIDES

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(Received April 8th, 1980)

SUMMARY

Kováts' isothermal retention indices on OV-1 columns are reported for several trimethylsilyl, *tert.*-butyldimethylsilyl, cyclotetramethylene-isopropylsilyl, cyclotetra-methylene-*tert.*-butylsilyl, acetyl and trifluoroacetyl derivatives of thymidine, uridine and 2'-deoxyadenosine, together with a few values for derivatives of 2'-deoxyuridine and adenosine. Retention increments for conversion of O-trimethylsilyl functions to other functions were found to be reproducible in most cases. Positional differences in the retention increments were observed for conversion to OH in the cases of thymidine, 2'-deoxyuridine and 2'-deoxyadenosine, or to O-trifluoroacetyl groups in the case of thymidine. Positional differences in the increments were also observed in the case of uridine, but were quite variable when unprotected OH groups were present. Uridine derivatives having a 2'-O-trifluoroacetyl group decomposed during gas chromatography. The ability of OV-1 columns to separate isomeric derivatives is also discussed.

INTRODUCTION

During studies on the synthesis of oligonucleotides it was necessary to find methods for producing, in high yield, selectively protected nucleoside monomers[1-7]. Preparation of silyl ethers formed from "sterically crowded trialkylsilyl" groups presented an attractive solution to the synthetic problems. The most useful of these groups were the *tert.*-butyldimethylsilyl (TBDMS), cyclotetramethylene-isopropylsilyl (TMIPS) and cyclotetramethylene-*tert.*-butylsilyl (TMTBS) groups. The first of these has been extensively used for synthetic and analytical purposes, and reagent formulations are available commercially.

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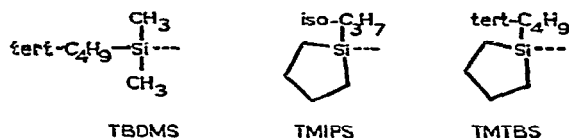
To aid in optimizing reaction conditions for preparation of protected nucleoside monomers, methods were developed for rapid, sensitive and precise analysis of reaction products. These methods were based on gas chromatography, with identity confirmation by mass spectrometry (MS). The silyl ethers formed from the TBDMS, TMIPS and TMTBS groups retain most of the desirable features of the trimethylsilyl (TMS) ethers, but are much more stable to hydrolytic conditions. This latter property is desirable if prior separations by liquid chromatography are necessary. A disadvantage of the use of the bulky silyl groups for gas chromatography (GC) is the inconveniently long retention times or high column temperatures resulting when several silyl groups are incorporated into the molecule.

During quantitative analysis of nucleosides and their partial TBDMS, TMIPS or TMTBS derivatives in reaction mixtures by gas chromatography some complications were encountered. In the case of the 2'-deoxynucleoside thymidine, injector port reactions were observed[8], while a 2' ↔ 3' rearrangement of the TBDMS group was observed during the gas chromatography of uridine derivatives having a 2'- or 3'-O-TBDMS group and an unprotected OH group in the 3'- or 2'-position[9]. Further derivatization by trimethylsilylation or acylation was recommended as a solution to such problems.

In this paper we report gas chromatographic retention data for the silyl and acyl derivatives of ribonucleosides and 2'-deoxynucleosides. The majority of the data apply to derivatives of thymidine and uridine, for which the synthetic work is most advanced. It is hoped that the regularities observed for these compounds can be extended to other nucleosides and can be used as a preliminary diagnostic test of the identity of compounds giving rise to specific GC peaks.
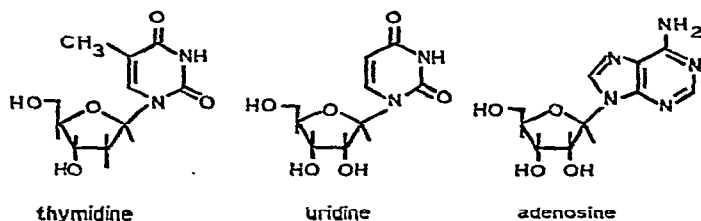
EXPERIMENTAL

Nucleoside derivatives

Nucleosides were purchased from Sigma (St. Louis, MO, U.S.A.). Full details of the preparation, isolation and characterization of TBDMS, TMIPS and TMTBS ether derivatives have been reported previously[4,6,7]. In brief, partial TBDMS, TMIPS or TMTBS derivatives were prepared by reacting a certain nucleoside with the silylating reagent (TBDMS-Cl, TMIPS-Cl, or TMTBS-Cl, 1 *M*, and imidazole, 2 *M*, in dimethylformamide) in slightly more than the stoichiometric amount, then isolating

and purifying the products. Excess silylating reagent was used to prepare fully silylated derivatives. When necessary, a 5'-O-silyl group could be removed selectively with 80% aqueous acetic acid. All products were characterized by elemental analysis, and, in the case of isomeric compounds, by chemical conversion into known compounds. For example, acetylation of 2',3'-bis-O-TBDMS-uridine followed by desilylation with tetra-*n*-butylammonium fluoride in THF yields 5'-O-acetyluridine.

Trimethylsilylation of partial O-TBDMS, O-TMIPS, or O-TMTBS derivatives was accomplished with Tri-Sil-Z (1.2 M trimethylsilylimidazole in pyridine; Pierce, Rockford, IL, U.S.A.). At room temperature, reactions were complete within 10 min. Under these conditions derivatization of the nucleobase moieties was insignificant, as verified by GC and MS. Trimethylsilylation of the uracil nucleobase was achieved by heating the nucleoside with neat bis(trimethylsilyl)trifluoroacetamide (BSTFA; Pierce) at 90°C for 2 h. Derivatization of the adenine nucleobase was accomplished by heating the nucleoside with BSTFA in pyridine (5:1) at 90°C for 1 h. Acetylations were accomplished with acetic anhydride–pyridine (1:2), and trifluoroacetylations with 1.5 M trifluoroacetylimidazole (Pierce) in pyridine. Reactions were complete within 30 min at room temperature. (Under these conditions trifluoroacetylation of the adenine nucleobase occurs.) Prior to GC, excess reagent and side-products were removed by evaporation *in vacuo*, and the residue was dissolved in dichloromethane for injection into the gas chromatograph.

Gas chromatography

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°C and 300°C, respectively. Columns 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., State College, PA, U.S.A.). Nitrogen carrier gas flow-rates were 30 ml/min and the column oven was operated isothermally. Kováts' isothermal retention indices[10] were determined on column A by mathematical interpolation of a plot of log of adjusted retention time *vs.* carbon number for *n*-alkanes (purchased from Applied Science Labs.) and have an estimated precision of ± 5 units.

RESULTS AND DISCUSSION

A non-polar stationary phase, such as OV-1, appeared to be the most suited for GC of nucleoside derivatives. Although other liquid phases were not examined in detail, initial experiments with partial O-TBDMS derivatives using the more polar stationary phase OV-17 did not appear promising. Thus, 3'- and 5'-O-TBDMS-thymidine did not chromatograph under any conditions studied. Kováts' isothermal retention indices[10], using an OV-1 stationary phase, for partial, mixed and full acyl-silyl derivatives of some 2'-deoxynucleosides are presented in Table I, and for some ribonucleosides in Table II.

Effect of column temperature

Most of the results reported in Tables I and II were obtained at a column temperature of 230°C which, in most cases, gave convenient retention times under

TABLE I

KOVÁTS' ISOTHERMAL RETENTION INDICES* FOR DERIVATIVES OF DEOXYNUCLEOSIDES

Nucleoside	3'-O-subst.	5'-O-substituent						
		H	TMS	TBDMS	TMIPS	TMTBS	Acetyl	TFA
Thymidine	H		2382	2621	2876	2909		
	TMS	2587		2621	2858	2892	2644	2437
	TBDMS		2616	2837, 2866[260]	3107[260]	3146[460]	2897	2681
	TMIPS	2851	2870	3114[260]	3356[460]	3396[460]	2917	2710
	TMTBS	2872	2881	3140[260]	3386[460]	3416[460]		
	Acetyl			2646	2892	2928	2439	2032[205]
	TFA			2403	2649	2684		2053
2'-Deoxyuridine	H		2336	2584				
	TMS							
	TBDMS	2558						
2'-Deoxyadenosine	H		2496	2739				
	TMS		2734	2737				
	TBDMS	2731		2799, 2826[260]				
	TMIPS			2954	3520[290]			
	TMTBS			2985[260]		3594[490]		
	Acetyl						2798	
N6-TMS-2'-deoxyadenosine	TMS		2607					
	TBDMS		2841	2795				
N6-TBDMS-2'-deoxyadenosine	TBDMS			2832, 3076[260]				
N6-TMIPS-2'-deoxyadenosine	TMIPS			3205	4070[290]			
N6-TMTBS-2'-deoxyadenosine	TBDMS			3278[260]				
	TMTBS			3598[260]		4183[390]		
N6-Acetyl-2'-deoxyadenosine	TBDMS			3058				
	Acetyl						3079	
N6-TFA-2'-deoxyadenosine	TFA			2574				2666
2'-Deoxycytidine	H	decomp.						
	TBDMS			decomp.				
2'-Deoxyguanosine	H	decomp.						
	TBDMS			decomp.				

* 10% OV-1, at 230°C (unless otherwise indicated by superscript).

TABLE II

KOVÁTS' ISOTHERMAL RETENTION INDICES* FOR DERIVATIVES OF RIBONUCLEOSIDES

Nucleoside	2'-O-subst.	3'-O-subst.	5'-O-substituent						
			H	TMS	TBDMS	TMIPS	TMTBS	Acetyl	TFA
Uridine	H	H	2628**		2576				
	H	TBDMS			2884**				
	TMS	TMS		2444	2682				
	TMS	TBDMS		2660	2891				
	TBDMS	H	2559**		2843**				
	TBDMS	TMS		2669	2899				
	TBDMS	TBDMS	2866	2879	3102 3128^{260}				
	TBDMS	Acetyl			2905			2905	2688
	TBDMS	TFA			2690			2689	2272
	TMIPS	TMIPS				3815^{490}			
	TMTBS	TMTBS					3916^{190}		
	Acetyl	TBDMS			2919			2718	
	Acetyl	Acetyl			2706			2509	
	TFA	TBDMS			decomp.				decomp.
	TFA	TFA			decomp.				decomp.
(O or N)-TMS-uridine	TMS	TMS		2449					
5-Methyluridine	TMS	TMS		2481					
	TBDMS	TBDMS			3133^{260}				
Adenosine	TMS	TMS		2573					
	TBDMS	TBDMS			3203 3237^{260} 3273^{290}				
N6-TMS-adenosine	TMS	TMS		2647					
N6-TBDMS-adenosine	TBDMS	TBDMS			3274^{260} 3475^{260} 3504^{290}				
N6-TMTBS-adenosine	TBDMS	TBDMS			3811^{290}				

* 10% OV-1, at 230° (unless otherwise indicated by superscript).

** Undergoes 2' ↔ 3' rearrangement (ref. 9).

TABLE III

RETENTION INCREMENTS FOR REPLACEMENT OF 3'- AND 5'-O-TBDMS SUBSTITUENTS OF DEOXYNUCLEOSIDES BY OTHER SUBSTITUENTS

Nucleoside	3'-O-subst.	5'-O-TBDMS → 5'-O-					
		H	TMS	TMIPS	TMTBS	Acetyl	TFA
Thymidine	H			255	288		
	TMS			237	271		
	TBDMS	−250	−239	241	280	−193	−400
	TMIPS	−233	−221	242	282	−187	−403
	TMTBS	−238	−214	246	276	−193	−400
	Acetyl		−229	246	282	−207	
	TFA			246	281		−350*
2'-Deoxyuridine	TBDMS	−241					
2'-Deoxyadenosine	TMS	−223	−220			−156*	
	TBDMS		−238				
N6-TMS-2'-deoxyadenosine	TMS		−225				
	TBDMS		−205				
Mean values ± standard deviation		−237 ± 10	−224 ± 12	245 ± 6	280 ± 6	−195 ± 9	−401 ± 2

Nucleoside	5'-O-subst.	3'-O-TBDMS → 3'-O-					
		H	TMS	TMIPS	TMTBS	Acetyl	TFA
Thymidine	H			264	285		
	TMS			254	265		
	TBDMS	−216	−234	248	274	−191	−434
	TMIPS	−201	−216	249	279	−185	−428
	TMTBS	−207	−219	250	270	−188	−432
	Acetyl		−224	253	273	−205	
	TFA			244	273		−384*
2'-Deoxyuridine	TBDMS	−215					
2'-Deoxyadenosine	TMS	−215	−241			−159*	
	TBDMS		−215				
N6-TMS-2'-deoxyadenosine	TMS		−234				
	TBDMS		−214				
Mean value ± standard deviation		−211 ± 7	−225 ± 11	252 ± 7	274 ± 7	−192 ± 9	−431 ± 3

* Not used in calculation of mean because the deviation was more than four times the mean deviation.

the conditions employed. For the nucleosides more highly substituted with the heavier silyl groups higher temperatures were necessary to achieve convenient retention times.

For a number of bis- and tris-TBDMS derivatives, Kováts' indices were measured at more than one temperature. Between 230 and 260°C, increases in Kováts' index of 31, 27, 31, 26 and 34 were observed for TBDMS derivatives of thymidine, 2'-deoxyuridine, 2'-deoxyadenosine, uridine and adenosine, respectively, yielding a mean value of 29.8 ± 3.3. Similarly, between 260 and 290°C the increase was 36 for 2',3',5'-tris-O-TBDMS-adenosine, and 29 for N6-TBDMS-2',3'5'-tris-O-TBDMS-adenosine. These observations were used to apply temperature corrections, when necessary, to retention indices measured at temperatures other than 230°C. As demonstrated below, these corrections apply well to the particular silyl and acyl derivatives studied here.

Retention increments for change of substituents

The results in Tables I and II were used to investigate the consistency (or otherwise) of the contribution of a substituent in a specific location in a nucleoside derivative to the total retention index. In Tables III and IV are presented retention increments for replacement of a TBDMS substituent in a molecule by another group, while keeping all other substituents the same. (TBDMS was chosen as the reference substituent so that the greatest number of retention increments could be calculated from the data in Tables I and II.) When possible, retention increments were determined at corresponding temperatures. Otherwise, an empirical correction of +30 units per 30°C was applied to values of Kováts' indices measured at temperatures other than 230°C (see above) before retention increments were determined. The upper part of Table III gives values for the replacement of a 5'-O-TBDMS substituent of 2'-deoxynucleosides by other groups. The consistency of the increments for a given type of replacement is encouraging. Accordingly, the mean values (rounded to integral values) and standard deviations (increased to next integral value) are also tabulated. The lower part of Table III gives results for similar replacements of 3'-O-TBDMS substituents of deoxynucleosides. Only in the following cases do the retention increments differ by more than two standard deviations from the mean values: (a) replacement of the 3'- or 5'-O-TBDMS group of 2'-deoxyadenosine by an acetyl group; (b) replacement of the second TBDMS group (3' or 5') of 3',5'-bis-O-TBDMS-thymidine by a trifluoroacetyl group.

The results in Table IV, calculated from those in Table II, give, in the same way, retention increments for replacement of 5'-, 3'- or 2'-O-TBDMS groups by other substituents. The increments for conversion into OH groups were very variable and, because of the high uncertainty and small number of experimental values, do not lead to a meaningful average. Otherwise, the standard deviations for the other conversions are small and all values in Table IV lie within two standard deviations of the mean.

The mean values shown in Tables III and IV were then used to calculate increments for conversion of O-TMS groups into other groups. The results are shown in Table V. The O-TMS substituent, rather than the unprotected OH group, was chosen as the new reference substituent because the TMS derivatives are perhaps the most common volatile nucleoside derivatives used for GC. Consequently, measured

TABLE IV

RETENTION INCREMENTS FOR REPLACEMENT OF 2'-, 3'- AND 5'-O-TBDMS SUB-
STITUENTS OF URIDINE BY OTHER SUBSTITUENTS

2'-O-subst.	3'-O-subst.	5'-O-TBDMS → 5'-O-			
		H	TMS	Acetyl	TFA
H	TBDMS	−256			
TMS	TMS		−238		
TMS	TBDMS		−231		
TBDMS	H	−284			
TBDMS	TMS		−230		
TBDMS	TBDMS	−236	−223	−197	−414
TBDMS	Acetyl			−216	
TBDMS	TFA				−418 .
Acetyl	TBDMS			−201	
Acetyl	Acetyl			−197	
Mean ± std. deviation		−259 ± 25	−231 ± 7	−203 ± 9	−416 ± 3

2'-O-subst.	5'-O-subst.	3'-O-TBDMS → 3'-O-			
		H	TMS	Acetyl	TFA
TMS	TMS		−216		
TMS	TBDMS		−209		
TBDMS	H	−307			
TBDMS	TMS	−210			
TBDMS	TBDMS	−259	−203	−197	−412
TBDMS	Acetyl			−216	
TBDMS	TFA				−416
Acetyl	TBDMS			−213	
Acetyl	Acetyl			−209	
Mean ± std. deviation		−259 ± 49	−209 ± 7	−209 ± 9	−414 ± 3

3'-O-subst.	5'-O-subst.	2'-O-TBDMS → 2'-O-			
		H	TMS	Acetyl	TFA
H	TBDMS	−267			
TMS	TMS		−225		
TMS	TBDMS		−217		
TBDMS	H	−238			
TBDMS	TMS		−219		
TBDMS	TBDMS	−267	−211		
TBDMS	Acetyl			−187	
Acetyl	TBDMS			−199	
Acetyl	Acetyl			−178	
Mean ± std. deviation		−257 ± 17	−218 ± 6	−188 ± 11	

Kováts' indices for these derivatives can be used as a basis, rather than hypothetical
values for underivatized nucleosides which do not chromatograph. The reliability of
the data in Table V was tested by using them to calculate expected retention indices
for bis- or tris-TMS derivatives of nucleosides based on the data in Tables I and II.
The results are shown in Table VI. For thymidine, after allowing for temperature
corrections, the largest deviation of the 35 values used for the calculations was only

TABLE V

RETENTION INCREMENTS FOR REPLACEMENT OF O-TMS SUBSTITUENTS OF NUCLEOSIDES BY OTHER SUBSTITUENTS

Subst. X	Position of O-TMS → O-X conversion				
	2'-Deoxynucleosides		Uridine		
	3'	5'	2'	3'	5'
H	14	−13	*	*	*
TBDMS	225	224	218	209	231
TMIPS	477	469			
TMTBS	499	504			
Acetyl	33	29	30	0	28
TFA	−206	−176	decomp.	−205	−185

* Variable.

12 units. Similarly, for uridine, the largest deviation of the 18 values used was only 8 units. Results for 2'-deoxyadenosine were more variable where, after temperature corrections, the largest deviations of the 10 values used were 29 and 23 units for acetyl derivatives. Estimation of reliability for the other nucleosides is uncertain because of the scarcity of experimental values.

TABLE VI

RETENTION INDICES, AT 230°C, FOR 3',5'-BIS-O-TMS-2'-DEOXYNUCLEOSIDES AND 2',3',5'-TRIS-O-TMS-RIBONUCLEOSIDES, CALCULATED FROM INCREMENTS IN TABLE V AND DATA OF TABLES I AND II

Nucleoside	No. of expl. values used	Calcd. retention index		Reliability*		
		Mean ± std. devn.	Max. devn.	Measured index	Mean error	Max. error
Thymidine**	35	2387 ± 5	12	2382	6	12
2'-Deoxyuridine	4	2345 ± 7	9	2336	12	14
2'-Deoxyadenosine	10	2515 ± 15	29	2496	24	48
N6-TMS-2'-deoxyadenosine	4	2607 ± 8	10	2607	7	10
Uridine***	18	2449 ± 5	8	2444	5	12
Adenosine	2	2559 ± 20	14	2573	—	—

* Reliability of retention index calculation of a derivative using measured index for bis- or tris-TMS nucleoside as basis.
** Omitting 3',5'-bis-O-TFA-thymidine.
*** Omitting derivatives with unprotected OH groups.

Table VI also lists the mean and maximum errors in calculating the retention index of a given compound using the increments in Table V and the experimentally measured value for the appropriate bis- or tris-TMS derivative. These calculations appear very reliable for thymidine and uridine derivatives; less so for those of 2'-deoxyadenosine. In the cases of thymidine and uridine at least, the substituent effects and temperature corrections appear to be internally self-consistent.

The retention index of 3',5'-bis-O-trifluoroacetylthymidine is of interest. At 230°C its calculated value is 2000 or 2005, depending on the choice of basis value

(*i.e.* 2382 or 2387) from Table VI. This represents a decrease of 48–53 units from the experimental value of 2053. Although the incorporation of the first trifluoroacetyl group into the molecule gives a normal increment, the incorporation of the second trifluoroacetyl group gives rise to an abnormal increment.

Table VII presents the few available retention increments for derivatization of the nucleobases. The variability of the results makes definitive statements difficult, but it is clear that the effects on retention increments of trimethylsilylation and, particularly, acetylation of adenine moieties differ considerably from those observed for sugar moieties.

TABLE VII

RETENTION INCREMENTS* FOR INTRODUCTION OF SUBSTITUENTS ONTO BASE MOIETIES OF NUCLEOSIDES

Substrate	Substituent introduced				
	TMS	*TBDMS*	*TMIPS*	*TMTBS*	*Acetyl*
2′,3′,5′-tris-O-TMS-U	5				
3′,5′-bis-O-TMS-dA	111				
3′,5′-bis-O-TBDMS-dA	91^{260}	251		613^{260}	
		293^{260}			
3′,5′-bis-O-TMIPS-dA			550^{290}		
3′,5′-bis-O-TMTBS-dA				589^{290}	
3′-O-TBDMS-5′-O-TMS-dA	107				
3′-O-TMS-5′-O-TBDMS-dA	95				
3′-O-TBDMS-5′-O-acetyl-dA					281
3′-O-Acetyl-5′-O-TBDMS-dA					263
2′,3′,5′-tris-O-TMS-A	74				
2′,3′,5′-tris-O-TBDMS-A	37^{260}	238^{260}		538^{290}	

* At 230°C unless otherwise indicated by superscript.

Separation of isomers

2′-Deoxynucleosides. From the results in Table V it it apparent that increments for replacement of TMS substituents by TBDMS, TMIPS, TMTBS or acetyl groups are very similar for the 3′- and 5′-positions. Consequently, isomer mixtures formed from mixed silyl substituents, or mixed acetyl–silyl substituents, will be extremely difficult (if not impossible) to separate on an OV-1 packed column. Increments for conversion of O-TMS groups into OH or O-trifluoroacetyl groups are significantly different for the 3′- and 5′-positions however, and separation and analysis of isomers on OV-1 columns becomes feasible. Fig. 1 illustrates the extent of separation between 3′- and 5′-O-TBDMS thymidines ($\Delta I = 34$) that was achieved on a 3.75-m packed column (5000 theoretical plates nominal). These compounds did suffer a slight amount of thermal decomposition during chromatography[8] and exhibited bad tailing when injected at trace levels. The fully protected derivatives behaved well. For quantitative analysis when it is known that only one of the isomers is present any of the derivatization methods (silylation, acetylation or trifluoroacetylation) can be used. However, for quantitative analysis of isomer mixtures, only trifluoroacetylation will give derivatives which are well separated on a packed column. Alternatively, it will be necessary to use a capillary column or GC–MS with selected ion recording

(the mass spectra of the isomers are characteristically different). Trifluoroacetylation is also the recommended method for analysis of the 3'- and 5'-O-TBDMS isomers of 2'-deoxyadenosine since the method yields products with $\Delta I = 2666 - 2574 = 92$.
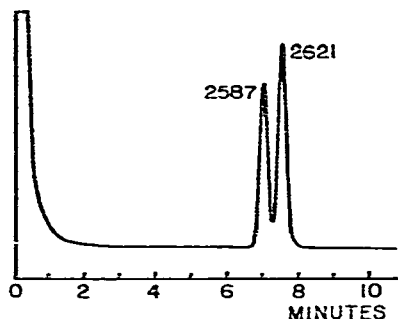

Fig. 1. Gas chromatogram of a mixture of 3'-O-TBDMS-thymidine ($I = 2587$) and 5'-O-TBDMS-thymidine ($I = 2621$). Conditions: 10% OV-1, 3.75 m × 2.4 mm I.D. glass column; 280°C; 30 ml/min nitrogen carrier gas.

Uridine. Inspection of Table V shows that the increments for substitution of TBDMS and acetyl groups for TMS groups at the 2'- and 5'-positions are similar to those for the deoxynucleosides. Increments for substitution at the 3'-position are significantly lower for both groups. Retention increments are quite variable when unprotected OH groups are present (possibly reflecting different degrees of shielding of the various OH groups from the liquid phase).

Acetylation of mixtures of partial O-TBDMS derivatives and total analysis by GC alone would require the use of capillary columns, since ΔI values for some isomer pairs are very small and would require a column with *ca.* 50,000 theoretical plates for effective separations. GC–MS with selected ion recording would be an effective method using packed columns. Trifluoroacetylation is unsuitable because the 2'-O-trifluoroacetyl group promotes decomposition during chromatography[9].

CONCLUSIONS

After application of temperature corrections, retention increments on OV-1 for conversion of O-TMS functions into OH, O-TBDMS, O-TMIPS, O-TMTBS, O-acetyl and O-trifluoroacetyl functions are internally self-consistent for thymidine and uridine. When positional contributions are taken into account, the maximum error in the calculation of the Kováts' index is 12 units for derivatives of either nucleoside. Similar calculations lead to a maximum error of 48 units for 2'-deoxyadenosine derivatives.

ACKNOWLEDGEMENT

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