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GAS–LIQUID CHROMATOGRAPHIC ANALYSIS OF TRIMETHYLSILYL DERIVATIVES OF PYRIMIDINE AND PURINE BASES AND NUCLEOSIDES

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SUMMARY

A systematic study of basic nucleic acid components and their biologically significant derivatives has been carried out. The optimum silylation conditions have been found and the chromatographic behaviour of the trimethylsilyl derivatives formed has been studied. Relations between the gas chromatographic behaviour and the structure of the substances analyzed were investigated on the basis of the retention indices measured on two stationary phases of different polarity. The relative molar responses were determined for the quantitative determination of the substances. The results make possible the determination of biologically active substances in pharmaceuticals.

INTRODUCTION

The pyrimidine and purine bases, uracil, thymine, cytosine, adenine and guanine, ordered in RNA and DNA chains, represent important information necessary for the harmonic biosynthesis of proteins. Modified bases and nucleosides, if artificially introduced in place of the original RNA components, can change the genetic code of amino acids and thus indirectly affect the composition and enzymatic properties of the proteins synthesized. Some derivatives of bases and nucleosides can thus suppress or completely stop carcinogenic cell growth and therefore are employed in the treatment of malignant tumours.

In view of the exceptional importance of these substances, it is imperative to know their structure, properties and composition. Many methods for the solution of these problems have been developed[1–32]. These are chiefly chromatographic methods[4–32], among which gas chromatography occupies an important place. The latter method enables the determination of nanogram amounts of the substances with a precision

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of better than ± 5% and with analysis times shorter than those required by other methods.

Gas chromatography requires that all the analyzed substances be sufficiently volatile. Compounds with higher molecular weights and polyfunctional groups cannot be analyzed directly as they are highly polar and poorly volatile. Nucleic acid components must be converted into less polar derivatives with lower boiling points before the gas chromatographic analysis itself. Methyl, acetyl and isopropylderivatives were tested first[12–14]; they were not very advantageous, since a single substance yielded several derivatives.

The first application of trimethylsilyl (TMS) derivatives of the basic components of nucleic acids in gas chromatography was carried out by Hancock and Coleman[15,16]. They succeeded in separating the basic nucleosides, although the TMS derivatives of adenosine and cytidine yielded several elution peaks, probably owing to incomplete silylation of the amino group. Hashizume and Sasaki[17–19] published works dealing with the gas chromatographic analysis of bases, nucleosides and nucleotides. They used a mixture of hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS) and pyridine for silylation. All bases and nucleosides tested yielded single derivatives after silylation performed by this method. However, the authors did not manage to separate TMS-3′-nucleotides from TMS-2′-nucleotides and TMS-cytidine monophosphates did not yield any chromatographic peak.

Gehrke and co-workers[20,21] first used the silylation reagent N,O-bis(trimethylsilyl)acetamide (BSA) in acetonitrile for the preparation of TMS derivatives, obtaining higher yields than those with HMDS–TMCS–pyridine. They found the optimum silylation conditions under which all the substances (except cytosine and 5-methylcytosine, which gave two derivatives) yielded single elution peaks on a column with the SE-30 stationary phase. The authors obtained even better results with the silylation reagent N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), enabling the use of shorter heating times during the silylation reaction. Muni *et al.*[22] measured the relative molar responses of selected bases and nucleosides; N^6-methyladenine and N^6-methyladenosine gave two chromatographic peaks after silylation with BSA.

Iwase *et al.*[23] studied the gas chromatographic analysis of hypoxanthine and guanine. They compared various silylation reagents for derivatization of these substances and found that trimethylsilylimidazole was unsuitable, while the TMS amines and amides showed good silylation effects. They further noticed that the presence of any ions in solution, except NH_4^+, considerably decreased the response of the TMS derivatives.

Nucleosides were studied in detail by Hancock[24], Jacobson *et al.*[25] and Butts[26]. During silylation of adenosine derivatives[24], small amounts of tetra(TMS) derivatives were formed in addition to stable tris(TMS) derivatives obtained by the silylation of the carbohydrate component. Even the TMS derivatives of guanosine and cytidine were prepared[25], which are two of the nucleosides silylated with the greatest difficulty. Better results were obtained with cytidine and desoxycytidine by converting the amino groups of the nucleosides into the methoxime derivatives with successive silylation of the carbohydrate component[26]. However, the application of this method to 2′- and 3′-cytidine monophosphate was unsuccessful. BSTFA has proven advantageous for silylation of less common nucleosides —dihydrouridine, pseudouridine, methylinosine, 6-thioguanosine and methylguanosine[27]. Relations between the structure and

the chromatographic behaviour on two stationary phases were studied by Hattox and McCloskey[28]. The good chromatographic properties of the TMS derivatives of nucleic acid components enabled their determination in RNA and DNA hydrolysates[21,29,30].

While relatively great attention has been paid to the gas–liquid chromatographic (GLC) analysis of the basic nucleic acid components, only very few papers have dealt with biologically important derivatives of pyrimidine and purine bases. Pacáková *et al.*[31] studied the qualitative analysis of 6-aza and 5-halogenoderivatives of pyrimidine bases while Pantarotte *et al.*[32] described the application of GLC to some bases and nucleosides in biological materials.

Athough detailed studies on the GLC analysis of the basic nucleic acid components have already been published, there still remain unsolved problems connected with defined conversion into volatile derivatives, followed by their determination. A number of important derivatives of basic pyrimidine and purine bases have not yet been studied by gas chromatography. Consequently, the systematic study of basic nucleic acid components and their biologically significant derivatives was carried out. The course of the silylation was monitored, relations between structure and the gas chromatographic data were studied and procedures for the quantitative determination have been worked out.

EXPERIMENTAL

Materials

The following chemicals were used: uracil, cytosine, 5-bromocytosine, 6-azathymine, thymine, 5,6-dihydrothymine, hypoxanthine, xanthine, adenine, guanine, uridine (Lachema, Brno, Czechoslovakia); 5-bromouracil, 5-iodouracil, 6-azauracil, thymidine, adenosine, desoxyadenosine, guanosine, cytidine, desoxycytidine (Calbiochem, Los Angeles, Calif., U.S.A.); 5,6-dihydrouracil, 5-methylcytosine hydrochloride (Sigma, St. Louis, Mo., U.S.A.); 5-fluorouracil (Hoffman La Roche, Basel, Switzerland); 6-azathymine (Fluka, Buchs, Switzerland); desoxyguanosine (Loba-Chemie, Wien, Austria); HMDS (Koch-Light, Colnbrook, Great Britain); TMCS (Lachema); BSTFA (Pierce, Rockford, Ill., U.S.A.); OV-17, OV-101 (Pierce); AW-DMCS Chromosorb W HP, 100–120 mesh (Carlo Erba, Milan, Italy); and pyridine, p.a. (Lachema).

Preparation of the TMS derivatives

The reagents for the preparation of the TMS derivatives were dried and the silylation reactions were performed in sealed glass ampoules. The solutions were stirred with a magnetic stirrer and heated on an oil bath regulated with a precision of $\pm$ 1°.

In the method employing HMDS, TMCS and pyridine, 0.5 ml of the silylation mixture (3:1:3) was added to *ca.* 1 mg of the dried sample and the mixture was heated at 150° for the required time.

In the method employing BSTFA, 0.5 ml of this reagent was added to the same amount of sample; up to 0.2 ml of pyridine were added, according to the sample (see below). The samples were then heated at 150° for 15 min to 3 h.

Gas chromatographic measurements

All measurements were carried out on a Packard gas chromatograph, Model 7409, equipped with a dual column system, an FID and with temperature programming. Glass columns, 2 m × 0.4 cm I.D., were used. The columns were packed with AW-DMCS Chromosorb W HP (100–120 mesh), coated with 3% OV-101 or 3% OV-17. The column temperatures were 160° for pyrimidine bases, 190° for purine bases and 260° for nucleosides; the injection block temperature was 240° for pyrimidines and purines, and 280° for nucleosides. The detector temperature was 250° for pyrimidines and purines and 280° for nucleosides. Argon carrier gas was used at a flow-rate of 60 ml/min.

RESULTS AND DISCUSSION

Study of the silylation of pyrimidine and purine bases and of nucleosides

The preparation of TMS derivatives of the bases and nucleosides is relatively simple. The silylation reaction proceeds unambiguously with many substances. More complicated reactions have been encountered with compounds containing the amino functional group, such as cytosine, adenosine and guanosine. In these cases several derivatives are formed.

However, derivatization can cause further errors in quantitative chromatographic analysis, owing to an irreproducible silylation reaction and instability of the TMS derivatives formed[33]. Even atmospheric humidity can cause decomposition. Therefore, defined conditions must be maintained during both silylation and chromatographic analysis.

In order to prevent hydrolysis, all silylation reactions had to be carried out in sealed ampoules and dried reagents had to be added with pre-dried syringes during sample preparation and injection. All gas chromatographic analyses had to be performed in an all-glass apparatus, as decomposition of TMS derivatives has been observed on contact with heated metallic parts[34].

Anomalous phenomena also occurred during the detection of the TMS derivatives of the nucleic acid components by the FID. If more than 25 μg of a TMS derivative were injected, the elution curve exhibited two peaks, this being similar to the situation encountered previously during the determination of tris-(TMS) phosphate. This is probably caused by temporary cooling of the flame during combustion of organosilicon substances[35].

Another source of error can arise through adsorption on the support. Therefore, exclusively silanized supports must be used. Under these conditions the peaks of the silylation reagents and of the analyzed TMS derivatives were sharp and symmetrical and hence suitable for quantitative analysis.

Optimization of the silylation reaction conditions

The course of the silylation is determined not only by the structure of the substances to be determined, but also by the kind and amount of the silylation reagent, the solvent employed and the reaction conditions.

Two silylation methods were compared for the preparation of the TMS derivatives. The method using HMDS–TMCS–pyridine (3:1:3) and the one using BSTFA. The advantage of the former method lies in the fact that both the silylation reagents

are relatively cheap; on the other hand, BSTFA is a stronger TMS-donor and silylation proceeds faster. The two derivatization methods were compared using cytosine, which is silylated with difficulty and for which differing silylation data have been published in the literature[21,36,37].

Cytosine samples were silylated by the two methods at a temperature of 150°, for 5–60 min, using a 100-fold molar excess of the silylation reagents[21]; the relative molar responses (RMR) were determined with respect to phenanthrene. The results obtained are given in Fig. 1.

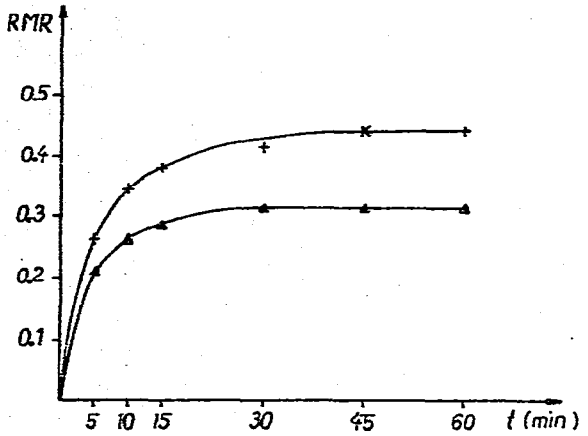


Fig. 1. The dependence of the RMR for cytosine on the silylation time using HMDS–TMCS–pyridine (3:1:3) (▲) and the BSTFA (+), without stirring.

It is obvious from the results that higher RMR values and thus higher yields of the silylation reaction were attained using the BSTFA method. A lower, but constant, RMR is attained after 30-min heating when HMDS and TMCS in pyridine are used. An increase in the RMR was observed even after 60-min. heating during silylation with BSTFA (see Fig. 1), owing to slow dissolution of cytosine in BSTFA. In order to increase the reaction rate, the silylation solution was stirred. As can be seen from Fig. 2, the heating time could then be shortened and the reaction yield increased. Stirring had no effect on silylation with HMDS and TMCS in pyridine.

The optimum conditions found for cytosine, *i.e.* silylation in a stirred solution with a 100-fold molar excess of BSTFA in a sealed ampoule at 150° for 15 min, were also employed for silylation of the other pyrimidine bases. In this way, the TMS derivatives of uracil, 5-fluorouracil, 5-bromouracil, 5-iodouracil, 5-nitrouracil, 5-hydroxymethyluracil, 6-azauracil, 5,6-dihydrouracil, thymine, 6-azathymine, 5,6-dihydrothymine, 5-methylcytosine, 5-bromocytosine and N-1-(2′-furanidyl)-5-fluorouracil (Ftorafur) were prepared.

The purine bases and nucleosides were silylated in an analogous manner. Adenine and adenosine were employed as model substances. The conversion of adenine and adenosine into the TMS derivative proceeded more slowly compared with that of cytosine. Therefore the reaction was hastened by adding pyridine (see Fig. 3). A constant RMR was attained after 15 min heating for adenine and after 30 min for adenosine.

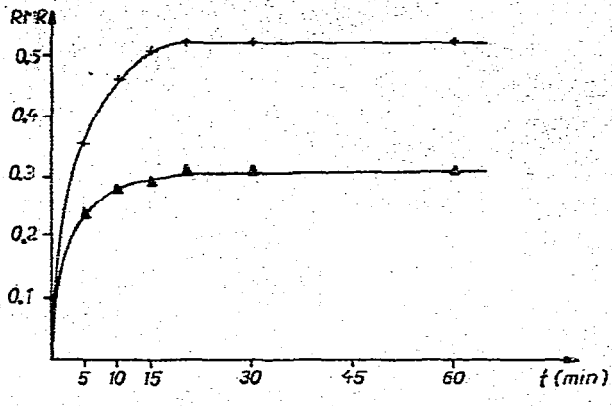


Fig. 2. The dependence of the RMR for cytosine on the silylation time using HMDS–TMCS–pyridine (3:1:3) (▲) and the BSTFA (+), with stirring.

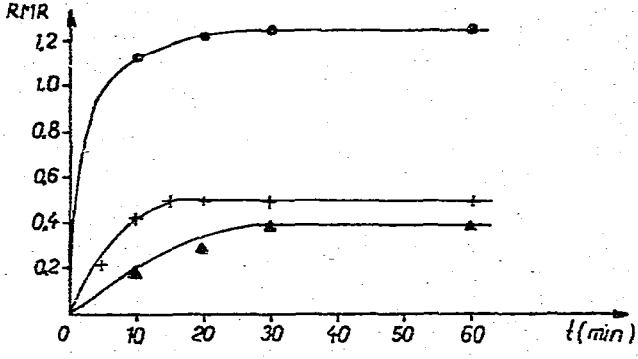


Fig. 3. The dependence of the RMR on the heating time for adenine with BSTFA–pyridine (+) and BSTFA (▲), and for adenosine with BSTFA–pyridine (●).

The conditions used for silylation of adenine and adenosine were also used in silylation of other purine bases (hypoxanthine, xanthine, guanine and allopurinol) and in the preparation of the TMS derivatives of ribonucleosides (uridine, cytidine, guanosine, 6-azauridine) and desoxyribonucleosides (thymidine, desoxycytidine, desoxyadenosine and desoxyguanosine).

Chromatographic behaviour of the TMS derivatives of the nucleic acid components

The prepared TMS derivatives were chromatographed on the OV-101 and OV-17 stationary phases, at the optimum temperatures of 160, 190 and 260° for pyrimidine bases, purine bases and nucleosides, respectively. The course of the silylation was verified by gas chromatography and the structure of these substances and their gas chromatographic behaviour were correlated using the retention indices measured on two stationary phases with different polarities. The data obtained were employed for the development of analytical procedures for separations of mixtures, and RMR values with respect to phenanthrene were determined for quantitative purposes. The retention data for all the substances studied are summarized in Tables I–III for pyrimidine bases (Table I), purine bases (Table II) and nucleosides (Table III).

TABLE I

RETENTION INDEXES OF TMS-PYRIMIDINE BASES

Stationary phases, OV-101 and OV-17; column temperature, 160°.

Compound	*Molecular weight*	$I_{OV\text{-}101}$	$I_{OV\text{-}17}$	ΔI
Uracil	112.1	1325.5	1448.4	122.9
5,6-Dihydrouracil	114.1	1328.2	1448.0	119.8
6-Azauracil	114.1	1449.9	1573.9	124.0
5-Fluorouracil	130.1	1317.9	1420.5	102.6
5-Bromouracil	191.0	1529.1	1655.2	126.1
5-Iodouracil	238.0	1595.8	1772.9	177.1
5-Hydroxymethyluracil	142.1	1673.0	1756.8	83.8
5-Nitrouracil	157.1	1649.8	1809.6	159.8
Thymine	126.1	1396.7	1507.9	111.2
5,6-Dihydrothymine	128.1	1395.9	1508.0	112.1
6-Azathymine	128.1	1459.8	1615.4	155.6
Cytosine	111.1	1509.6	1696.4	186.8
5-Methylcytosine	125.1	1536.3	1706.9	170.6
5-Bromocytosine	190.0	1615.6	1760.0	144.4
Ftorafur	200.2	1318.7	1420.0	101.3

TABLE II

RETENTION INDEXES OF TMS-PURINE BASES

Stationary phases, OV-101 and OV-17; column temperature, 190°.

Compound	*Molecular weight*	$I_{OV\text{-}101}$	$I_{OV\text{-}17}$	ΔI
Hypoxanthine	136.1	1798	2037	239
Adenine	135.1	1849	2073	224
Xanthine	152.1	2010	2261	251
Guanine	151.1	2106	2298	192
Allopurinol	136.1	1611.7	1722.7	110

TABLE III

RELATIVE RETENTION OF TMS-NUCLEOSIDES

Stationary phases, OV-101 and OV-17; column temperature, 260°.

Compound	$r_{1,2(OV\text{-}101)}$	$r_{1,2(OV\text{-}17)}$
Thymidine	1.00	1.00
6-Azauridine	—	0.66*
Uridine	1.17	1.00
Adenosine	2.19	1.51
Desoxyadenosine	1.72	1 62
Guanosine	2.70	2.26
Desoxyguanosine	2.57	2.50
Cytidine	2.70	4.16

* Column temperature, 225°.

Pyrimidine bases

Uracil and its derivatives. The TMS derivative of uracil can easily be determined chromatographically on both the stationary phases, because of its high volatility. The halogen derivatives of uracil yield a single derivative on silylation, which can also be chromatographed on OV-101 and OV-17. The retention indices of the individual halogen derivatives on both stationary phases increase with increasing molecular weight (see Table I). 5-Fluorouracil is an exception; it is eluted before uracil on both stationary phases. Faster elution of the TMS derivative of 5-fluorouracil is caused by its higher volatility, due to introduction of the fluorine atom into the uracil molecule.

5-Nitro- and 5-hydroxymethyluracil were also successfully converted into a single TMS derivative, thus enabling their gas chromatographic analysis. The elution order of the TMS-derivatives was 5-hydroxymethyluracil followed by 5-nitrouracil on the more polar phase, OV-17, and was reversed on the less polar phase, OV-101. In order to explain the retention behaviour, the retention indices on the two columns were compared. The more polar nitro group caused the higher retention of the TMS derivative with respect to uracil on OV-17 compared to OV-101. (The difference in the retention indices of the TMS derivatives of 5-nitrouracil and uracil is 361.2 on OV-17, while it is 324.3 units on OV-101). The longer retention time of the TMS derivative of 5-hydroxymethyl uracil on the non-polar phase with respect to the TMS derivative of uracil can be explained by assuming that tris(TMS) derivative is formed during derivatization due to silylation of the hydroxymethyl functional group. The molecular weight is thus increased and the polarity decreased. Both stationary phases can be employed for separation of the TMS derivatives of 5-hydroxymethyl- and 5-nitrouracil.

The TMS derivative of 6-azauracil yields a single sharp non-tailing peak. The retention indices of the aza derivative on both stationary phases, compared with those of the TMS derivative of uracil, indicate that the replacement of the C-6 carbon by nitrogen in the uracil molecule leads to equal increases in the retention indices on the two phases. Separation of the TMS derivatives of uracil, 5-fluoro-, 5-bromo-, 5-iodouracil and 6-azauracil on the OV-17 stationary phase is depicted in Fig. 4.

5,6-Dihydrouracil is readily silylated with formation of a single derivative. The retention indices on the two phases used were virtually identical (see Table I). In contrast to the data given by Butts[36], it was impossible to separate the TMS derivative of 5,6-dihydrouracil from TMS-uracil, even with temperature programming.

Ftorafur, which is used as an anticarcinogenic substance, was also successfully converted into its TMS derivative. It was eluted in a single sharp, non-tailing peak on both stationary phases used; this behaviour has enabled analytical application to pharmaceuticals[38].

Thymine and its derivatives. The TMS derivative of thymine resembles that of uracil because of its volatility and is eluted in a single sharp, non-tailing peak on both OV-101 and OV-17. It can be seen from the difference in their retention indices on the two phases (see Table I) that the difference in the molecular weights of thymine and uracil, due to the methyl group, has a more pronounced effect on the non-polar phase than on the phase of medium polarity.

6-Azathymine, similar to 6-azauracil, can also be converted into the TMS derivative yielding a single, well-developed elution peak. The different chromatographic

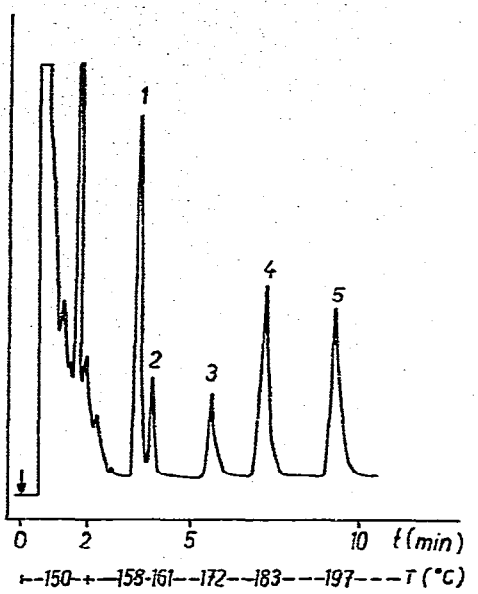


Fig. 4. Chromatogram of a mixture of the TMS derivatives of 5-fluorouracil (1), uracil (2), 6-azauracil (3), 5-bromouracil (4) and 5-iodouracil (5). Stationary phase, OV-17; temperature program, 2 min at 150°, then at 7°/min to 200°.

behaviour, *i.e.* the higher retention of the TMS derivative of 6-azathymine on the more polar phase ($\Delta I_{\text{6-aza-T}} - \text{6-aza-U} = 41.5$, compared with a ΔI value of 9.9 for the same substances on OV-101), can be explained by the increased basicity of the 1,3,6-triazine ring owing to the effect of the methyl group.

5,6-Dihydrothymine exhibits similar behaviour to 5,6-dihydrouracil. In agreement with theoretical assumptions, its TMS derivative cannot be separated from that of thymine (see Table I). This finding is again at variance with the results published by Butts[36], who found a difference in the retention indices on phases OV-101 and OV-17 equal to 141 units.

The separation of a mixture of the TMS derivatives of thymine and 6-azathymine is illustrated in Fig. 5.

Cytosine and its derivatives. Cytosine, 5-methylcytosine and 5-bromocytosine were the pyrimidine bases most difficult to silylate, because of the amino group bound to the C_4 carbon. During the chromatographic process, TMS-cytosine was eluted in a single peak, which was sharper and tailed less on the OV-17 column. The longer elution time on this column also permits perfect separation of cytosine from the initial peak of the silylation reagent under the given conditions. The formation of a single volatile derivative with the same retention indices was verified in the work of Butts[36]. On the other hand, Gehrke and Ruyle[21] found that two derivatives are formed during silylation with BSTFA in acetonitrile. The more volatile compound (corresponding to silylation of both the amino and the hydroxyl groups) was identical with the derivative prepared in the present paper using HMDS and TMCS in pyridine or using BSTFA.

The differences in the retention indices of TMS-cytosine and TMS-uracil can

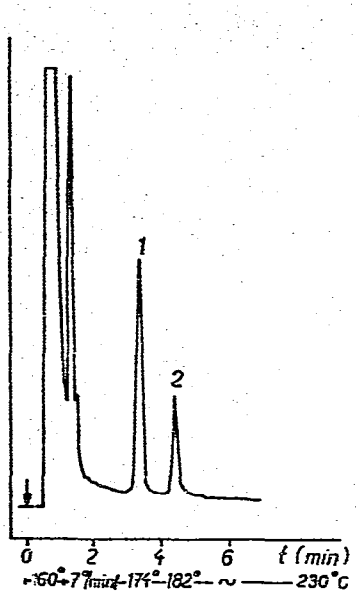


Fig. 5. Chromatogram of a mixture of the TMS-derivatives of thymine (1) and 6-azathymine (2). Stationary phase, OV-17; temperature program, 1 min at 160°, then at 7°/min to 200°.

be explained on the basis of their structure. The replacement of the uracil hydroxyl group by the cytosine amino group leads to an increase in the retention indices of TMS-cytosine compared with TMS-uracil, on non-polar phase OV-101 and, especially, on medium polar phase OV-17. The increased retention on OV-17 can be explained by the fact that only a single hydrogen is silylated in the amino group.

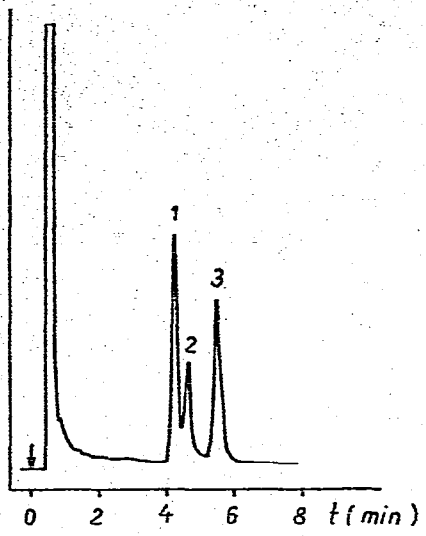
5-Methylcytosine was available as the hydrochloride and therefore pyridine was employed to liberate the base from the salt form during silylation with BSTFA. In agreement with Butts[36], a single volatile derivative was obtained, while Gehrke and Ruyle[21] obtained two volatile derivatives, similar to cytosine. The methyl group, analogous to thymine, increases the retention of the TMS derivative of 5-methylcytosine on the non-polar stationary phase.

Among halogen derivatives, 5-bromocytosine was studied and was relatively easy to convert into the TMS derivative and to determine chromatographically. The separation of TMS-cytosine and its 5-methyl and 5-bromo derivatives is depicted in Fig. 6.

Purine bases

The principal purine bases, xanthine, hypoxanthine, adenine and guanine and derivative allopurinol, were studied. All the substances were more difficult to dissolve in the silylation reagents. The prepared TMS derivatives were chromatographed on stationary phases OV-101 and OV-17. The retention indices are given in Table II.

The retention indices on both stationary phases increased with increasing number of hydroxyl and amino groups on the purine ring, in the order, hypoxanthine, adenine, xanthine and guanine. Replacement of the hydroxyl group of hypoxanthine and xanthine by the amino group, analogous to cytosine, led to lenghtening of retention times at a constant molecular weight. The separation of all the principal bases is very good on the two stationary phases (see Fig. 7).



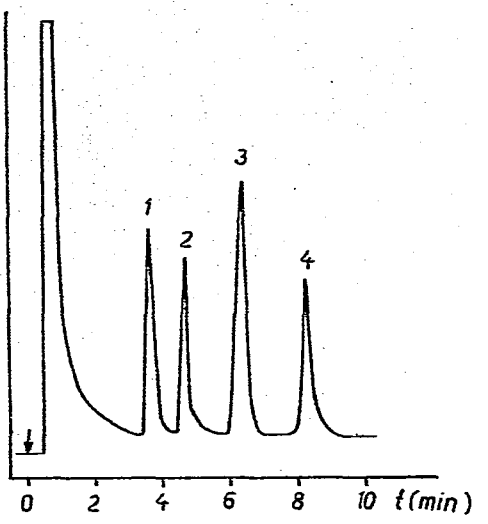


Fig. 6. Chromatogram of a mixture of the TMS derivatives of cytosine (1), 5-methylcytosine (2) and 5-bromocytosine (3). Stationary phase, OV-17; temperature, 160°.

Fig. 7. Chromatogram of a mixture of the TMS derivatives of purine bases hypoxanthine (1), adenine (2), xanthine (3) and guanine (4). Stationary phase, OV-17; temperature, 190°.

The unambiguous silylation course of allopurinol and good chromatographic properties of the TMS derivative formed have been utilized in its determination in pharmaceuticals[38].

Nucleosides

Similar to the purine bases, silylation of nucleosides with BSTFA required the presence of pyridine to hasten dissolution. All the nucleosides studied yielded single TMS derivatives, which gave rise to sharp peaks on the chromatogram. Cytidine was exceptional; its peak showed slight tailing on the polar phase, because of its high retention time. The relative retention times with respect to thymidine were measured on both stationary phases (see Table III).

Ribonucleosides were eluted on the more polar phase in the order, uridine, adenosine, guanosine and cytidine and their separation was very good (see Fig. 8). Guanosine and cytidine had identical retention times on the non-polar phase and could not be separated even when the temperature was programmed.

The elution order of desoxyribonucleosides was the same on the two columns, namely, thymidine, desoxyadenosine and desoxyguanosine. Desoxycytidine did not yield a TMS derivative even after heating for several hours, although it dissolved in the silylation reagents.

Desoxyribonucleosides have shorter elution times than the corresponding ribonucleosides on the non-polar stationary phase, while the opposite is true on the more polar phase, OV-17 (see Table III). The pyrimidine nucleosides, except cytidine, are eluted before the purine nucleosides on both stationary phases.

6-Azauridine was again studied from the point of view of using its TMS derivative in analytical application to pharmaceuticals[38]. It was relatively easy to prepare

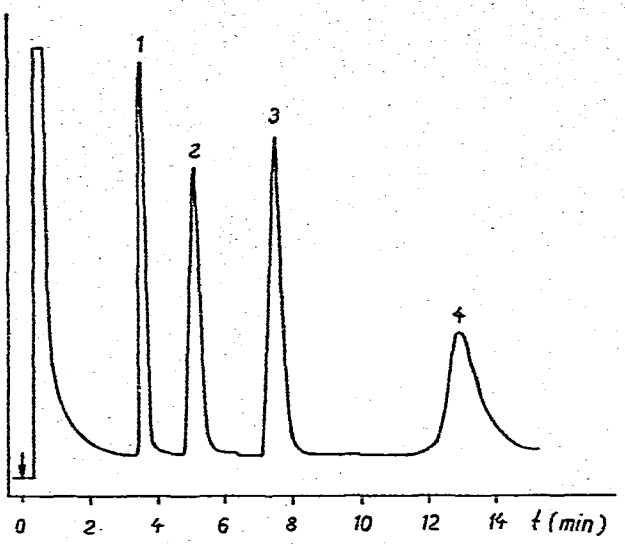


Fig. 8. Chromatogram of a mixture of the TMS derivatives of ribonucleosides uridine (1), adenosine (2), guanosine (3) and cytidine (4). Stationary phase, OV-17; temperature, 260°.

a single TMS derivative, which exhibited favourable chromatographic properties on phase OV-17.

Relative molar responses of some pyrimidine and purine bases and of nucleosides

In order to quantitatively determine the TMS derivatives of the studied substances, their RMR values had to be determined. The RMR values related to phenanthrene are given in Table IV.

As is evident from Table IV, a great majority of the RMR values for the derivatives of pyrimidine and purine bases lie between 0.5 and 0.6. Substantially lower RMR

TABLE IV

RELATIVE MOLAR RESPONSE OF TMS-BASES AND NUCLEOSIDES

Compound	*RMR*	*Compound*	*RMR*
Uracil	0.580	Hypoxanthine	0.548
5,6-Dihydrouracil	0.542	Adenine	0.504
6-Azauracil	0.113	Xanthine	0.548
5-Fluorouracil	0.514	Guanine	0.118
5-Bromouracil	0.685		
5-Iodouracil	0.669	Thymidine	0.517
5-Hydroxymethyluracil	0.363	6-Azauridine	0.672
5-Nitrouracil	0.083	Uridine	0.901
Thymine	0.606	Adenosine	1.263
5,6-Dihydrothymine	0.604	Desoxyadenosine	0.556
6-Azathymine	0.162	Guanosine	0.580
Cytosine	0.527	Desoxyguanosine	0.358
5-Methylcytosine	0.553	Phenanthrene	1.000
5-Bromocytosine	0.654		
Phenanthrene	1.000		

values were found for the TMS derivatives of 5-nitrouracil, 6-azauracil and 6-azathymine, *i.e.* for substances containing another heteroatom in their molecules. The low RMR value for TMS-guanine can be explained by incomplete conversion during silylation or by adsorption in the chromatographic system.

Higher response values of nucleosides in the FID, compared with the pyrimidine and purine bases, can be ascribed to the presence of ribose or desoxyribose in their molecules.

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